Original article

Phytochemical composition, antioxidant, in vitro and in silico studies of active compounds of *Curculigo latifolia* extracts as promising elastase inhibitor

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ABSTRACT

Curculigo latifolia is a plant in the Hypoxidaceae family commonly used in herbal medicine. The study objective was to evaluate the antioxidant and anti-elastase properties of *C. latifolia* extracts in vitro and silico as a candidate for antiaging active ingredients. This study identified secondary metabolites of the hexane (HE), ethyl acetate (EAE), and ethanol extracts (EE) from the root (R), stem (S), and leaf (L) organs by LC-ESI-MS and evaluated in vitro antioxidant and inhibitor elastase activity. An antioxidant evaluation was performed using ABTS, Beta Carotene Bleaching (BCB), and Ferric Reduction Antioxidant Power (FRAP). Evaluation of anti-elastase was carried out using elastase and followed by an in silico study of molecular docking using the target protein elastase (1B0F). Fifteen *C. latifolia* metabolites were identified in *C. latifolia* extracts, most of which were phenolic compounds. In antioxidant testing, REE, REAE, SEE, and SEAE extracts showed potent antioxidant activity based on the ABTS, BCB, and FRAP methods. In anti-elastase testing, it was found that SEE, REE, REAE, and RHE extracts gave powerful inhibition of elastase activity (in the ranges of 16.89 to 27.91 μg/mL). The in-silico study demonstrated the potential of the identified metabolites to bind to the target protein 1B0F involved in remodeling the skin aging process. This research concludes that the extracts from *C. latifolia* have the potential to serve as an active antiaging source.

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1. Introduction

Aging is a complex and continuous biological system involving changes in the cellular and molecular structure of the body. Intrinsic and extrinsic factors influence the maturation process of the skin. As the body ages, the intrinsic factor causes fine wrinkles

and a thinned epidermis (Purohit et al., 2016; Zhang and Duan, 2018). Contrary to internal aging, extrinsic aging leads to deep wrinkles, weak skin structure, and hyperpigmentation, primarily due to prolonged sun exposure (Lago and Puzzi, 2019; Xia et al., 2015). As the skin ages, wrinkles appear, and the skin's elasticity is diminished, resulting in progressive dermal atrophy. Several factors contribute to the development of dermal atrophy, including a decrease in the extracellular matrix (ECM), which includes collagen and elastic fibers (Lukitaningsih et al., 2020).

Elastin is an ECM fibrous protein that plays a role in providing elasticity and repair to tissues. Collagen is a fundamental molecular unit in forming skin tissue produced from procollagen. In the dermis, dermal fibroblasts produce procollagen under the influence of transforming growth factor-β (TGF-β) and activator protein-1 (AP-1), where TGF-β regulates collagen production, and AP-1 regulates collagen breakdown. The presence of intrinsic and extrinsic effects, such as chronic UV radiation, promotes the break-

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down and decreased protein synthesis due to the upregulation of matrix metalloproteinases (MMPs), including the decrease in elastase synthesis. This process causes tissue damage during photoaging and premature aging (Jadoon et al., 2015; Varani et al., 2000).

An increase in skin damage necessitates chemoprevention and therapy strategies. One promising strategy is by utilizing natural materials. In today's era, treatment based on natural materials is increasingly popular. Most people feel safe in its use due to the minimal side effects. A study by WHO stated that about 80% of the world population strongly believes in herbal medicine because of the therapeutic effects of natural ingredients. The increasing use of natural ingredients in medicine encourages investigation of chemical compounds that can provide pharmacological potential (Saleem et al., 2021; Zengin et al., 2018).

Curculigo latifolia is a plant from the Hypoxidaceae family widely distributed in China, Japan, Nepal, Malaya, India, Australia, and Africa. The original distribution of this plant comes from India, Myanmar, Thailand, Peninsular Malaysia, Singapore, Philippines and some parts in Indonesia such as Sumatra, Lingga Islands, Bangka Island, Borneo, Java, and Celebes (Wang et al., 2021). Scientifically, *C. latifolia* has been found to possess antioxidant, antidiabetic, and antibacterial properties (Farzinebrahimi et al., 2016; Ooi et al., 2018; Umar et al., 2021). The bioactivities of *C. latifolia* are strongly supported by the content of secondary metabolites found in these plants. Previous reports showed that the roots of *C. latifolia* contain phenolic compounds like phloridzin, pomiferin, scandenin, and mundulone through the LC-MS analysis (Zabidi et al., 2019). As reported in a previously study, *C. latifolia* contains phenolic glycosides such as orchioside derivatives, curculigoside derivatives, and triterpenes (cycloartane) curculigosaponin derivatives determined based on the UHPLC-Q-Orbitrap HRMS. The leaves were reported to contain phenolic glycoside and curculigosaponin products (cycloartan triterpenes) (Umar et al., 2021).

Although *C. latifolia* has been reported to have various pharmacological effects, this study explored the antiaging effect through antioxidant activity and elastase inhibition. Some previous information stated that the leaf and root extracts of *C. latifolia* had antioxidant effects but were carried out with limited methods. This study explored the antioxidant activity using the ABTS radical reduction method, lipid peroxidation (BCB assay), and iron reduction (FRAP Assay). Antioxidant compounds in *C. latifolia* extract have a role in reducing the activity of skin-degrading enzymes, one of which is elastase. A study of the phytochemical content by LC-ESI-MS in the root, stem, and leaf extracts of *C. latifolia* was also conducted in order to enrich the data information and study its elastase inhibitory activity with an in silico approach through molecular docking.

2. Materials and methods

2.1. Plant collection and extraction

C. latifolia plant was obtained from the Research Institute for Spices and Medicinal Plants (BALITTRO), Bogor City, West Java, Indonesia (-6.576994315224358° N, 106.78637511741873° E). The specimen of *C. latifolia* was identified by Dr. Anang Setiawan Ahmad and stored at the Herbarium Bogoriense, Center for Biological Research, National Research and Innovation Agency (BRIN), Cibinong, West Java, with voucher code B-132/V/DI.05.07. Plant parts of *C. latifolia* were (the roots, stems, and leaves) cleaned and dried at 40 °C (72 h). Dry materials were powdered and extracted by maceration in stages with solvent variations starting from non-polar to polar solvents (*n*-hexane, ethyl acetate, and 70% ethanol, respectively). Solvents from each extract was evaporated to obtain *n*-hexane extract of root (HRE), stem (HSE), and leaf

(HLE). Ethyl acetate extract of root (REAE), stem (SEAE), leaf (LEAE). Ethanol extracts of roots (REE), stems (SEE), and leaves (LEE). The extracts obtained were then subjected to further testing.

2.2. Phytochemical screening by LC-ESI-MS

The extracts of *C. latifolia* were analysed by LC-ESI-MS. LC-ESI-MS analysis was carried out using Waters Acquity UPLC I-Class and XEVO G2-XS QTof. Chromatographic separation was performed using an ACQUITY UPLC® BEH C18 (1.7 m, 2.1 x50 mm). The mobile phase used a mixture of A (H_2O + 0.1% Formic Acid) and B: (ACN + 0.1% Formic Acid) with gradient concentration. The ionization was carried out in a positive ion mode in m/z range 100–1200 amu, with a capillary voltage of 2 kV and the source temperature of 120 °C, the desolvation temperature of 500 °C. The fragmentation amplitude was set to 40 eV, and MS2 data were acquired in positive ion mode (Hanafi et al., 2018).

2.3. Antioxidant analysis

The antioxidant activity of each extract was analyzed with different methods including ABTS (Nur et al., 2021b), lipid peroxidation by beta-carotene bleaching (Nur et al., 2021a), and FRAP assays (Dravie et al., 2020).

2.3.1. Radical ABTS reduction assay
A radical scavenging method based on ABTS was modified by Nur et al. (2021b). A mixture of ABTS (7 mM in 10 mL distillate water) and potassium persulfate (2.45 mM in 10 mL distillate water) was incubated for 12–16 h in the dark to form an ABTS radical solution. The solution mixture was then diluted with ethanol to 50 mL. A working solution of ABTS was prepared by mixing it with ethanol (1:10). 1 mL of ABTS working solution was diluted to 5 mL with ethanol and incubated for 30 min as a blank solution. The absorbance was measured with a UV–visible spectrophotometer (745 nm; Shimadzu UV-1900). The serial volumes of each *C. latifolia* extract were reacted with 1 mL of ABTS working solution, and the volumes were made up to 5 mL with ethanol. An absorbance measurement at 745 nm was performed after incubating the mixture for 30 min in the dark at room temperature. The positive control in this study used standard quercetin and ascorbic acid.

IC_{50} values were calculated by comparing the percentage of inhibition of ABTS radicals across different concentrations of sample solution with the formula:

% Inhibition = (Abs. blank – Abs. sample/Abs. blank) $\times$ 100%.

