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Research article

Antioxidant and anti-aging enzyme activities of bioactive compounds isolated from selected Zingiberaceae plants

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Abstract

Bioactive compounds and antioxidant and enzyme inhibition activity levels were evaluated for three plants in the Zingiberaceae family in Thailand. Quantification of the phenolic and curcumin contents revealed that the largest component of all extracts was phenolic compounds (213.16–317.64 mg/g extract). The major curcuminoids in *Curcuma longa* L., *Curcuma aromatica* Salisb. and *Zingiber montanum* (J.Koenig) Link ex A.Dietr. were bisdemethoxycurcumin (BDMC; 27.31 mg/g extract), curcumin (16.59 mg/g extract), and curcumin (0.12 mg/g extract), respectively. The extracts from *C. longa*, *C. aromatica* and *Z. montanum* all showed antioxidant activity that was assessed using 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl ($DPPH^{\bullet}$) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging assay. In addition, all extracts had copper chelating activity of more than 50%, with the extract from *C. longa* having the highest chelating activity (76.45%). All extracts from the three plants inhibited tyrosinase and elastase activity with half maximal inhibitory concentration values of 290.33–1,373.68 µg/mL and 69.61–3386.23 µg/mL, respectively. In addition, collagenase inhibition activity was observed in all extracts. The findings from this study showed that extracts from *C. longa*, *C. aromatica* and *Z. montanum* have potential antioxidant activity and can act as anti-tyrosinase, anti-collagenase and anti-elastase agents. The extracts could be further applied in cosmeceuticals as an active ingredient for anti-aging products.

Introduction

The Zingiberaceae family contains tropical or subtropical plants that can be found in South East Asia (Saensouk et al., 2016). This family consists of about 120 species that have been used as folk medicine for the treatment of diseases (Srivilai et al., 2017). The phytochemicals from Zingiberaceae species have been extracted from several plant parts, especially the rhizome (Rachkeeree et al., 2020). The rhizome from this family is also known as turmeric in which the main bioactive compounds are essential oils and curcuminoids composed of curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (Funk et al., 2010; Lee et al., 2014; Kukula-Koch et al., 2018). The extracts have health-promoting properties that have been added to beverages, processed foods, nutraceuticals and cosmetics (Kieliszek et al., 2020; Rachkeeree et al., 2020). Previous research has proven that curcuminoids have antioxidant, antiviral, antibacterial, and anti-cancer properties (Zhou et al., 2019). In Thailand, the rhizome extracts are widely used in dietary intake as well as in traditional medicine and herbs, including extracts from *C. longa* L., *C. aromatica* and *Z. montanum* (Rachkeeree et al., 2020). According to Anjusha and Gangaprasad (2014), the rhizome extracts with ethanol contained curcumin at 0.0175 g/100 g

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for *C. longa* and ethyl acetate extracts of *Z. montanum* had a curcumin content in the range 0.634–0.89 g/100 g. Phytochemical compounds such as flavonoids, tannin, saponin, sterols and terpenoids were observed in extracts of *C. aromatica* (Anjusha and Gangaprasad, 2014). The bioactive compound in rhizome extracts showed various bioactivities, particularly antioxidant activity and enzyme inhibition activity (Verma et al., 2018). The antioxidant of extract from *C. longa* increased with increasing curcumin content with values of 80 µM Trolox/g extract. Extracts of *Z. montanum* have demonstrated *in vitro* antioxidant activity and were anti-inflammatory. The antioxidative compounds isolated from the rhizomes of *Z. montanum* were complex curcuminoids, such as cassumunins A, B and C (Masuda and Jitoe, 1994) and cassumunarins A, B and C (Jitoe et al., 1994). The extracts from *Z. montanum* showed inhibition of metal ions, including $CuCl_2$, $CoCl_2$, $HgCl_2$ and $ZnCl_2$ (Jamir and Seshagirirao, 2018). Thokchom and Sharma (2012) reported that methanol extract from *Z. montanum* displayed DPPH$^{\bullet}$ radical scavenging activity with a half maximal inhibitory concentration (IC_{50}) value of 0.34 mg/mL. In addition, *Z. montanum* extract exhibited a high DNA protection rate of 81.87%. Plants in the Zingiberaece family in Thailand have been used for antifungal, anti-bacteria, anti-inflammatory or food ingredients (Rachkeeree et al., 2020). Thus, three selected plants (*C. longa* L., *Curcuma aromatica* Salisb. and *Zingiber montanum* (J.Koenig) Link ex A.Dietr.) were studied for their bioactive compounds and antioxidant, anti- tyrosinase, anti-collagenase and anti-elastase abilities. The findings from the current research could contribute to the discovery of useful bioactive compounds.

