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Full Paper

Bioactive compounds of aqueous extracts of flower and leaf of *Etlingera elatior* (Jack) R.M.Sm. for cosmetic application

Nattawut Whangsomnuek [1], Lapatrada Mungmai [2], Kriangsak Mengamphan [3,4] and Doungporn Amornlerdpison [3,4,*]

[1] Interdisciplinary Agriculture Program, Maejo University, Chiang Mai, Thailand
[2] Cosmetic Science, School of Pharmaceutical Sciences, University of Phayao, Phayao, Thailand
[3] Center of Excellence in Agricultural Innovation for Graduate Entrepreneur, Maejo University, Chiang Mai, Thailand
[4] Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand
* Corresponding author, e-mail: doungpornfishtech@gmail.com

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Abstract: Aqueous extracts of flower and leaf of *Etlingera elatior* were examined for amino acid content and phenolic compounds and were evaluated for their antioxidant activities via scavenging of ABTS, DPPH and superoxide radicals. The extracts were also assessed for the activity of tyrosinase enzyme and collagenase inhibition to determine possible skin benefits. The total phenolic contents of the flower and leaf extracts were determined to be 38.68 ± 0.45 and 246.52 ± 0.26 mg GAE/g respectively. LC-MS analysis showed that the major components of both extracts are isoquercetin, catechin and gallic acid. In addition, the amino acid content of these extracts, which includes lysine and leucine, features them as potentially effective in improving the formation of collagen and reducing wrinkle appearance when used on skin. The antioxidant activity results establish that both the flower and leaf extracts can be used as natural antioxidants. Both of the extracts were found to inhibit tyrosinase and collagenase activities, thus exhibiting potential for being used as active ingredients for anti-wrinkle and whitening purposes in cosmetic applications.

Keywords: *Etlingera elatior*, antioxidant, phenolic content, amino acids content, tyrosinase, collagenase

INTRODUCTION

Skin aging is a dermatological change influenced by several factors including age, hormonal changes, environmental exposure and metabolic processes [1]. Skin-aging effects, caused by environmental factors, particularly UV radiation exposure, damage skin through the generation of reactive oxygen species (ROS). ROS are related to extracellular matrix (ECM) protein degradation

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in the epidermal and dermal layers, which include collagen and elastin. Matrix metalloproteinases (MMPs) are enzymes involved in ECM protein degradation and include collagenase and gelatinase. The effect of ECM protein degradation impacts the integrity of the skin structure, resulting in wrinkles, dryness and roughness [2-3]. Further, inhibiting ECM protein degradation has been found to combat the skin-aging process [4].

Etlingera elatior (torch ginger) is a plant of the Zingiberaceae family and is widely cultivated in South-east Asia [5]. It grows of up to 5-6 m tall from underground, and the width of the stout rhizome is 3-4 cm in diameter. The leaves are entirely green, feature a lanceolate shape and are up to 81 cm in length. The tall stems, formed by sheaths of leaves, are pseudostems that grow from underground rhizomes. The waxy inflorescences are shaped like spearheads when young, and when blossoming, the bracts are larger and feature prominent red and pink colouration. The fruits are shaped like pineapples and have green to reddish colouration, housing many black seeds inside [6-8].

Extracts of both flowers and leaves of this plant have been shown to possess cosmeceutical activities that can improve some skin problems [5]. The methanolic extract of the flower contains flavonoids, terpenoids, saponins, tannins and carbohydrates [9]. The ethanolic extract of the leaf contains a group of phenolic compounds including chlorogenic acid and caffeoylquinic acid, which possess antioxidant capacity, tyrosinase inhibiting activity and antibacterial properties [10]. The flower extract exhibits antioxidant, antibacterial and anticancer activities [11]. In addition, the hydroglycolic extract of the flower also has skin whitening and anti-aging properties via tyrosinase and collagenase inhibitory activities [12].