2.3.2. Lipid peroxidation inhibition by beta carotene bleaching (BCB) assay
Assays for BCB were performed in accordance with Nur et al. (2021a) with slight modifications. To create beta carotene emulsion (BCE), beta carotene powder (20 mg) was dissolved in chloroform (0.2 mL), followed by linoleic acid (0.2 mL) and tween 20 (2 mL). After mixing well, the remaining chloroform was evaporated. To form a transparent emulsion, the mixture was diluted with distilled water to 100 mL. A serial volume of each extract solution was prepared by mixing 1 mL of BCE with 5 mL of ethanol. After incubation at 50 °C for 20 and 120 min, the absorbance at 461 nm was measured by a spectrophotometer (Shimadzu UV-1900). A blank solution was prepared (1 mL of BCE in 5 mL of ethanol), and its absorption was measured in the same procedure. Quercetin and butyl hydroxytoluene (BHT) were used as positive controls.

The difference between the degradation of the sample and a blank solution was used to calculate antioxidant activity. The absorbance results were then processed to find the rate of degradation based on the formula (**ln (a/b)** $\times$ **1/t),** where **Ln** is a corre-

sponds to the natural logarithm, **a** is an absorbance value after 20 min of incubation, **b** is an absorbance value after 120 min, and **t** is the incubation time (120 min). IC_{50} values are determined by calculating the inhibition of lipid peroxidation at each concentration of sample solution using the formula:

$$Inhibition(\%) = \frac{degradation\ rate\ of\ blank - degradation\ rate\ of\ sample}{degradation\ rate\ of\ blank} x100$$

2.3.3. FRAP (Ferric reducing antioxidant power) assay

This study involved the reduction of iron using the FRAP method (Dravie et al., 2020). FRAP reagent was made by reacting acetate buffer (pH 3.6), Tris Pyridyl Triazine (TPTZ, 1 mM), and $FeCl_3$ (0.02 M) in a ratio of 10:1:1. Each extract solution (0.1% w/v) was mixed with 2 mL of FRAP reagent, and distilled water was added to make up the volume (5 mL). Incubation at room temperature for 30 min was followed by an absorbance measurement at 595 nm (Shimadzu UV-1900). The FRAP value for each extract was calculated using quercetin as the standard curve. The quercetin equivalent value per gram of extract (µMQEV/g extract) was used to estimate its antioxidant capability (Nur et al., 2019).

2.4. Elastase inhibition assay

Testing the elastase inhibitory activity of each extract of *C. latifolia* according to the modified assay protocol based on several studies conducted by Anggraini et al. (2020), Elgamal et al. (2021) dan Putri et al. (2019). Each extract of *C. latifolia* (10 mg) was weighed and dissolved in tris-buffer HCl up to 10 mL (1000 µg/mL). A concentration of 10–1000 µg/mL of the test solution was achieved by diluting the sample solution in series. As much as 20 µL of sample solution (10–1000 µg/mL) was inserted into 96 wells, added 120 µL of Triz-HCl buffer pH 8 to the well. The test solutions were added to 20 µL (0.22 U/mL) elastase (Sigma Aldrich SLBV 9311) and then incubated for 20 min at 25 °C. After incubation, the mixture was added of N-succinyl-(Ala-Ala-Ala) nitroanilide of 30 µL (Sigma Aldrich, S4760) to the well plate. After incubating at 25 °C for 50 min, the absorbance was measured at 405 nm with a 96-well Microplate Reader (Promega Glomax) (Anggraini et al., 2020).

2.5. In silico study by docking molecular

Molecular docking analysis of the fifteen compounds produced by the LC-ESI-MS analysis was carried out using the procedure described by Nursamsiar et al. (2020). In silico analysis of this enzyme's structure was conducted using the protein data bank (https://www.rcsb.org/structure/1B0F). Results of molecular docking were analysed regarding various evaluation parameters, i.e., the orientation of the ligand structure, hydrophobic interactions, hydrogen bonds formed, and the value of free energy from each ligand.

2.6. Data analysis

In vitro experiments were performed three times, with results reported as mean ± SD. To determine the significance level of the mean values, a one-way ANOVA was performed (Tukey HSD). The correlation between the antioxidant activity of each sample against elastase inhibition was analyzed through Pearson's correlation (Minitab 20 version). The in silico models were analyzed by Autodock Tools 4.2 program package.

3. Result

3.1. Extraction yields

The extraction process of plant organs of *C. latifolia* was carried out by maceration using solvents of different polarity to obtain their phytochemical profile. Based on the "like dissolved like" principle, *n*-hexane, ethyl acetate, and ethanol (70% v/v) were used to extract compounds that have low, semi, and high polarity from each plant organ, respectively (Chormey and Bakirdere, 2018; Zhuang et al., 2021). The ability of the solvents to extract compounds can be determined based on the yield percetages obtained. Results show that compared to *n*-hexane, ethyl acetate, and ethanol (70%v/v) gave the highest yield, obtaining yields of 48.32%, 46.75%, and 34.46%, from roots, stems, and leaves, respectively.

3.2. Phytochemical screening

The phytochemicals of *C. latifolia* were analysed using LC-ESI-MS. Figs. 1 to 3 showed the LC-ESI-MS profiles of the root, stem, and leaf extracts. According to their mass accuracy and fragmentation pattern, as many as 15 compounds were identified. Ten of which were of phenolic, and four others were terpenoid/steroid derivates (Fig. 1). Information on compound names, retention times, and *m*/*z* fragmentation (positive molecule ion) can be seen in Table 1.

3.3. ABTS radical reducing activity

ABTS radical scavenging activity in the extract positively correlated with their concentrations. The radical scavenging activity of ABTS increases with increasing sample concentration. An inhibitor concentration of 50% (IC_{50}) was used to measure the capacity of *C. latifolia* extract to lessen ABTS radicals. The test results in Fig. 4 show that the ethanol and ethyl acetate extracts in the roots, stems, and leaves of *C. latifolia* were highly active antioxidants with an IC_{50} value of 50 µg/mL, while the *n*-hexane extract in each organ had weak antioxidant activity (IC_{50} > 150 µg/mL). Among all *C. latifolia*, REE extract demonstrated the strongest antioxidant activity with an IC_{50} value of 9.79 µg/mL, significantly different from ascorbic acid (6.51 µg/mL) and three times weaker than the antioxidant power of quercetin (3.6 µg/mL) as a positive control.