Materials and Methods

Plant materials and sample extraction

Rhizomes of *C. longa*, *C. aromatica* and *Z. montanum* were purchased from Vejpongosot Holding Co., Ltd, Thailand. The samples were cut into small pieces (6 mm × 6 mm), and then samples were extracted with 50% ethanol (sample:solvent, 1:5 weight per volume). The mixture was agitated using an orbital shaker at 150 rpm for 24 hr. After that mixture was passed through filter paper (Whatman, No.1) and then the supernatant was evaporated using a rotary evaporator at 45ºC. The yield of extracts was calculated based on the fresh weight of the plant (Equation 1):

$$\% \text{ Yield of extracts} = \frac{\text{Weight of extracte}}{\text{Weight of plant}} \times 100 \quad (1)$$

The extracts were dried using a spray drying method and stored in a dry place until analysis.

Determination of total phenolic compounds

The total phenolic compounds were determined using Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977), with some modifications. Each extract (1 mg/mL) of 20 µL was mixed with 100 µL of 10% Folin-Ciocalteu reagent in a 96-well plate and was neutralized with 80 µL of 7.5% sodium carbonate solvent. Each mixture was kept at room temperature for 1 hr; then, the color was measured using an ultraviolet-visible (UV/Vis) spectrophotometer in a microplate reader at a wavelength of 760 nm. The total phenolic content was expressed as the concentration of gallic acid equivalent which was calculated using the linear equation of gallic acid (40–200 µg/mL).

Quantification and qualification of curcuminoids high-performance liquid chromatography

The curcuminoids in extracts were performed using high-performance liquid chromatography (HPLC; Agilent 1200; USA) equipped with a diode array detector according to Perko et al. (2015) with slight modifications. An extract sample (10 µL) was injected into the column (Agilent Eclipse XDB-C18; USA) with dimensions 150 nm × 4.6 mm, 5 µm practical size and the flow rate was 1 mL/min. The mobile phase was 2% acetic acid in water (solvent A) and 2% acetic acid in acetonitrile (solvent B) in a gradient system which in the ratios 90:10, 80:20, 75:25 and 65:35 at 3, 8, 13 and 18-40 min, respectively. The absorbance was measured at 420 nm.

Color and appearance of extracts

The color of the rhizome extracts was measured using a colorimeter (Portable colorimeter, NH 300; PRC) according to Jiménez-Aguilar et al. (2011). The color presentation followed the CIE L^* a^* b^* system which is the color space defined by the Commission internationale de l'éclairage (Yuan et al., 2007), where the L^* value represents the lightness of color with a range from 0 (black) to 100 (white), a^* is the chroma of green (–) to red (+) and b^* is the chroma of blue (–) to yellow (+). Hue is a color appearance that is expressed in degrees from 0 to 360. The values of red, yellow, green, and blue were 0, 90, 180 and 270, respectively.

Antioxidant properties

Determination of 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH$^{\bullet}$) radical scavenging activity was carried out as proposed by Brand-Williams et al. (1995), with slight modification. Briefly, a 96-well plate was filled with 50 µL of extracts mixed with 150 µL of DPPH solution (0.16 mM). The plate was incubated in a dark place at room temperature for 30 min. After that, absorbance was measured at 515 nm (UV/Vis spectrophotometer in a microplate reader). Ascorbic acid and Trolox were used as positive controls. The percentage of DPPH radical scavenging was calculated from linear regression analysis using Equation 2:

$$\text{DPPH radical scavenging (\%)} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100 \quad (2)$$

where A_{blank} is the absorbance of the DPPH solution and A_{sample} is the absorbance of the extract.