Many studies have been carried out on assessing the biological and pharmaceutical properties of the flower or leaf of *E. elatior* using methanol, ethanol, acetone or water/propylene glycol as extracting solvent. The biological activities of *E. elatior* flowers and leaves have been reported focusing on their antioxidant, antibacterial and anticancer activities [9-16]. However, comparative investigation of the aqueous extracts of flower and leaf for cosmetic applications has not been reported. This research therefore seeks to determine the relevant bioactive compounds (amino acids and phenolic compounds) and also analyse the biological activities (antioxidant, anti-tyrosinase and anti-collagenase) of the aqueous extracts of *E. elatior* flower and leaf to evaluate their suitability for cosmetic ingredients.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's reagent and sodium carbonate were purchased from BDH Prolabo Chemicals (France). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitrotetrazolium blue chloride, phenazine methosulfate, β-nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH), potassium persulfate, sodium phosphate monobasic, sodium phosphate dibasic, N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), tyrosinase from mushroom, and methanol were purchased from Sigma-Aldrich (USA). 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid, kojic acid, 3,4-dihydroxy-L-phenylalanine (L-DOPA), and ammonium formate were procured from Sigma-Aldrich (Germany). Collagenase type I from *Clostridium histolyticum* and (-)-epigallocatechin gallate (EGCG) were obtained from Merck (USA). Acetonitrile was purchased from Labscan (Ireland). All other reagents were of analytical or chromatographic grade.

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Sample Preparation

E. elatior flowers and leaves were purchased from a local cultivator in the Reso district of Narathiwat province, Thailand. They were rinsed several times with distilled water, cut into small pieces (protected from sunlight), and air-dried with a hot-air oven at 50ºC. Dried samples were ground into fine powder using a high-speed disintegrator and then stored in amber zip-lock bags at room temperature.

Sample Extraction

A powdered sample (100 g) was warmed (50ºC) in distilled water (1,000 mL) for 8 hr. The mixture was then centrifuged at 4,000 rpm for 5 min. at ambient temperature and filtered through Whatman No. 1 filter paper to separate the residue. The filtrate was concentrated by evaporation under reduced pressure and lyophilised with a freeze dryer (Labconco, Model 7750020, Denmark) to obtain a dried flower or leaf extract.

Total Phenolic Content

The total phenolic content of an extract was determined by Folin-Ciocalteu method [17]. In brief, 200 µL of the extract at different concentrations were added with 1,000 µL of 10% (v/v) Folin-Ciocalteu's reagent and 800 µL of sodium carbonate (7.5% w/v). The mixture was incubated for 60 min. at ambient temperature. Next, the absorbance of the mixture was measured at 765 nm using a spectrophotometer (Thermo Scientific™, Evolution 260 Bio, Finland). The total phenolic content was expressed as gallic acid equivalent (GAE) in mg/g of dried extract (mg GAE/g extract) via calculation from a calibration curve of gallic acid.

Analysis of Phenolic Compounds

Phenolic compounds in an extract were determined using a liquid chromatography–mass spectrometry (LC-MS) system (Agilent 1100 series, LC/MSD SL, USA). The following column was used: LiChroCART (150 x 4.6 mm), Purospher STAR 120 RP-18e (5 µm). Phenolic compounds were determined as described by Peñarrieta et al. [18] with some modifications. The mobile phase consisting of acetonitrile (solvent A) and 10 mM ammonium formate buffer (pH 4) with formic acid (solvent B) was used. The elution flow rate was 1.0 mL/min. The elution of solvent was performed employing the following sequence: 100% of solvent B from 0 - 5 min.; 0% → 20% of solvent A from 5 - 10 min.; 20% of solvent A from 10 - 20 min.; and 20% → 40% of solvent A from 20 - 60 min. The results, acquired with a diode array at wavelengths of 270, 330, 350 and 370 nm, were interpreted and quantified using apigenin, catechin, eriodictyol, gallic acid, hydroquinin, isoquercetin, kaempferol, quercetin, rutin and tannic acid as standard compounds.

Amino Acids Analysis

Sample extracts were sent for amino acids analysis at the Central Laboratory Co. (Chiang Mai, Thailand), where an in-house method based on AOAC official method [19] was used. The products from performic acid oxidation with acid hydrolysis-sodium metabisulfite method were analysed by GC (Agilent Model 6890N, USA) and MS (Agilent Model 5973 inert, USA), and the ion exchange chromatographic procedure was performed with a Zebron ZB-AAA capillary column (Phenomenex, USA).