3.4. BCB (Beta carotene bleaching) assay

Antioxidant activity testing the usage of the BCB technique was primarily based on the potential of antioxidant compounds from *C. latifolia* extract to prevent the rate of beta-carotene degradation because of the formation of peroxide radicals attributable to the oxidization of linoleic acid. In this study, the antioxidant capabilities of each extract of *C. latifolia* were expressed as IC_{50} (Table 2). The process of inhibiting the amount of beta-carotene degradation relies upon on increasing the concentration of the sample. The higher the concentration, the higher the inhibition amount of beta-carotene degradation. The test results for the ability of antioxidants to inhibit the beta-carotene degradation rate by lipid peroxidation showed that each *C. latifolia* extract had a significantly different antioxidant capacity among the samples (P < 0.05, n = 3). In Table 2, REAE and SEAE samples (IC_{50} < 20 µg/mL) showed high antioxidant properties compared to other extracts and quercetin as a positive control. However, compared with the BHT (IC_{50} = 0.55 µg/mL) as a positive control, the REAE and SEAE samples showed low antioxidant properties. BHT was used as a comparison because BHT is known to have specific antioxidant activity in preventing lipid peroxidation (Abdul Karim et al.,
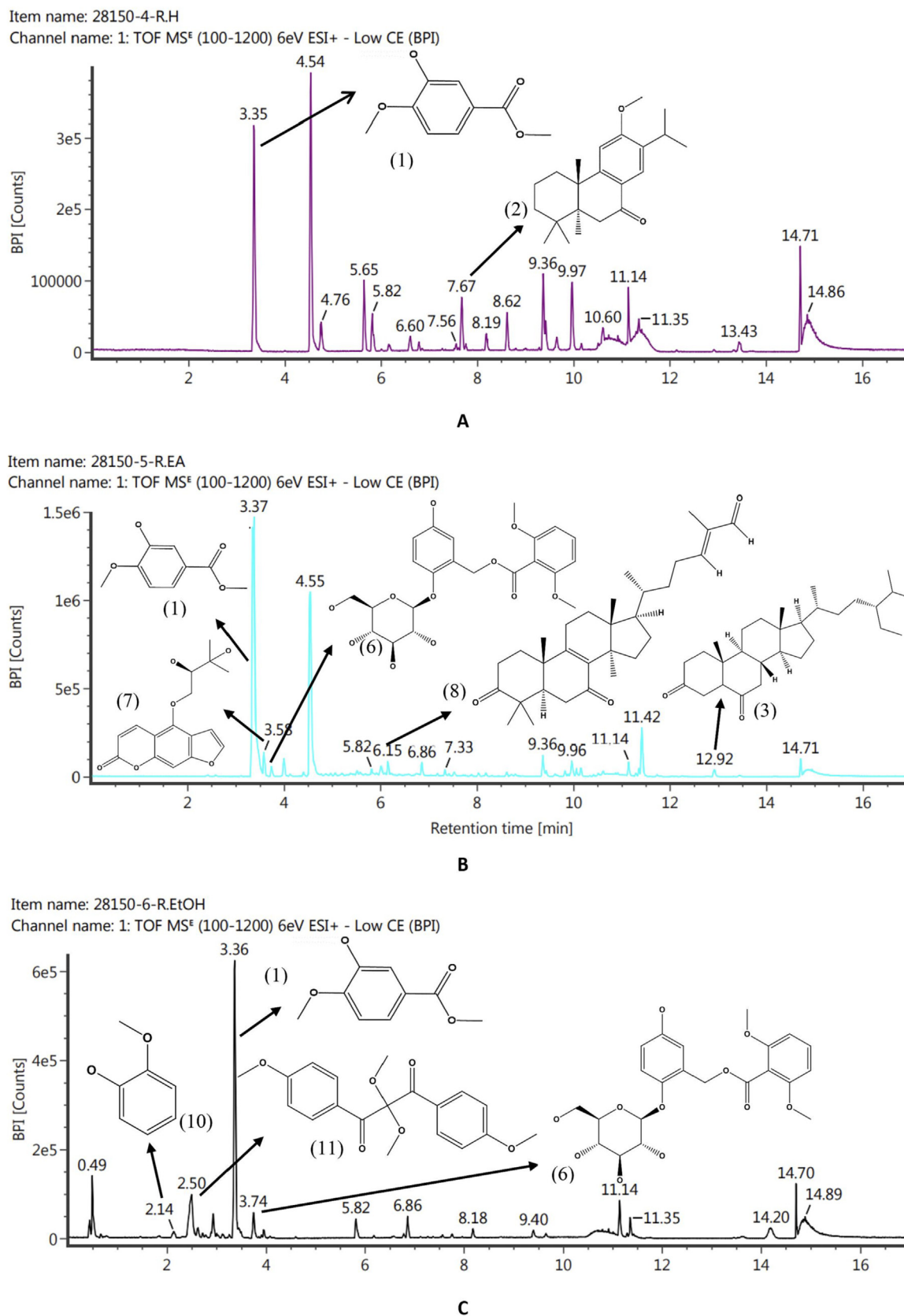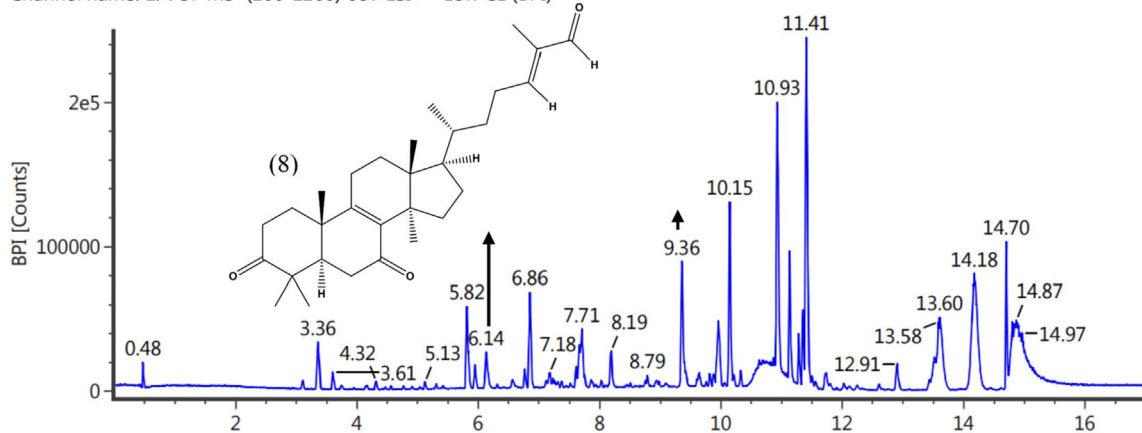
Fig. 1. LC-ESI-MS/MS profile accompanied by the structure of compounds identified from root extract. Fig. 1A (RHE) was predicted to contain methyl-3-hydroxy-4-methoxybenzoate (1) and sugiol (2). Fig. 1B (REAE) was predicted to contain the compounds methyl-3-hydroxy-4-methoxybenzoate (1), stigmastan-3,6-dione (3), curculigoside (6), aviprin (7), and lucialdehyde B (8). Fig. 1C (REE) was predicted to contain the compounds methyl-3-hydroxy-4-methoxybenzoate (1), curculigoside (6), guaiacol (10), and smilaxin (11).
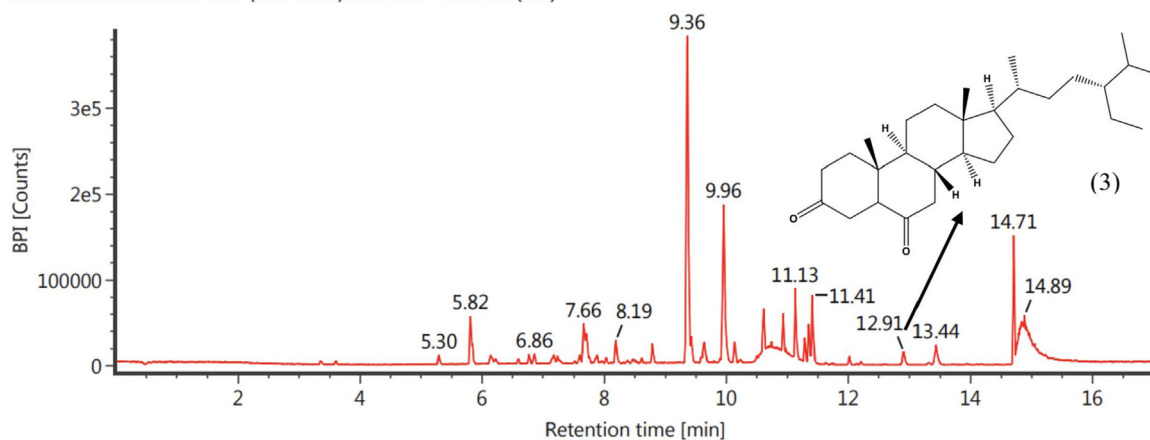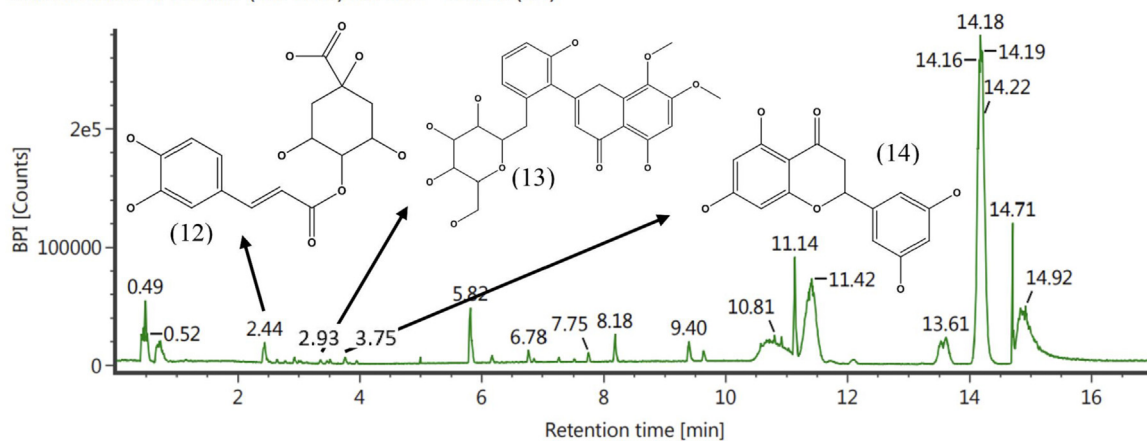
Fig. 2. LC-ESI-MS/MS profile accompanied by the structure of compounds identified from stem extract. Fig. 2D (SHE) was predicted to contain stigmastan-3,6-dione (3). Fig. 2E (SEAE) was predicted to contain lucialdehyde B (8), and Fig. 2F (SEE) was predicted to contain the compounds 5,2′,6′-trihydroxy-7,8-dimethoxy-flavone-2′-O-β-D-glucopyranoside (13), 5,7,3′,5′-tetrahydroxyflavanone (14), and quercetin (15).
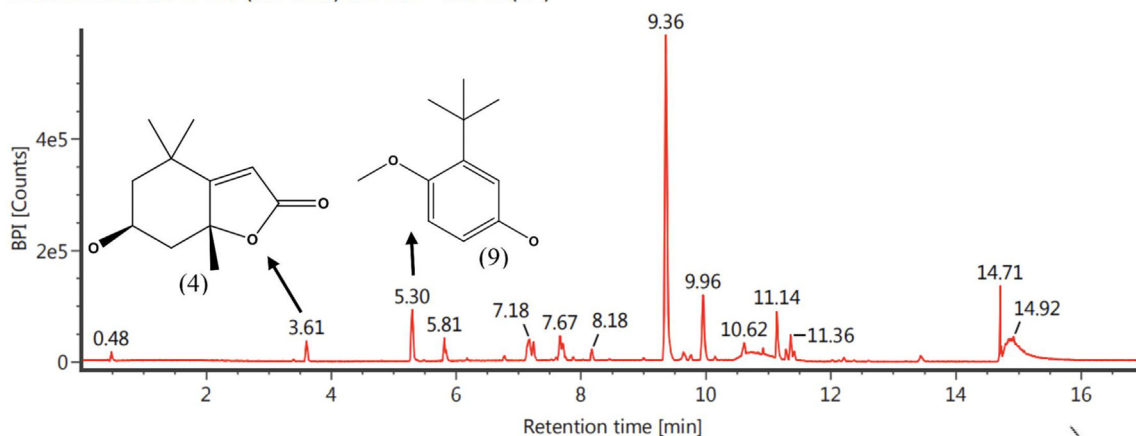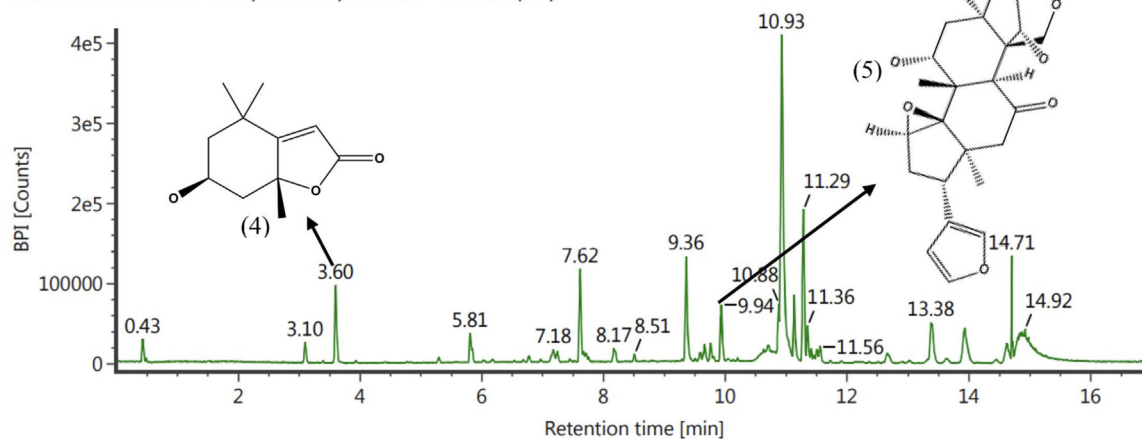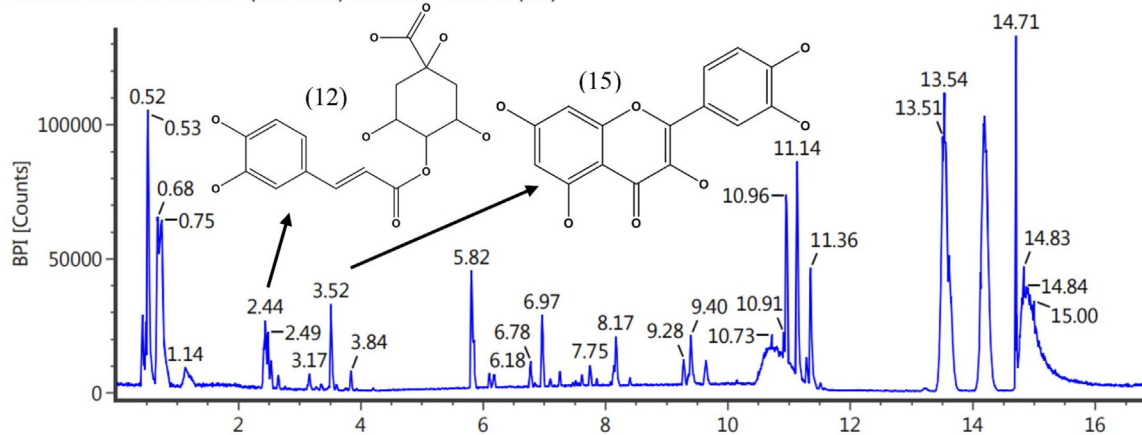
Fig. 3. LC-ESI-MS/MS profile accompanied by the structure of compounds identified from leaf extract. Fig. 3G (LHE) was predicted to contain digiprolactone (4) and 3-*tert*-butyl-4-methoxyphenol (5). Fig. 3H (LEAE) was predicted to contain digiprolactone (4) and azedarachin C (5). Fig. 3I was predicted contain 4-*O*-Caffeoylquinic acid-1 (12) and quercetin (15).
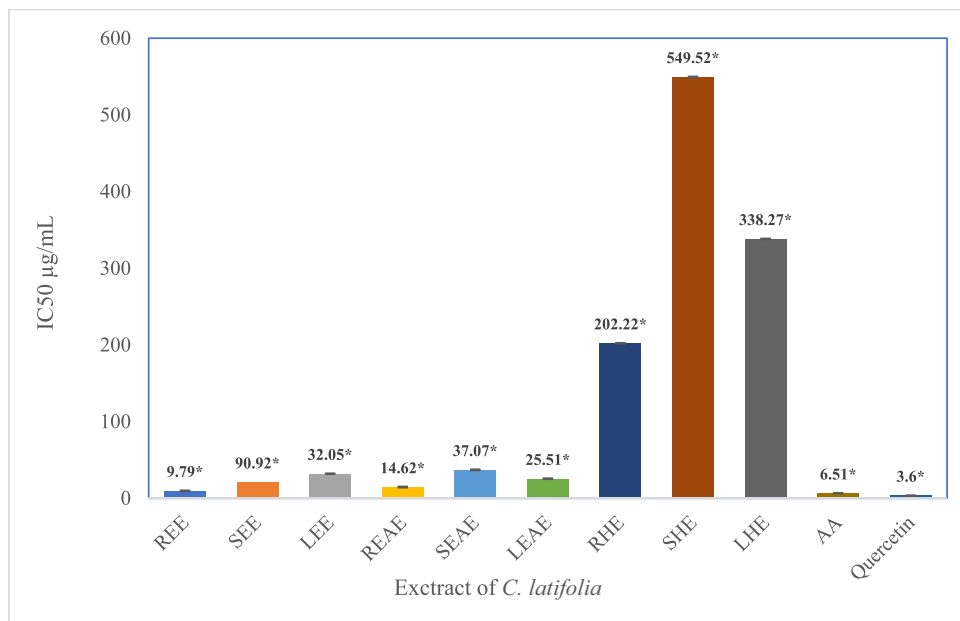
Fig. 4. Test the antioxidant activity of each *C. latifolia* extract by reducing ABTS radicals. This study used ascorbic acid (1–7.5 μg/mL) and quercetin (1–5 μg/mL) as positive controls. *indicates that the data are significantly different with a Tukey HSD of $p \leq 0.05$ (n = 3).