The inhibition concentration (IC_{50}) was calculated using regression analysis between concentrations (10–450 µg/mL) and the percentage inhibition.

Determination of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging activity followed the method of Cai et al. (2004), with some modifications. The $ABTS^{\bullet+}$ solution (7 mM of $ABTS^{\bullet+}$ in distilled water) was mixed with potassium persulfate ($K_2S_2O_8$; 2.45 mM in distilled water) in the ratio 2:1. The mixture was incubated in a dark place at room temperature for 16 h. The $ABTS^{\bullet+}$ radical solution was diluted with ethanol in the ratio 1:15 to obtain absorbance of 0.7 ± 0.05 at 734 nm. After that, the $ABTS^{\bullet+}$ radical solution (150 µL) was added to extracts (50 µL) in a 96-well plate. The mixture was incubated at room temperature for 6 min, then absorbance was measured at 734 nm. The percentage of $ABTS^{\bullet+}$ scavenging activity was calculated using Equation 3 and the IC_{50} values were determined:

$$\text{ABTS radical scavenging (\%)} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100 \quad (3)$$

where A_{blank} is the absorbance of the ABTS solution and A_{sample} is the absorbance of the extract.

Determination of copper chelating activity

The copper chelating activity of extracts was investigated according to Carrasco-Castilla et al. (2012). Each extract of 10 µL (1 mg/mL) was mixed with 290 µL of 50 mM sodium acetate buffer (pH 6) and 6 µL of 4 mM of pyrocatechol violet in a centrifuge tube. Then $CuSO_4 \bullet 5H_2O$ (10 µL) was added and incubated for 2 min. The absorbance of the mixture was measured at 632 nm using a microplate reader. The copper chelating activity was calculated using Equation 4:

$$\text{Chelating activity (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (4)$$

Measurement of cytotoxicity on skin fibroblast

The effect of extracts on skin fibroblast cytotoxicity was evaluated using (3- (4,5-dimethylthia- zol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay as described by Chirumamilla et al. (2017). The human skin fibroblast ATCC no. CRL 1947 (CCD-986SK) was cultured in RPMI 1640 medium with 5% fetal calf serum incubated under 5% CO_2 in air atmosphere. Then cells were trypsinized (0.05% Trypsin- ethylene-diamine-tetraacetic acid) and resuspended with growth medium. The medium at a density of 2.5×10^4 cell/ml in 200 µL of medium was seeded in 96-well plates that were then incubated for 24 h in the 5% CO_2 incubator. After that, the extracts with different concentrations (0 µg/mL, 1.562 µg/mL, 3.125 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL) were prepared in 2 µL of dimethyl sulfoxide (DMSO) and incubated for 72 hr. After this incubation period, 10 µL of MTT were added to each well, and the mixture was then incubated at 37 ºC for 4 hr. The MTT medium was removed and 150 µL of DMSO were added followed by 25 µL of glycine buffer (pH 10.5). The plate was wrapped and then shaken mechanically to dissolve the formazan product. The absorbance was read at 540 nm and calculated using Equation 5:

$$\text{\% Survival} = \frac{OD_{sample}}{OD_{control}} \times 100 \quad (5)$$

where OD_{sample} is the absorbance of the extract with the cell line and $OD_{control}$ is the absorbance of the cell line without extract.

Determination of tyrosinase inhibition

Tyrosinase inhibition was investigated as described by Karkouch et al. (2017). Initially, each extract was dissolved in 50 mM sodium phosphate buffer (pH 6.8). The positive control (A) consisted of 160 µL of 50 mM sodium phosphate buffer mixed with 20 µL of 2.5 mM L-3,4-dihydroxyphenylalanine (DOPA) and 20 µL of tyrosinase (100 units/mL). The negative control (B) contained 180 µL of 50 mM sodium phosphate buffer and 20 µL of 2.5 mM L-DOPA. Each sample, consisting of each concentration of extract (20 µL), 140 µL of 50 mM sodium phosphate buffer, 20 µL of tyrosinase and 20 µL of 2.5 mM L-DOPA was mixed as sample (C). The blank sample (D) was the separate mixtures of extracts at various concentrations, 160 µL of 50 mM sodium phosphate buffer and 20 µL of 2.5 mM L-DOPA. All mixtures were reacted in 96-well plates incubated at 25ºC; then, the absorbance was measured at 475 nm. The percent inhibition was calculated using Equation 6:

$$\text{Tyrosinase inhibition (\%)} = \frac{(A-B) - (C-D)}{(A-B)} \times 100 \quad (6)$$

where A is the absorbance of the positive control, B is the absorbance of the negative control, C is the absorbance of the sample and D is the absorbance of a blank sample.