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Antioxidant Activities

ABTS radical scavenging

ABTS radical-scavenging activity was determined as described by Re et al. [20] with some modifications. The $ABTS^{\bullet+}$ was generated by mixing an equal volume of 7 mM solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand for 12 hr in the dark at room temperature. Before performing the assay, the $ABTS^{\bullet+}$ solution was diluted to achieve a final absorbance of 0.7 ± 0.5 at 734 nm. Then each extract of different concentrations (200 µL) was mixed with 1,800 µL of the $ABTS^{\bullet+}$ solution and allowed to stand for 6 min. at ambient temperature (distilled water was used for the control reaction). The absorbance at 734 nm was measured. Trolox was utilised as a reference substance. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) in mg/g of dried extract (mg TEAC/g extract) and calculated from a calibration curve of Trolox.

DPPH radical scavenging

DPPH radical scavenging was evaluated as described by Mensor et al. [21] with some modification. Each extract of different concentrations (400 µL) was mixed with 2,000 µL of DPPH (0.3 mM) in methanol. After 20 min. of incubation at ambient temperature in the dark (with methanol used in the control reaction), the absorbance at 517 nm was measured. Gallic acid was used as a reference substance. The results were expressed as gallic acid equivalent antioxidant capacity (GEAC) in mg/g of dried extract (mg GEAC/g extract).

Superoxide radical scavenging

Superoxide anion radical scavenging was measured using nitrotetrazolium blue chloride reduction method described by Nishikimi et al. [22] with slight modification. All solutions were prepared with 100 mM phosphate buffer (pH 8). The reaction mixture contained 100 µL of the extract, 1000 µL of 156 µM nitrotetrazolium blue chloride, 1000 µL of 468 µM NADH and 100 µL of 60 µM phenazine methosulfate, and it was incubated at ambient temperature for 5 min. (with phosphate buffer used in the control reaction). Subsequently, absorbance at 560 nm was measured. Gallic acid was used as a reference substance. The results were expressed as mg GEAC/g extract.

Enzymatic Assays

Tyrosinase inhibition

Tyrosinase inhibition was assessed with the dopachrome method as reported by Masuda et al. [23] with some modifications. Solution of tyrosinase (200 U/mL) from mushrooms and 2.5 mM L-DOPA were prepared with 20 mM phosphate buffer (pH 6.8). Tyrosinase reactions were performed in a 96-well plate, with each well containing 20 µL of extract of different concentrations, 40 µL of tyrosinase solution and 140 µL of phosphate buffer. The 96-well plate was allowed to stand for 10 min., and then the reaction was started by adding 40 µL of L-DOPA solution (with phosphate buffer employed in the control reaction). The 96-well plate was then incubated at ambient temperature for 20 min., and absorbance of the solution was measured at 492 nm using a microplate reader (Biochrom EZ Read 400, UK) with kojic acid as reference substance. Each sample testing was completed with a blank plate of test sample without the tyrosinase solution.

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Collagenase inhibition

The collagenase inhibition activities of the extracts were measured using the method described by Zakiah et al. [2] with slight modification. Each extract (50 µL) of different concentrations, 50 µL of 50 mM of tricine buffer (pH 7.5) and 50 µL of *Clostridium histolyticum* collagenase (type I) solution (125 U/mL) were added to a 96-well plate, which was permitted to stand for 15 min. A tricine buffer was used in the control reaction. The reaction was commenced by adding 50 µL of 0.5 mM of FALGPA and collagenase inhibition activity was measured by continuously monitoring the decrease in absorbance of FALGPA at 340 nm using a microplate reader. EGCG was utilised as reference substance.

The capacity of an extract to scavenge radicals and inhibit enzyme activity were obtained using the following equation:

$$\text{Inhibition activity (\%)} = [(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$$

In this equation $Abs_{control}$ is absorbance of the control without sample extract and Abs_{sample} is absorbance reading in the presence of sample extract.