Table 1
Phytochemical screening of *C. latifolia* extracts using an LC-ESI-MS.

No	RT (min)	Observed MS (m/z)	Molecular ion	Compounds	Molecular Formula	Extracts	References
1	3.36	183.06	M + H, +Na	Methyl-3-hydroxy-4-methoxybenzoate	$C_9H_{10}O_4$	HRE, REAE, REE	(Seghiri et al., 2006)
2	7.68	301.21	M + H	Sugiol	$C_{20}H_{28}O_2$	HRE	(Chao et al., 2005; Simoneit et al., 2019)
3	12.91	429.37	M + H	Stigmastan-3,6-dione	$C_{29}H_{48}O_2$	HSE, REAE	(Lim et al., 2005)
4	3.61	197.12	M + H	Digiprolactone	$C_{11}H_{16}O_3$	HLE. LEAE	(Lestari et al., 2022)
5	9.94	609.27	M + H	Azedarachin C	$C_{32}H_{42}O_{10}$	LEAE	(Chao et al., 2005; Priyanto et al., 2023)
6	3.75	489.14	M + H, +Na	Curculigoside	$C_{22}H_{26}O_{11}$	REAE, REE	(Zhao et al., 2014)
7	3.58	305.102	M + H, +Na	Aviprin	$C_{16}H_{16}O_6$	REAE	(Ishihara et al., 2001)
8	6.14	453.34	M + H	Lucialdehyde B	$C_{30}H_{44}O_3$	SEAE, REAE	(Gao et al., 2010)
9	5.30	181.12	M + H	3-*tert*-butyl-4-methoxyphenol	$C_{11}H_{16}O_2$	HLE	(Wiley and Sons., 2023)
10	2.14	125.06	M + H	Guaiacol	$C_7H_8O_2$	REE	(Dorfner et al., 2003)
11	2.49	317.102	M + H	Smilaxin	$C_{17}H_{16}O_6$	REE	(Woo et al., 1992)
12	2.44	355.102	M + H, +Na	4-*O*-Caffeoylquinic acid-1	$C_{16}H_{18}O_9$	SEE, LEE	(Li et al., 2016)
13	2.93	492.13	M + H, +Na	5,2′,6′-Trihydroxy-7,8-dimethoxy-flavone-2′-*O*-β-D-glucopyranoside	$C_{23}H_{24}O_{12}$	SEE	(Nurul Islam et al., 2013)
14	3.76	288.6	M + H, +Na	5,7,3′,5′-Tetrahydroxyflavanone	$C_{15}H_{12}O_6$	SEE	(Yang et al., 2016)
15	3.52	303.05	M + H	Quercetin	$C_{15}H_{10}O_7$	LEE	(Chen et al., 2015)

Table 2
Antioxidant activity of *C. latifolia* extract by beta carotene bleaching assay.