Elastase inhibitory activity

The elastase inhibitory activity was determined by the reaction of the extracts at various concentrations with porcine pancreatic elastase (PE-E.C.3.4.21.36) and succinyl-Ala-Ala-Ala-p-nitroanilide (AAAPVN) according to Kim et al. (2004), with slight modification. Briefly, each extract was mixed with porcine pancreatic elastase in a 96-well plate; then, the mixture was incubated at room temperature for 15 min. AAAPVN was added to the mixture to make a final volume of 250 µL/well and incubated at room temperature. Then, the elastase inhibitory activity was determined spectrophotometrically at 381 nm and 402 nm on a microplate reader. The inhibitory activities of extracts were reported as IC_{50} values which were compared with epigallocatechin gallate (EGCG).

Collagenase inhibitory activity

The anti-collagenase activity was determined using an EnzChek® collagenase/gelatinase kit (Molecular Probes; USA) as reported in Pientaweeratch et al. (2016), with some modifications. The DQ™ collagen 1 and DQ™ gelatin made up a substrate of collagenase activity that was isolated from *Clostridium histolyticum*. The concentration of extract was tested using a fluorescence-based 96-well plate assay. The

fluorescence intensity of the sample was measure using a fluorescence microplate reader (Infinite® 200 PRO; Switzerland) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed using the SAS version 9 software (SAS Institute; USA). The values were expressed as mean ± SD. Analysis of variance was applied to the data. Duncan's multiplerange test was used to determine significant differences between means ($p < 0.05$).

Results and Discussion

Yield percentage and bioactive compounds

The rhizome of the three Zingiberaceae plants (*C. longa*, *C. aromatica* and *Z. montanum*) were extracted with ethanol and their crude extracts were dried using a spray drier. The yield and chemical characterization are presented in Table 1. The yields of *C. longa*, *C. aromatica* and *Z. montanum* extracted with ethanol were 7.99%, 5.92% and 5.57%, respectively. The yield variation depended on variety. Furthermore, the total phenolic content was analyzed using an oxidation reaction between the hydroxyl group in the crude extracts and Folin-Ciocalteu reagent. As shown in Table 1, the total phenolic contents in the extracts from *C. longa*, *C. aromatica* and *Z. montanum* were 317.64 mg/g extract, 295.47 mg/g extract and 213.16 mg/g extract, respectively.

Curcuminoids are natural polyphenol compounds derived from rhizome extract from the Zingiberaceae. Total curcumin (curcumin, DMC and BDMC) was 73.90 mg/g extract, 32.01 mg/g extract and 0.12 mg/g extract of extract from *C. longa*, *C. aromatica* and *Z. montanum*, respectively. The curcumin profile and quantity were examined using HPLC (Fig. 1). In the present study, in total three compounds were isolated and identified as the main constituent in extracts which were curcumin, DMC and BDMC. In *C. longa*, the highest content was for BDMC (27.31 mg/g extract) followed by curcumin (25.17 mg/g extract) and DMC (21.42 mg/g extract). The major compound in *C. aromatica* was curcumin (16.59 mg/g extract) which was similar to *Z. montanum* (0.12 mg/g extract). In addition, DMC (13.69 mg/g extract) and BDMC (1.73 mg/g extract) were detected in *C. aromatica* as secondary compounds. These results were in good agreement with Himesh et al. (2011) who reported that the most important constituents, including curcumin, DMC and BDMC, were observed from ethanolic extract of *C. longa* and *C. aromatica*. Although BDMC and DMC were not observed in *Z. montanum*, they were found in *C. longa* L. and *C. aromatica*. Nagano et al. (1997) reported that cassumunin A and B, and cassumunarin A, B and C were the main constituents in *Z. montanum*. In the current study, the three Zingiberaceae species were extracted with 50% ethanol to avoid undesirable compounds including chlorophyll, fats and waxes according to Oztekin and Martinov, (2014). In addition, an ethanol-water mixture (40–60% volume per volume) was more efficient for the extraction of bioactive compounds from plant than a mono-solvent system (Spigno et al., 2007; Chew et al., 2011; Waszkowiak and Gliszczyńska-Świgło, 2016).