Statistical Analysis

Values are expressed as mean ± standard deviation (S.D.). Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows (IBM, USA) using one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test. Statistical significance was determined to be $p < 0.05$.

RESULTS AND DISCUSSION

Phenolic Content

The yields of aqueous extracts from both the flower and leaf of *E. Elatior*, together with their phenolic content are shown in Table 1. The results indicate that the total phenolic content of the leaf extract is approximately six time higher in comparison to the flower extract. Generally, phenolic compounds from plants exhibit significant free radical scavenging compared to vitamin C, E and carotenoids [24-25].

Table 1. Yield and total phenolic content of *E. elatior* extracts

Part	Yield of extract (% w/w)	Total phenolic content (mg GAE/g extract)
Flower	23.90 ± 4.09	38.68 ± 0.45
Leaf	24.30 ± 1.98	246.52 ± 0.26

Ten phenolic compounds in the flower and leaf extracts of *E. elatior* were assayed, viz apigenin, catechin, eriodictyol, gallic acid, hydroquinin, isoquercetin, kaempferol, quercetin, rutin and tannic acid. The results are shown in Table 2. Chromatograms of the extracts are shown in Figure 1.

Previous studies have reported gallic acid as exhibiting significant antioxidant activity, preventing oxidative stress caused by ROS and suppressing cellular melanin synthesis in B16F10 cells through inhibition of tyrosinase activity [26, 27]. Catechin is a metal chelator and may bind with the Zn^{2+} ion inside the collagenase structure, thereby replacing the binding of the substrate, and

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may also be involved in inhibiting the activities of tyrosinase and elastase enzymes [28, 29]. Eriodictyol is also well known for its antioxidant and anti-inflammatory activities [30], as well as its anti-tyrosinase activity with an IC_{50} value of approximately 150 μM [31]. Choi and Shin [32] reported that quercetin is a potent tyrosinase inhibitor and also inhibits melanogenesis. Furthermore, quercetin has anti-inflammatory benefits as well as antioxidant and anti-allergen properties [33, 34]. Apigenin has been reported to be a collagenase and hyaluronidase inhibitor with anti-aging properties [35]. Phenolic constituents were shown to inhibit *Propionibacterium acne*-induced inflammatory response by suppressing interleukin production [36]. Diverse phenolic compounds such as tannic acid, rutin and isoquercetin are regarded to have antioxidant properties [37, 38]. The present study thus shows that phenolic compounds in *E. elatior* flowers and leaves can be important ingredients that offer a variety of properties for promoting healthy skin and enhancing skin appearance when incorporated in skin care products.