Sample	Percentage of inhibition (%) in a concentration (μg/mL) of:						IC_{50} (μg/mL)
	6.25	12.5	25	50	100	200	
REE	4.11 ± 0.05	10.79 ± 1.08	28.13 ± 0.56	48.11 ± 1,96	73.03 ± 0.88	–	62.05[*b]
SEE	6.80 ± 0.23	8.91 ± 0.4	17.76 ± 0.13	42.98 ± 0.53	74.91 ± 1.22	–	64.97[*a]
LEE	22.34 ± 0.87	29.67 ± 1.35	39.38 ± 2.13	42.50 ± 0.98	59.43 ± 0.39	–	70.55[*b]
REAE	38.44 ± 1.36	45.83 ± 0.97	59.93 ± 0.39	67.83 ± 1,3	82.15 ± 1.12	–	14.81[*e]
SEAE	39.96 ± 1.33	48.23 ± 1.10	50.55 ± 0.64	67.29 ± 0.75	80.44 ± 0.88	–	19.42[*d]
LEAE	8.66 ± 0.77	9.69 ± 0.89	37.77 ± 1.25	56.58 ± 5.19	82.16 ± 5.16	–	51.34*
RHE	–	2.25 ± 0.14	9.96 ± 0.26	23.96 ± 0.61	44.65 ± 1.42	86.29 ± 4.60	115.1[*h]
SHE	–	17.09 ± 1.12	18.63 ± 0.74	23.50 ± 0.40	47.23 ± 0.48	75.48 ± 1.27	118.7[*g]
LHE	–	15.08 ± 0.92	19.53 ± 0.59	40.76 ± 0.87	63.17 ± 0.87	85.49 ± 0.61	94.92*
BHT*	IC_{50} (0.55)*						
Quercetin*	IC_{50} (43.38)*						

Note: *Concentration series on positive control BHT (0.2 to 1 μg/mL) and quercetin (10 to 50 μg/mL). The percentage inhibition (%) of lipid peroxidation at each concentration is described as mean ± SD (n = 3). With a Tukey HSD of $p \leq 0.05$, the data indicate a significant difference (*). [abc.]There was no extensive difference in data from the alphabetical order at $p \leq 0.05$ (Tukey HSD).

2014). At the same time, quercetin is a phenolic and flavonoid compound that is spread in natural ingredients, has potent antioxidant activity, and was used as a comparison to determine its action in preventing the oxidation process of beta carotene.

3.5. Ferric reducing antioxidant capacity (FRAP) assay

To generate the standard calibration curve used in this study (Fig. 5). Quercetin is a phenolic-flavonoid compound that can represent the compound content of *C. latifolia*, which can reduce the Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ so that the results can be equivalent to its antioxidant capacity (Lim et al., 2007; Nur et al., 2019). Generally, each extract from *C. latifolia* can reduce iron complexes to Fe^{2+}.

The standard curve of quercetin (Fig. 5) obtained a linear regression value (r^2) 0.9967 with 3 repetitions which showed good linearity so that it could be used to calculate the antioxidant power by reduction of Fe^{3+} to Fe^{2+} from *C. latifolia* extracts. Table 3 shows that the REAE extract had a significantly higher reducing ability ($p \leq 0.05$) with a value of 833.33 ± 8.35 µMQEV/g among sample, followed by REE (462.12 ± 7.57), SEAE (422.98 ± 4.16), SEE (316.92 ± 9.53) and LEE (209.34 ± 7.04) extract. Meanwhile, LEAE, RHE, SHE, and LHE extracts provided a reduced power of < 100 µMQEV/g. The antioxidant capacity in reducing iron from REAE samples showed a reducing power close to that of ascorbic acid (997.26 ± 10.42 µMQEV/g extract) as a positive control.

3.6. In vitro anti-elastase assay

The in vitro anti-elastase test results (Table 4) showed that SEE, RHE, REE, and REAE extracts provide obtained powerful inhibitory action with IC$_{50}$ values of 16.89, 19.22, 23.84, and 27.91 µg/mL, respectively. A similar action was also discovered for quercetin with an IC$_{50}$ value of 4.88 µg/mL. The SEAE and LEE extracts showed strong activity with IC$_{50}$ values > 50 µg/mL, while SHE extracts a moderate activity with IC$_{50}$ values of 106.35 µg/mL. In contrast, LEAE and LHE extracts gave weak activity in inhibiting the action of elastase (IC$_{50}$ > 150).

3.7. Pearson's correlation between antioxidant and anti-elastase

The existence of a correlation between the antioxidant activity of each *C. latifolia* extract on elastase inhibition was performed through Pearson correlation statistical analysis. The correlation results of each antioxidant method on elastase inhibition can be seen in Table 5. The analysis results show that each extract's antioxidant activity correlates with elastase inhibition.
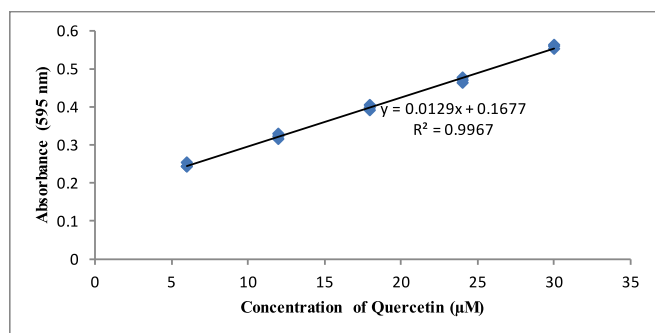

Fig. 5. The quercetine calibration curve (6–30 µM), each data point were analyzed triplicates (n = 3).

Table 3
Antioxidant activity in reducing iron using the FRAP method.

Extract Samples	Reduction Power (µMQEV/g extract) (595 nm)
REE	462.12 ± 7.57*
SEE	316.92 ± 9.53*
LEE	209.34 ± 7.04*
REAE	833.33 ± 8.35*
SEAE	422.98 ± 4.16*
LEAE	88.38 ± 1.16*
RHE	37.27 ± 2.48*
SHE	18.61 ± 1.70*i
LHE	14.57 ± 0.61*h
Ascorbic Acid	997.26 ± 10.42*

Note: Reduction power are represented as mean ± SD with three times replication (n = 3). *The data demonstrate a significant difference at the $p \leq 0.05$ level (Tukey HSD). abc..Alphabetical differences showed that the data were not extensive different at the $p \leq 0.05$ level (Tukey HSD).

3.8. In silico anti-elastase assay

The Parameters in this docking study were amino acid residues, hydrogen bonds, and free bond energy (ΔG). Fig. 6 shows the compound (ligand) structure of *C. latifolia* obtained from the predicted LC-MS/MS for each extract. Primarily based at the visualization results, it is able to be seen that all compounds from *C. latifolia* extract were able to interact with the active site of elastase (Table 6). The data in Table 6 suggests that the compounds in the extract of *C. latifolia* have bond-free energies (ΔG) in the ranges of −4.18 to −8.27 kcal/mol concerning the active site of the elastase. Eight of these molecules form bonds with the amino acids Gly 193, Val 216, and Ser 195, key amino acids in elastase target proteins and capable of restate some of the reported interactions with native ligands (Fig. 7).

4. Discussion

The current study focused on screening the antiaging activity of extracts from several parts of the *C. latifolia* plant based on antioxidant activity and in vitro and molecular docking approaches to anti-elastase activity. Three solvents (*n*-hexane, ethyl acetate, ethanol 70% v/v) were used to extract the phytochemical compounds in the dried plant parts of *C. latifolia*. The percentage yields value of *C. latifolia* extracts differs based on the polarity of the solvent used. This study considered that the use of ethanol as a solvent was effective in extracting components from each extract. The polarity index of ethanol is 5.2, while its dielectric constant is 24.55, which allow it to extract compounds with high polarity. In this study, ethanol (70% v/v) was used, indicating a water content of 30% v/v so that the polarity of the solvent was increasing (Abarca-Vargas et al., 2016; Hikmawanti et al., 2021). The use of ethanol solvents has been reported to attract components of the carbohydrate group, phenolic flavonoids, alkaloids, steroids, and terpenoids to allow a large percentage of yield value compared to the use of other solvents such as ethyl acetate and *n*-hexane, which have low polarity (Lapornik et al., 2005; Zabidi et al., 2019).