Table 1 Extraction yield, total phenolic and curcuminoid contents in the extracts of *Curcuma longa*, *Curcuma aromatica* and *Zingiber montanum*

Sample	Yield (%)	Total phenolic content (mg/g extract)	Total curcuminoid content (mg/g extract)
C. longa	7.99 ± 0.1[a]	317.64 ± 4.7[a]	73.90 ± 1.4[a]
C. aromatica	5.92 ± 0.1[b]	295.47 ± 2.7[b]	32.01 ± 0.1[b]
Z. montanum	5.57 ± 0.1[c]	213.16 ± 2.7[c]	0.12 ± 0[c]

Values are expressed as mean ± SD ($n = 3$); means in the same column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Extract color and appearance

The color of extracts was measured using the CIELAB and hue systems. Table 2 shows there was a significant difference in lightness (L^*), redness (a^*) and yellowness (b^*) in all crude extracts. The crude extract from *Z. montanum* had the highest L^* value (72.31) followed by *C. longa* (53.36) and *C. aromatica* (52.85). These L^* results confirmed that the higher content of curcumin possesses produced a more intense color. In addition, the yellowness in the different crude extracts was significantly different at 26.54, 25.92 and 32.49 in *C. longa*, *C. aromatica* and *Z. montanum*, respectively, which indicated the curcumin content. Pothitirat and Gritsanapan, (2006) reported that the main yellow bioactive substances in the rhizomes are curcumin, demethoxy compounds, DMC and BDMC. The color of extracts was also reported by the hue system that described red, green, blue and yellow using a single number. The result showed that the color of all crude extracts ranged from 52.46 to 71.13 which indicate a yellow color (Table 2). The highest hue value (71.13) was found in an extract from *Z. montanum* that was related to the yellow color in the CIE system, confirming that the color of extracts was dependent on the curcumin content which presented as a yellow color.

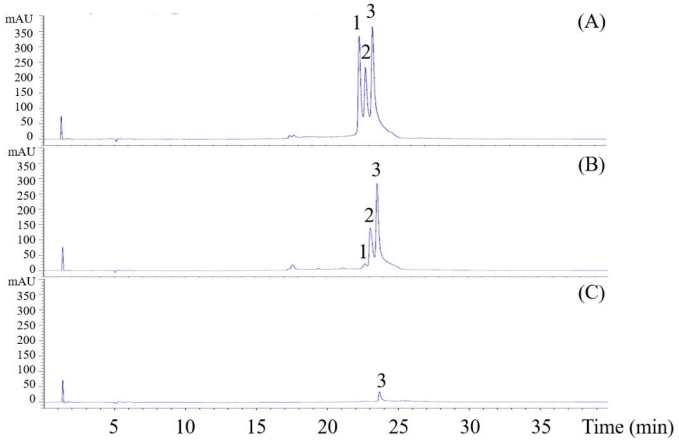

Fig. 1 Curcuminoid profiles in all extracts: A) *Curcuma longa*; B) *Curcuma aromatica*; C) *Zingiber montanum*, where peak number 1 = bisdemethoxycurcumin (BDMC), peak number 2 = curcumin, demethoxycurcumin (DMC) and peak number 3 = curcumin

Table 2 Color and appearance of crude extracts from *Curcuma longa*, *Curcuma aromatica* and *Zingiber montanum*