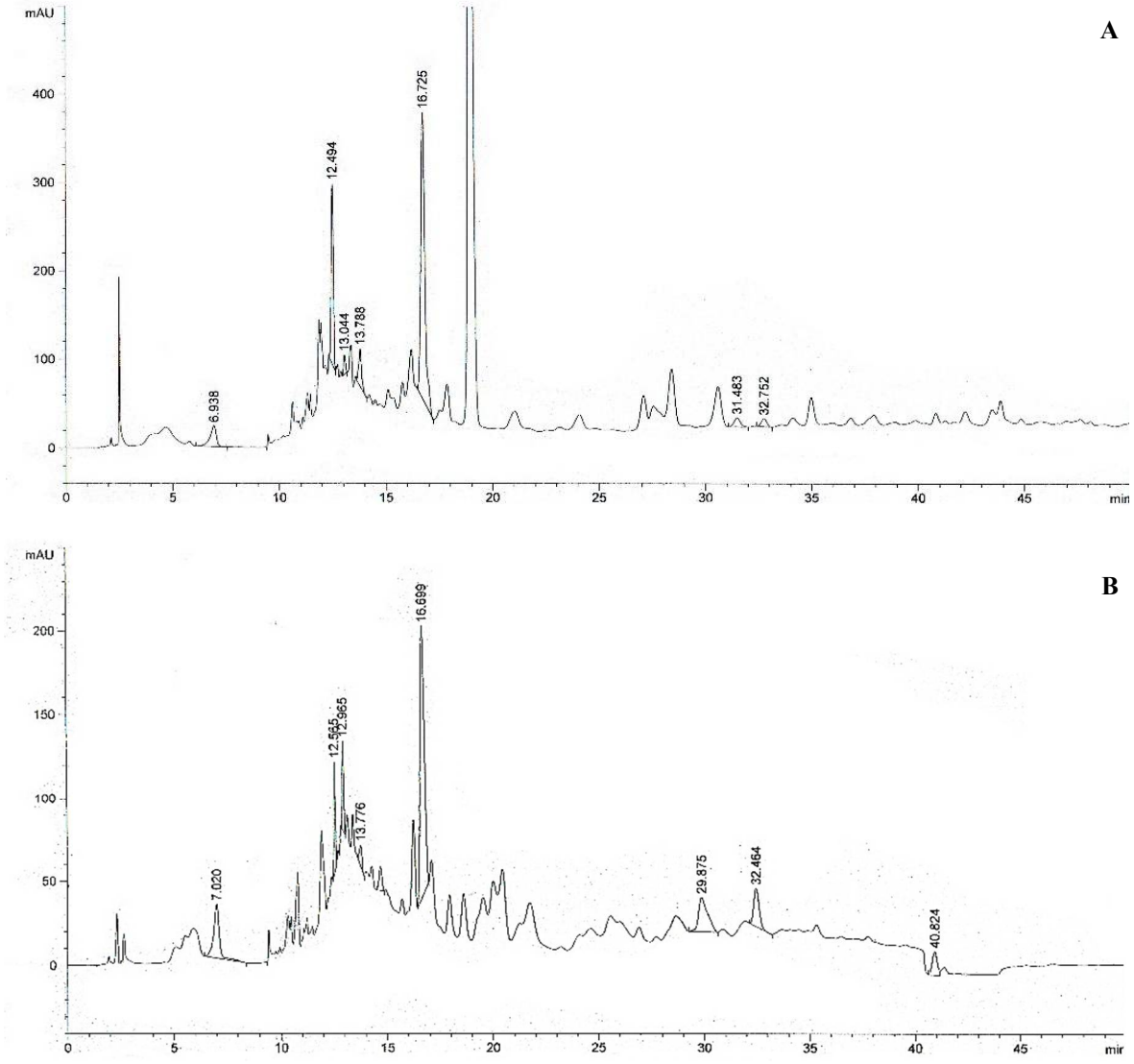

Figure 1. LC-MS chromatograms of *E. elatior* flower extract (A) and leaf extract (B) showing peaks of gallic acid (RT 6.94 and 7.02 min.), catechin (RT 12.50 and 12.57 min.), tannic acid (RT 13.04 and 12.97 min.), rutin (RT 13.79 and 13.78 min.), isoquercetin (RT 16.73 and 16.70 min.), eriodictyol (RT 31.48 and 29.88 min.), quercetin (RT 32.75 and 32.46 min.) and apigenin (RT 40.82 min.)

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Table 2. Phenolics content of *E. elatior* flower and leaf extracts (mg/100 g dried extract)

Compound	Flower	Leaf	Compound	Flower	Leaf
Apigenin	ND	4.72	Isoquercetin	55.36	69.37
Catechin	40.69	65.75	Kaempferol	ND	ND
Eriodictyol	1.78	3.39	Quercetin	12.64	31.24
Gallic acid	35.47	50.01	Rutin	11.66	24.16
Hydroquinin	ND	ND	Tannic acid	15.17	49.10

Note: ND = non-detectable

Amino Acids Analysis

Amino acids have been demonstrated to play an important role as antioxidants and as cosmetic ingredients with skin improvement functions [39, 40]. In this study the amino acids content of both the flower and leaf extracts were analysed (Table 3). The flower extract exhibits overall higher amino acids content than that of the leaf extract, with the highest values for lysine, glutamic acid and aspartic acid in that order. Of the 19 amino acids present in the *E. elatior* extracts, cysteine, glycine, histidine, leucine, lysine, proline and tyrosine have been reported to have cosmetic properties [41-45], with cysteine, glycine and histidine exhibiting anti-inflammatory property [41]. Proline is involved in correcting wrinkles by enhancing the elasticity of the stratum corneum [44]. A combination of proline and branch-chain amino acids has benefits in recovering the biosynthesis of collagen after UV exposure, while lysine and leucine amino acids are involved in the formation of collagen [42-45]. Several amino acids present in *E. elatior* thus seem to exhibit cosmetic properties valuable for restoring and resolving skin-aging problems.