Based totally on the results of the LC-ESI-MS analysis, the majority of the compounds were obtained from the ethanol extract of each organ of *C. latifolia* (Table 1). In the root (Fig. 1) of *C. latifolia*, curculigoside is dominant. Comparable results were also found in (Ooi et al., 2016; Umar et al., 2021), which identified curculigoside compounds in the ethanolic extract of *C. latifolia* roots and leaves. However, the compounds methyl-3-hydroxy-4-methoxybenzoate found in HRE, REAE, and REE, sugiol (HRE), aviprin (REAE), guaiacol (lignin), and smilaxin found in REE were reported in the first time in the roots of the *C. latifolia* and stigmastan-3,6-dione and lucyaldehyde B in the roots and stem of *C. latifolia* (Fig. 2). Some

Table 4
The anti-elastase activity of *C. latifolia* extract.

Extract	Percentage of inhibition (µg/mL) in a concentration of:							IC$_{50}$ (µg/mL)
	2.34	4.69	9.375	18.75	37.5	75	150	
REE	31.76 ± 4.22	36.91 ± 0.59	45.14 ± 0.27	49.09 ± 0.73	52.87 ± 3.29	57.90 ± 2.04	–	23.84*
SEE	36.14 ± 1.78	40.88 ± 1.05	44.12 ± 6.41	51.44 ± 1.62	56.21 ± 1.84	60.75 ± 3.77	–	16.89*g
LEE	35.29 ± 0.78	37.91 ± 1.49	40.21 ± 3.25	44.35 ± 3.06	48.45 ± 4.79	51.32 ± 2.69	–	58.31*e
REAE	26.12 ± 5.95	31.38 ± 4.06	39.85 ± 1.41	45.72 ± 1.53	52.04 ± 4.78	61.12 ± 1.59	66.06 ± 1.01	27.91*
SEAE	24.66 ± 4.31	27.87 ± 3.28	33.10 ± 2.34	41.34 ± 3.00	45.84 ± 2.22	51.35 ± 0.86	59.12 ± 2.47	58.11*c
LEAE	13.82 ± 4.21	17.49 ± 2.65	21.13 ± 2.93	26.95 ± 3.77	30.26 ± 3.27	36.13 ± 4.68	45.37 ± 2.03	>150*
RHE	31.37 ± 6.86	36.72 ± 3.33	46.93 ± 1.08	48.96 ± 1.79	54.39 ± 1.10	61.27 ± 0.59	68.79 ± 1.68	19.22*b
SHE	14.91 ± 4.41	19.07 ± 3.72	23.91 ± 0.19	33.54 ± 3.63	40.36 ± 1.08	48.14 ± 1.69	52.60 ± 8.00	106.35*
LHE	3.69 ± 1.91	7.70 ± 6.81	12.8 ± 7.19	17.4 ± 5.79	23.2 ± 1.64	27.1 ± 2.97	30.0 ± 1.35	>150*
Quercetin	IC$_{50}$ (4.88 µg/mL)							

Note: Concentration series on positive control quercetin (1.5 to 13.5 µg/mL). The percentage inhibition (%) of elastase at each concentration is expressed as mean ± SD (n = 3). *indicates the data is extensive different with p ≤ 0.05 level (Tukey HSD). [abc..]Alphabetical differences indicated that the data were not extensive different with a level of p ≤ 0.05 (Tukey HSD).

Table 5
The Pearson's correlation coefficient of BCB, ABTS, FRAP and anti-elastase activity.

Correlation	*r*-values			Anti-elastase
	BCB assay	ABTS assay	FRAP assay	
BCB	1			
ABTS	0.796	1		
FRAP	−0.814	−0.691	1	
Anti-Elastase	0.279	0.615	−0.495	1

of these compounds have pharmacological effects, including curculigoside, a phenolic compound, has been widely reported to have an antioxidant effect (Ooi et al., 2016; Wang et al., 2012; Zhang et al., 2019) and act as an anti-aging agent in inhibiting MMP-1 (Lee et al., 2009). Methyl-3-hydroxy-4-methoxybenzoate (Wang et al., 2016), aviprin (Zahri et al., 2012), guaiacol (Azadfar et al., 2015), and sugiol (Bajpai et al., 2014) also been reported to have an antioxidant effect. Phenolic derivatives in the stem of *C. latifolia* were also found in the form of 4-*O*-caffeoylquinic acid-1, 5,2′,6′-tri hydroxy-7,8-dimethoxy-flavone-2′-*O*-β-D-glucopyranoside, and 5,7,3′,5′- tetrahydroxyflavanone and they are confirmed that compounds have the anti-radical capacity (Ganzon et al., 2018; Sun et al., 2007; Wang et al., 2018). In addition to the stem, 4-*O*-caffeoylquinic acid-1 (phenolic) compounds were found in the *C. latifolia* (LEE) leaf. The leaf of *C. latifolia* (Fig. 3) also contains quercetin (flavonoid) compounds (LEE), which have very potent antioxidant bioactivity (Chen et al., 2015) and terpenoid derivatives such as digiprolactone (HLE, LEAE) and azedarachin C (LEAE). The profile of chemical compounds produced from each extract of *C. latifolia* plant organs strongly supports the development of *C. latifolia* in delivering natural products that have pharmacological effects and are beneficial in medicine.

The phenolic content in *C. latifolia* extract acts as an antioxidant in inhibiting the activity of free radicals. In this study, antioxidant activity was quantified using different methods to confirm further the compounds' role in *C. latifolia* as antioxidants. In the ABTS method, antioxidant activity is measured by the potential of antioxidant compounds to contribute proton radicals to free radical compounds (Ahmad Wani and Tirumale, 2018; Morales and Paredes, 2014; Nur et al., 2021b). In the BCB method, Antioxidant potential compounds from the extract C. latifolia work by preventing beta carotene decomposition when linoleic acid is oxidized to hydroperoxide radicals at 50 °C. Hydroperoxide radicals formed attack the double bonds in beta carotene resulting in oxidation, which causes the loss of chromophore groups that give the color orange of beta carotene (Amiri, 2014; Bogacz-Radomska and Harasym, 2018). Both ABTS and BCB methods work in reducing radical reactions through the proton coupled electron transfer

mechanism. The strength of antioxidant activity in the ABTS and BCB methods was determined based on the IC$_{50}$ value. An extract with a smaller IC$_{50}$ value has greater antioxidant activity. The IC$_{50}$ values that are in the range of < 50 µg/mL, 50–100 µg/mL, >100–150 µg/mL, and > 150 µg/mL indicate very strong, strong, moderate, and weak activity, respectively (Nur et al., 2021a, 2021b).

The ABTS method found that the ethanol and ethyl acetate extracts from the parts of the roots, stems, and leaves of *C. latifolia* tended to have powerful activity (Fig. 4). This result is different from the results described by Ooi et al. (2018) which stated that the methanol extract from *C. latifolia* had an IC$_{50}$ value of 431 ± 36.9 in ABTS assay with a weak category. At the same time, the extract on the stems and leaves of this plant has not been reported.

A BCB study indicated that the ethyl acetate extract of *C. latifolia* plant's roots, stems, and leaves were the most effective antioxidants. This was followed by ethanol extract and *n*-hexane extract, respectively (Table 2). These compounds can penetrate the lipid phase of the BCE and make it simpler to counteract hydroperoxide radicals from the linoleic acid oxidation process (Nur et al., 2021a). The phenolic compounds are methyl-3-hydroxy-4-methoxybenzoate, curculigoside, quercetin, 4-*O*-caffeoylquinic acid-1, 5,2′,6′-tri hydroxy-7,8-dimethoxy-flavone-2′-*O*-β-D-glucopyranoside, and 5,7,3′,5′- tetrahydroxy-flavanone are thought to work in inhibiting lipid peroxidation in the initiation phase and also in the propagation phase (Marco, 1968; Nickavar and Esbati, 2012). Studies on the anti-radical capacity of *C. latifolia* plant extracts in inhibiting the rate of beta-carotene degradation have not been reported. Therefore, this study provides information on the antioxidant bioactivity profile of *C. latifolia* extract in inhibiting lipid peroxidation by the beta-carotene bleaching method, which is relevant to peroxidation that occurs biologically (Aminjafari et al., 2016; Koleva et al., 2002).