Sample	Color of extract				Appearance
	*L**	*a**	*b**	Hue	
C. longa	53.36 ± 0.02[b]	20.29 ± 0.03[a]	26.54 ± 0.07[b]	52.61 ± 0.09[b]	
C. aromatica	52.85 ± 0.18[c]	19.92 ± 0.13[b]	25.92 ± 0.20[c]	52.46 ± 0.04[b]	
Z. montanum	72.31 ± 0.22[a]	11.11 ± 0.11[c]	32.49 ± 0.19[a]	71.13 ± 0.08[a]	

Values are expressed as mean ± SD (n = 3); means in the same column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Antioxidant activity

DPPH radical scavenging activity

In the present study, the antioxidant activity of extracts from *C. longa*, *C. aromatica* and *Z. montanum* was determined using their DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging activity. and based on the IC_{50} values that refer to the ability of an antioxidant to reduce a 50% concentration of free radicals. The lower the IC_{50}, the higher the antioxidant activity, whereas the higher the IC_{50}, the lower the antioxidant activity. In the present study, the lowest IC_{50} value was in ascorbic acid (173.53 µg/mL) as a reference compound. The IC_{50} value for the DPPH radicals in the ethanol crude extracts from *C. longa*, *C. aromatica* and *Z. montanum* were 212.43 µg/mL, 227.42 µg/mL, and 233.37 µg/mL, respectively (Table 3). The DPPH scavenging activity can be explained by the phytochemical constituents acting directly as free radical scavengers, hydrogen donors and peroxide decomposers (Moyo et al., 2012). The crude extracts from the three Zingiberaceae plants contained very high amounts of phenolic compounds which resulted in stabilizing free radicals due to their being strong electron donors (Chumark et al., 2008). However, ascorbic acid had the lowest IC_{50} which showed the highest scavenging activity.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

ABTS assay is a method that measures the capacity of an extract to scavenge the ABTS radical cation via electron donation or hydrogen atom transfer (Apak et al., 2016). The results showed that the IC_{50} values of the extracts from *C. longa*, *C. aromatica* and *Z. montanum* were 272.40 µg/mL, 470.75 µg/mL and 543.34 µg/mL, respectively (Table 3). These values confirmed that the phenolic compound is the important molecule that can reduce ABTS$^{\bullet+}$ via hydrogen atom transfer or electron donation. In addition, the IC_{50} values of ABTS in all crude extracts displayed a similar pattern to DPPH. Furthermore, the DPPH scavenging activities of all crude extracts were better than for the ABTS assay, indicating that the ethanolic extracts showed a variety of antioxidant activities such as ABTS$^{\bullet+}$ and DPPH$^{\bullet}$. However, the best IC_{50} values were reported in L-ascorbic acid.

Determination of copper-chelating activity

Copper is an essential part of some enzymes and acts as an active site of the enzyme (Wilcox et al., 1985; Solomon et al., 2014). As observed in Table 4, the extract concentrations (1 mg/mL) of *C. longa*, *C. aromatica* and *Z. montanum* were estimated to chelate 76.45%, 70.24% and 54.96%, respectively, which were significantly different. The copper chelating activity and antioxidant activity of extracts increased with increased bioactive compound content, including the total contents of phenolics and curcumin. Curcumin can bind to Cu^{2+} that could function as a chelator (Picciano and Vaden, 2013). The chelating activity of metal ions is based on the electron donor group and the metal ion that produce stable complexes. Messner et al. (2017) reported that curcumin bound most strongly to copper, strongly suggesting metal chelation. The present results showed that extracts from *C. longa*, *C. aromatica* and *Z. montanum* were powerful copper-chelating agents.

Table 3 Antioxidant radical (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) scavenging activity of the extracts from *Curcuma longa*, *Curcuma aromatica* and *Zingiber montanum*

Sample	Antioxidant activity IC_{50} (µg/mL)	
	DPPH$^{\bullet}$	ABTS$^{\bullet+}$
C. longa	212.43 ± 10.22[c]	272.40 ± 27.79[c]
C. aromatica	227.42 ± 3.82[b]	470.75 ± 14.98[b]
Z. montanum	233.37 ± 7.04[a]	543.34 ± 34.88[a]
L-ascorbic acid	173.53 ± 3.30[d]	255.05 ± 0.98[d]

DPPH$^{\bullet}$ = 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; ABTS$^{\bullet+}$ = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); IC_{50} = half maximal inhibitory concentration