Table 3. Amino acid contents of *E. elatior* flower and leaf extracts (mg/100 g dried extract)

Amino acid	Flower	Leaf	Amino acid	Flower	Leaf
Alanine	261	74.35	Lysine	2891	138.50
Aspartic acid	1177	94.67	Methionine	<5.00	<5.00
Cysteine	35	24.65	Phenylalanine	685	141.67
Glutamic acid	1250	202.82	Proline	216	28.02
Glycine	176	41.16	Serine	394	122.65
Histidine	437	19.36	Threonine	146	12.05
Hydroxylysine	<5.00	<5.00	Tryptophan	60	9.99
Hydroxyproline	29	7.79	Tyrosine	372	26.08
Isoleucine	581	83.64	Valine	523	57.41
Leucine	838	112.74			

Antioxidant Activity

Results of ABTS assay are presented in Figure 2A and Table 4. The leaf extract has approximately 24fold greater ABTS radical-scavenging capacity than that of the flower extract. Of note, the ABTS-scavenging activity of the leaf extract closely resembles that of Trolox, the reference substance.

Results of DPPH radical-scavenging assay are shown in Figure 2B and Table 4, which show that the leaf extract also demonstrates higher antioxidant capacity than the flower extract. The

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flower and leaf extracts exhibit IC_{50} values of 0.44 ± 0.00 and 0.09 ± 0.00 mg/mL respectively. In a previous study of *E. elatior* flower, Lachumy et al. [9], using this assay, determined the IC_{50} values of methanolic and ethanolic extracts to be 9.14 mg/mL and 0.04-0.14 mg/mL respectively [11].