Testing of antioxidant activity on *C. latifolia* extract was also performed the use of the FRAP technique. In the FRAP assay, the compounds in the extract redox react with iron complexes as a substrate. In principle, the FRAP test measures the ability of *C. latifolia* compounds to oxidize Fe^{3+} to Fe^{2+} under acidic conditions
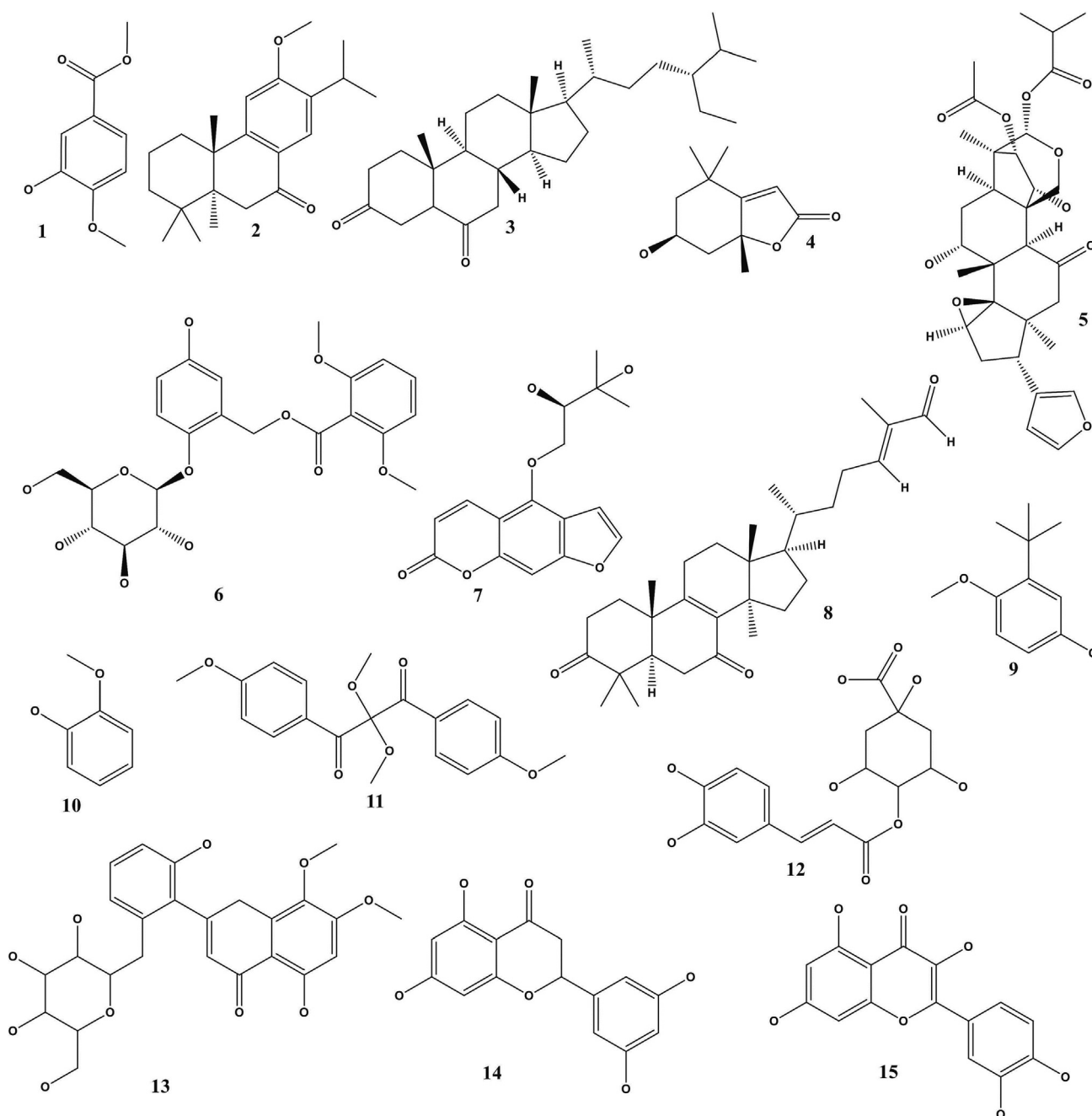
Fig. 6. Chemical structure of the compound *C. latifolia* extract. Names of chemical compounds is according to the order in Table 1 and Table 6.

(Aminjafari et al., 2016; Rabeta and Nur Faraniza, 2013). In FRAP method, the antioxidant strenght of each extract was determined that the root organ of *C. latifolia* plants tended to have extreme activity in reducing Fe^{3+} to Fe^{2+} complexes (Table 3). Based totally on the results of the phytochemical screening, the majority of compounds identified in the root extracts were phenolic compounds (Table 1). Hydroxyl groups in phenolic aromatics can chelate iron and reduce it by the redox reaction (Simamora et al., 2020; Spiegel et al., 2020). Testing of antioxidant activity using the FRAP assay on *C. latifolia* extract has not been widely informed. This test describes biological events in the body through the Fenton reaction, which aims to inhibit the conversion process of hydrogen peroxide produced in the metabolism into hydroxyl radicals, which negatively affect the body (Caillet et al., 2007; Kulisic et al., 2004; Nur et al., 2021b). The same thing for *n*-hexane extract

showed a lower effect than other extracts among the ABTS, BCB, and FRAP methods. This result may be influenced by the lack of phenolic in the *n*-hexane extract.

The three antioxidant quantification methods that have been carried out tend to provide similar activity between samples with a Pearson correlation coeffiicient (r) of 0.691 to 0.814 (Table 5). Antioxidant activity in the BCB method against ABTS and FRAP is strongly correlated with r-values of 0.796 and −0.814, which means that the BCB/ABTS method has similar activity up to 79.6% (strong correlation) and BCB/FRAP reaches 81.4% (strong correlation). The moderate correlation occurs in the ABTS/FRAP method with an r-value of 0.691, which means that the similarity of activities reaches 69.1%.

This study also tested the anti-elastase activity of *C. latifolia* extract. Inhibition of elastase activity is one of the parameters to

Table 6
The value of bond-free energy and amino acid residues that bind to the elastase (1B0F).

No.	Compounds	Bond-free energy (kcal/mol)	H-bond/van der waals Interaction*
Native ligand	(1-{3-methyl-2-[4-(morpholine-4-carbonyl)-benzoyl-amino]-butyryl} pyrrolidine-2-carboxylic acid-(3,3,4,4,4-pentafluoro-1-isopropyl-2-oxo-butyl)-amide	−7.69	**Gly 193, Val 216** **Ser 195**
1.	Methyl-3-hydroxy-4-methoxybenzoat	−4.71	**Gly 193, Ser 195** **Val 216**
2.	Sugiol	−6.50	**Gly 193, Ser 195** Ser 214
3.	Stigmastan-3,6-dione	−8.27	Arg 217, Gly 218
4.	Diprolactone	−5.67	Val 216
5.	Azedarachin C	−5.30	**Gly 193, Ser 195** Phe A41
6.	Curculigoside	−4.90	**Gly 193, Ser 195, Val 216**, Phe215
7.	Aviprin	−6.46	**Gly 193, Ser 195,** **Val 216**
8.	Lucialdehyde B	−6.81	**Ser 195**, Arg 217
9.	3-Tert-butyl-4-methoxyphenol	−5.76	**Gly 193, Ser 195** **Val 216**
10.	Guaiacol	−4.18	**Gly 193, Ser 195** Asp 194
11.	Smilaxin	−5.26	**Ser 195** **Val 216** Phe 192
12.	4-*O*-caffeoylquinic acid-1	−5.27	**Ser 195, Val 216** (phi-aril), **Gly 193**
13.	5,2,6-Trihydroxy-7,8 dimethoxy-flavone-2-*O*-β-D-gluc	−4.27	**Gly 193, Ser 195,** **Val 216**, Asp 194 Ser 214
14.	5,7,3,5-Tetrahydroxyflavanone	−6.34	**Ser 195, Val 216**
15.	Quercetin	−6.06	**Gly 193, Ser 195** **Val 216**

Note: *no bold letter in the column indicates as fol other amino acid residues that interact with hydrogen/van der waals on the *C. latifolia* compounds and are not the same as co-crystals.
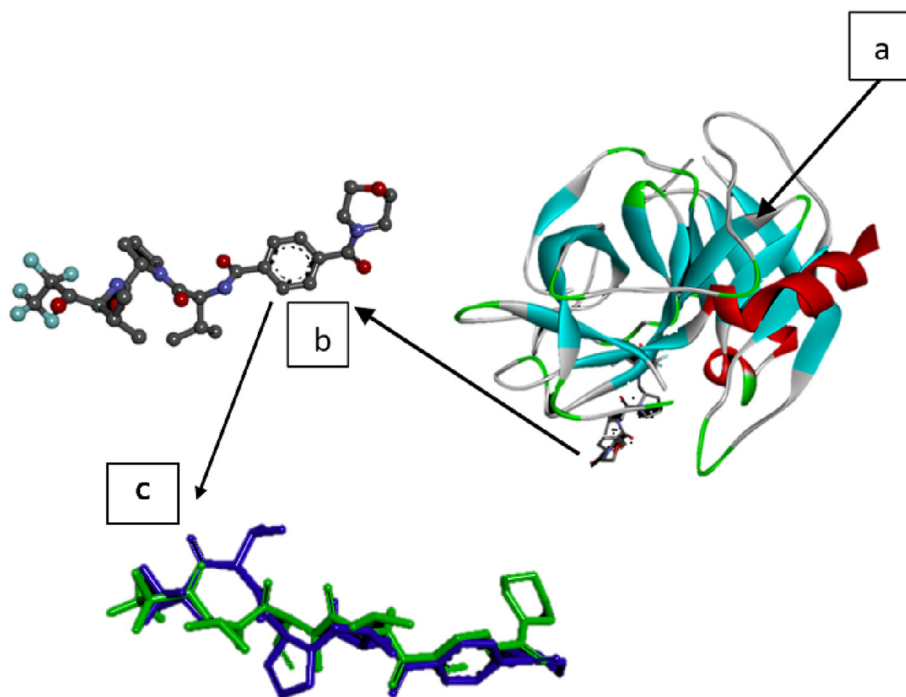
Fig. 7. Elastase Receptor (1B0F) Structure **(a)**, the native ligand (1-{3-methyl-2-[4-(morpholine-4-carbonyl)-benzoyl-amino]-butyryl} pyrrolidine-2-carboxylic acid-(3,3,4,4,4-pentafluoro-1-isopropyl-2-oxo-butyl)-amide **(b)** and re-docking native ligand (co-crystal) result in elastase pocket for validate the method with RMSD value of 1.678 Å **(c).**