Values are expressed as mean ± SD (n = 3); means in the same column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Table 4 Tyrosinase inhibitory activity and Cu^{2+} chelating activity of the extracts from *Curcuma longa*, *Curcuma aromatica* and *Zingiber montanum*

Plant	Tyrosinase inhibitory IC_{50} (µg/mL)	Cu^{2+} Chelating activity (%)
C. longa	290.33 ± 2.60[c]	76.45 ± 1.91[a]
C. aromatica	484.53 ± 1.54[b]	70.24 ± 0.80[b]
Z. montanum	1373.68 ± 3.20[a]	54.96 ± 1.26[c]

IC_{50} = half maximal inhibitory concentration.

Values are expressed as mean ± SD (n = 3); means in the same column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Cytotoxicity

The effects of various crude extracts derived from *C. longa*, *C. aromatica* and *Z. montanum* on the viability of skin fibroblast are shown in Figure 2. The evidence showed that skin fibroblast incubated with crude extracts from the selected plants had viability rates of more than 50% at all concentrations. The extracts from *Z. montanum* in all tested concentrations resulted in >90% of cell viability which is defined as not cytotoxic (De Felice et al., 2009). The fibroblasts treated with crude extracts from *C. longa*, *C. aromatica* and *Z. montanum* at different concentrations of 12.5 µg/mL, 25 µg/mL and 50 µg/mL had significantly different viability rates and the viability rate decreased with increasing concentration of each extract. However, skin fibroblast treated with crude extracts from *C. aromatica* had the lowest viability rate (50%) at 100 µg/mL (IC_{50} = 80.53 µg/mL). The phenolic compounds are naturally occurring in the extracts from *C. longa*, *C. aromatica* and *Z. montanum* and at high concentrations, phenolics are highly toxic, leading to apoptosis of skin fibroblast cells (Cohen et al., 2009).

Tyrosinase inhibitory activity

Tyrosinase is a copper-containing enzyme that catalyzes the production of melanin (Wilcox et al., 1985). The substrate of tyrosinase is L-3,4-dihydroxyphenylalanine (L-DOPA) which induces the melanogenic pathway (Slominski et al., 1989). Tyrosinase converts L-DOPA to dopaquinone that generates melanin and brown melanin by interacting with cysteine (Li et al., 2019). In the present study, all crude extracts were investigated for tyrosinase enzyme activity. As shown in Table 4, the extract from *C. longa* was the most potent tyrosinase inhibitor, followed by *C. aromatica* and *Z. montanum* with IC_{50} values of 290.33 µg/mL, 484.53 µg/mL, and 1373.68 µg/mL, respectively. Mukherjee et al. (2001) reported that the methanolic extract of *C. longa* rhizome had high tyrosinase inhibition because the hydroxyl group in the structure of the phenolic compounds has a structural resemblance with the substrate of tyrosinase (L-DOPA) (Uchida et al., 2014). Therefore, it is subject to competitive inhibition.

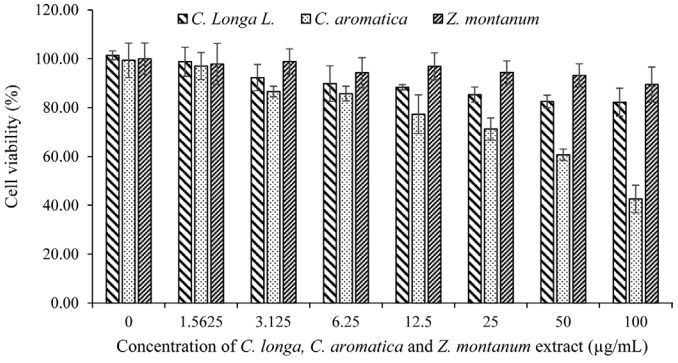

Fig. 2 Cell viability of skin fibroblast after incubation with extracts from *Curcuma longa, Curcuma aromatica* and *Zingiber montanum,* where error bars = ± SD