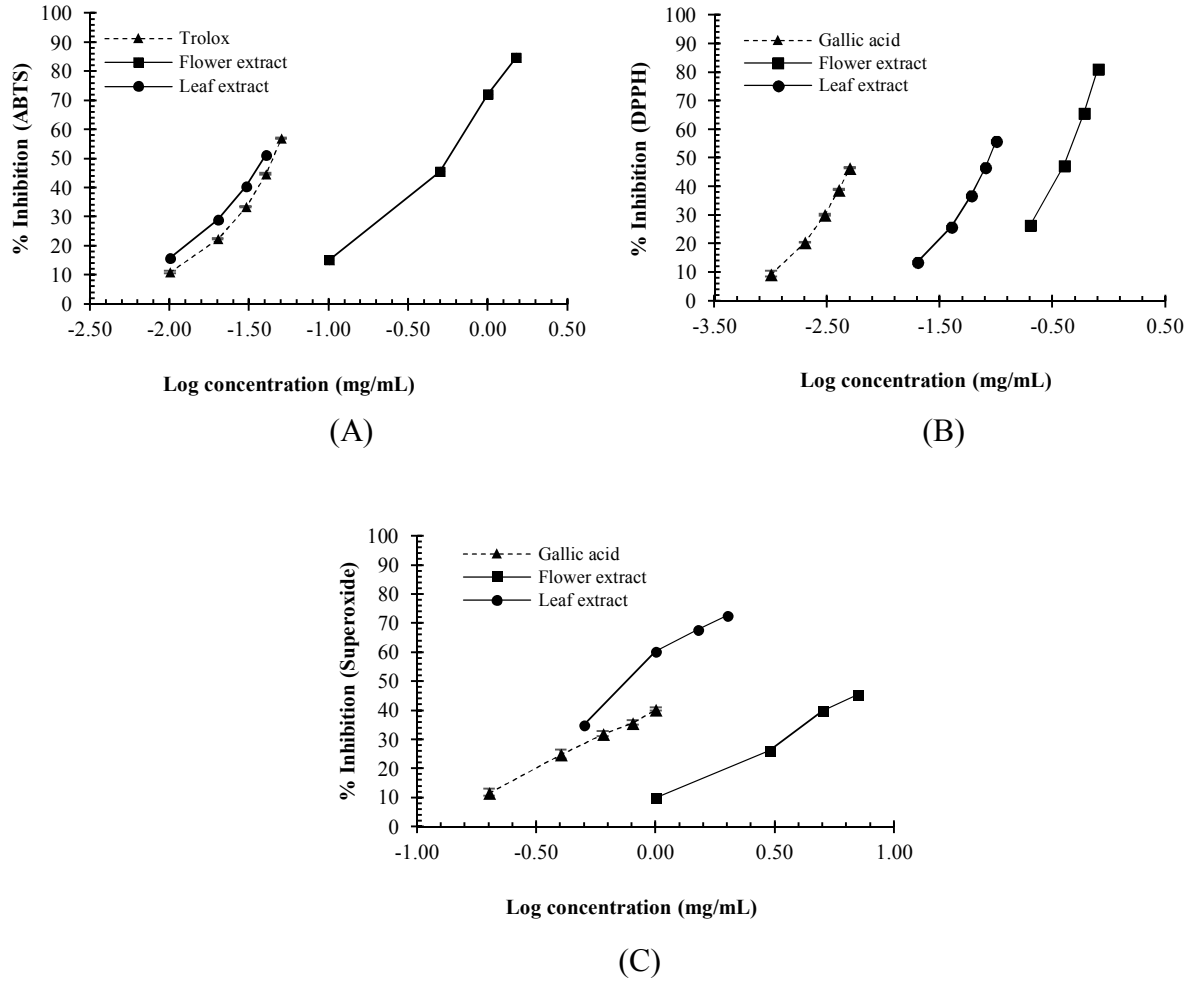

Figure 2. Antioxidant activities: ABTS (A), DPPH (B) and superoxide (C) radical-scavenging activities of *E. elatior* flower and leaf extracts

Table 4. Antioxidant capacities of *E. elatior* flower and leaf extracts

Part	ABTS assay		DPPH assay		Superoxide assay	
	mg TEAC /g extract	IC_{50} (mg/mL)	mg GEAC /g extract	IC_{50} (mg/mL)	mg GEAC /g extract	IC_{50} (mg/mL)
Flower	43.41±0.56	0.69±0.01[a]	9.85±0.08	0.44±0.00[a]	219.65±4.38	6.39±0.13[a]
Leaf	1030.74±10.82	0.04±0.00[b]	56.86±0.65	0.09±0.00[b]	314.12±12.79	0.88±0.03[c]
Trolox	-	0.04±0.00[b]	-	-	-	-
Gallic acid	-	-	-	0.01±0.00[c]	-	1.37±0.05[b]

Note: Values are expressed as mean ± S.D.; n = 3. For each column of IC_{50} values, means not sharing the same letter are significantly different at $p < 0.05$.

The superoxide anion radical is well known as initial radical that plays an important role in acutely damaging cells and tissues by forming other ROS such as singlet oxygen and hydroxyl radical [46]. As found in Figure 2C, the leaf extract exhibits higher superoxide anion-scavenging

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capacity than that of the flower extract, with approximately seven times smaller IC_{50} (Table 4). Skin-aging processes and dermatological conditions are most commonly the result of oxidative stress from many endogenous and exogenous factors such as metabolism, pollution and exposure to UV radiation [47, 48]. Antioxidants have been reported to be involved in preventing cellular damage by harmful free radicals, interrupting the autoxidation chain reactions and protecting cellular defence mechanisms that take place during the oxidation process, which results in slowing the skin-aging process and reducing the severity of dermatological conditions [24, 49, 50].