Elastase inhibitory activity

Elastase is an enzyme belonging to the protease family whose function is to break up elastin that is important for skin elasticity (Thring et al., 2009). As presented in Table 5, the elastase inhibition activity of extracts from the Zingiberaceae plant was determined and compared with that of EGCG. The results showed that all extracts displayed strong elastase inhibition activity. The inhibition activity of the extracts from *C. longa*, *C. aromatica* and *Z. montanum* on elastase activity were expressed as IC_{50} values of 69.61 µg/mL, 160.63 µg/mL and 3386.23 µg/mL, respectively. In the present study, extracts from *C. longa* had the highest elastase inhibition activity, though it was not as high as for EGCG (57.32 µg/mL) as the standard. These results suggested that phenolic compounds and curcuminoids in the crude extracts had a potential antiaging property through elastase inhibition activity.

Collagenase inhibitory activity

Matrix metalloproteinase (MMP) is known as collagenase, gelatinase and stromelysins (Philips et al., 2011) and is responsible for degrading collagen in human skin by collagenase or MMP-1 cleaving the collagen structure. They also reported that gelatinase or MMP-2 degrade membrane collagens and decrease denatured structural collagens. The results of extracts against MMP-1 and MMP-2 activity are shown in Table 5. The EGCG had the highest inhibition of collagenase (MMP-1 and MMP-2) when compared to the extract of *C. longa, C. aromatica and Z. montanum*. The IC_{50} values for MMP-1 and MMP-2 were in the ranges 90.46–4953.01 µg/mL and 88.77–4677.67 µg/mL, respectively. However, the highest inhibition against MMP-1 and MMP-2 was observed in EGCG with IC_{50} values of 61.45 µg/mL and 60.65 µg/mL, respectively. The inhibition activity values of EGCG and all extracts were not significantly different for MMP-1 and MMP-2. The extracts from *C. longa*, *C. aromatica* and *Z. montanum* successfully inhibited MMP-1 and MPP-2, perhaps due to chelating activity. Philips et al. (2011) reported that copper could stimulate MMP activity and subsequent collagen deposition. Therefore, MMP-1 and MMP-2 inhibition activity resulted from the chelating activity of Cu^{2+}. In addition, the extract from *Z. montanum* had the lowest MMP-1 and MPP-2 inhibition activity at concentrations of 4953.01 µg/mL and 4677.67 µg/mL, respectively. Furthermore, the extracts from *C. longa* and *C. aromatica* had significant antioxidant activity and tyrosinase and elastase inhibition activity.

Table 5 Elastase inhibitory and collagenase (MMP-1 and MMP-2) inhibitory of the extracts from *Curcuma longa*, *Curcuma aromatica* and *Zingiber montanum*

Plants	Elastase inhibitory	Collagenase inhibitory IC_{50} (µg/mL)	
	IC_{50} (µg/mL)	MMP-1	MMP-2
C. longa	69.61 ± 0.75^{c}	90.46 ± 0.20^{c}	88.77 ± 0.70^{c}
C. aromatica	160.63 ± 0.59^{b}	219.62 ± 0.55^{b}	201.66 ± 0.57^{b}
Z. montanum	3386.23 ± 5.13^{a}	4953.01 ± 1.65^{a}	4677.67 ± 2.79^{a}
EGCG	57.32 ± 0.09^{d}	61.45 ± 0.41^{d}	60.65 ± 0.62^{d}

IC_{50} = half maximal inhibitory concentration; MMP = matrix metalloproteinase; EGCG = epigallocatechin gallate

Values are expressed as mean ± SD (n = 3); means in the same column followed by different lowercase superscripts are significantly ($p < 0.05$) different.

The present results indicated that the phenolic compound and curcuminoids were the main compounds found in the ethanolic extracts of *C. longa*, *C. aromatica* and *Z. montanum*. Although curcumin, DMC and BDMC were found in *C. longa* and *C. aromatica*, they were not observed in *Z. montanum*. The main curcuminoid compound in *Z. montanum* was curcumin. The most potent levels of antioxidant activity towards the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, copper chelating activity and enzyme inhibition activity were observed in all extracts due to the presence of phenolic compounds. Hence, the extracts from the three plants belonging to the Zingiberaceae family could have potential application as active ingredients in nutraceuticals and cosmeceuticals.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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