Enzymes Assay

Tyrosinase inhibition activity

Tyrosinase is a crucial rate-limiting enzyme in the direct synthesis of melanin, which prevents skin damage caused by ROS or UV radiation exposure. However, overproduction of melanin pigment may cause hyperpigmentation, the primary cause of skin melanoma, melasma, age spots, skin darkening and other issues [51]. Tyrosinase is involved in catalysing the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to DOPA quinone in the biosynthetic pathway of melanin [52]. Therefore, cosmetic products containing tyrosinase inhibitors are becoming more common for their skin-whitening properties. In this study mushroom tyrosinase was used to determine the role of *E. elatior* in the process of melanogenesis. Different concentrations of the flower and leaf extracts (consisting of 1, 5 and 10 mg/mL) and reference compound (kojic acid, 1 mg/mL) were incubated with the tyrosinase solution, and the reactions were stopped by adding L-DOPA. The results show that all concentrations of the flower and leaf extracts inhibit tyrosinase activity in a dose-dependent manner (Figure 3A). The tyrosinase inhibition activities at 10 mg/mL are 24.37 ± 0.52% and 31.48 ± 1.28% respectively, with IC_{50} values of 25.77 ± 0.88 and 18.08 ± 0.74 mg/mL respectively.

Collagenase inhibition assay

Collagenase is a proteolytic enzyme belonging to the Matrix metalloproteinases group and is involved in breaking down collagen [53]. Thus, inhibiting collagenase activity delays the skin-aging process that produces wrinkles, leading to an appearance of youthful, healthy and glowing skin [54, 55]. Different concentrations of *E. elatior* flower and leaf extracts (2, 3 and 4 mg/mL) were assessed, along with the reference substance EGCG (0.05 mg/mL). Collagenase from *C. histolyticum* was employed to measure the anti-aging properties of *E elatior*. The results demonstrate that both the flower and leaf extracts significantly inhibit collagenase activity in a dose-dependent manner, as shown in Figure 3B. The collagenase inhibition activities at 4 mg/mL are 51.73 ± 0.20% and 41.54 ± 0.75% respectively. The IC_{50} values are 3.89 ± 0.17 and 5.02 ± 0.47 mg/mL respectively.

Our findings indicate that while the flower extract exhibits more anti-collagenase activity than the leaf extract, the reverse is true for the anti-tyrosinase activity (i.e. the leaf extract showing greater activity).

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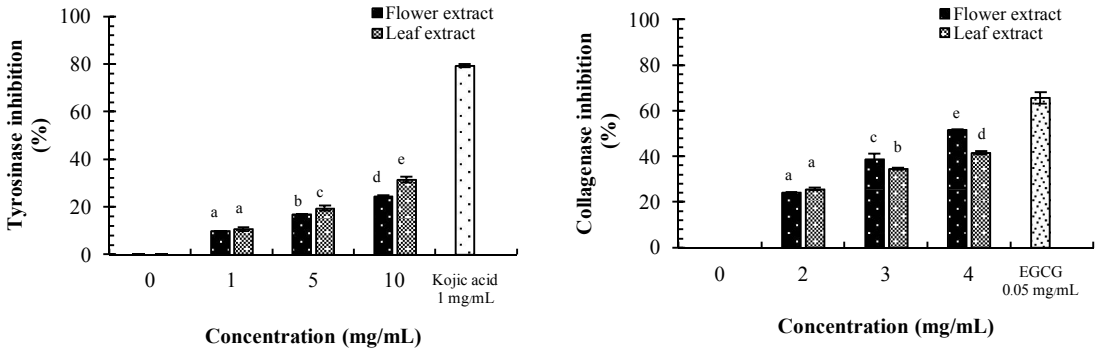

(A) (B)

Figure 3. Inhibition activities of *E. elatior* flower and leaf extracts on tyrosinase (A) and collagenase (B) (Values are expressed as mean ± S.D.; n = 3. Column not sharing the same letter are significantly different at $p < 0.05$.)

CONCLUSIONS

The results presented herein provide evidence that both the flower and leaf of *E. elatior* can potentially be a natural source of active ingredients with anti-aging and anti-wrinkle properties in cosmetic products. Further studies to confirm these findings in animals should be pursued. Moreover, to explore possible benefits and safety concerns related to *E. elatior* extracts in terms of human cosmetic applications, studies in humans should also be carried out in the future.

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