determine the ability of a compound to inhibit skin aging. The inhibition of elastase by the inhibitor can be observed visually through the yellow color fading that occurs due to the inhibition of the interaction between elastase and *N*-succinyl-(Ala-Ala-Ala) nitroanilide as a substrate by compounds from the extract of *C. latifolia* (Desmiaty et al., 2021). Extracts from the roots of *C. latifolia*

(HRE, REAE, and REE) confirmed the most efective inhibition of elastase with an IC$_{50}$ value < 50 µg/mL (Table 4). Although the IC$_{50}$ value was significantly different (p ≤ 0.05) from quercetin as a positive control, the HRE, REAE, and REE extracts had the same potency, which was in the powerful category (IC$_{50}$ value < 50 µg/ mL). The results of identification carried out by LC-ESI-MS (Table 1) showed that extracts from the root of *C. latifolia* were generally a phenolic group of compounds. The previous studies from Szewczyk et al. (2020) and Pientaweeratch et al. (2016) confirmed that phenolic compounds can inhibit skin-degrading enzymes, through hydrogen binding from the elastase's active site and chelating ions from metalloenzymes.

The relationship study between anti-elastase and antioxidant activity (Table 5) showed that ABTS/FRAP was moderately correlated with anti-elastase activity, which reached 61.5% (r-value of 0.615) and 49.5% (r-value of 0.495), respectively. Unlike the case with anti-elastase/BCB, it shows a weak correlation with an r-value of 0.279, which means that anti-elastase activity is only influenced by 27.9% of the antioxidant capacity by the BCB test. However, the effect of the antioxidant action of each extract of *C. latifolia* on anti-elastase activity still needs to be further studied to confirm the relationship between the two mechanisms.

In the present study, the mechanism of interaction and correlation of the phenolic compound of *C. latifolia* to its bioactivity on elastase was described in silico. In silico anti-elastase activity of compounds (Fig. 6) from *C. latifolia* extract was carried out by molecular docking to predict the interactions between ligands (compounds in *C. latifolia*) against skin-degrading enzymes' target proteins (1B0F) (Cregge et al., 1998). The molecular structure of the target protein elastase, native ligan (co-crystal), and overlay of co-
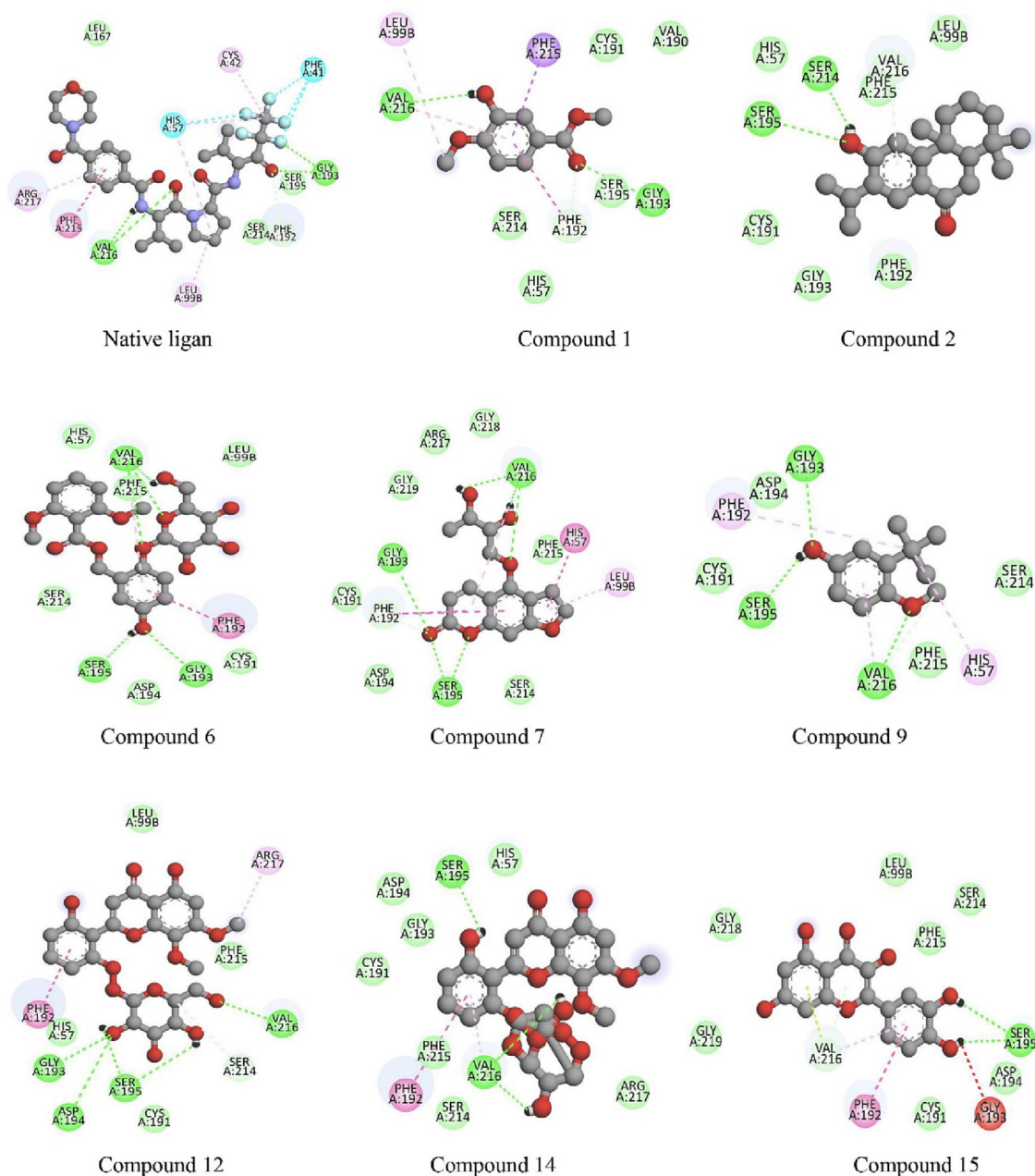
Fig. 8. 2D interactions of co-crystals 1B0F (native ligands) and compounds 1 (methyl-3-hydroxy-4-methoxybenzoat), 2 (sugiol), 6 (curculigoside), 7 (aviprin), 9 (3-*tert*-butyl-4-methoxyphenol), 12 (4-O-caffeoylquinic acid-1), 14 (5,7,3,5-tetrahydroxyflavanone), and 15 (quercetin) with amino acid residues of elastase (1B0F). Numbering of a chemical compound according to the order in Table 5.

crystal re-docking result can be seen in Fig. 7 (7a-c). Re-docking of the native ligand into the target protein pocket was carried out to validate the docking method so that the Root Means Standard Deviation (RMSD) value was obtained. RMSD value, which is<2 Å, indicates that the molecular docking method parameters provide results closer to the experimental results (Fig. 7c). The validation results show that the RMSD value of 1.678 Å indicating that the molecular docking method parameters used meet the requirements. Smaller RMSD indicates a closer alignment of the crystallographic ligand position with the re-bonded ligand position (Kontoyianni et al., 2004; Nursamsiar et al., 2020).

The compounds that showed the best interactions were compounds 2, 7, 14, 15, 9, and 12, with bond-free energy values of −6.60, −6.46, −6.34, −6.06, −5.76, and −5.27 kcal/mol, respectively (Table 6). It was found that the six compounds interact similarly with co-crystals and have free energy values that are close to those for the co-crystals binding to the target protein (Fig. 8). However, compounds 3 and 8 have bond-free energies close to the bond-free energies of co-crystals, but the binding pattern to key amino acids is different from that of co-crystals. The more negative the bond free energy (ΔG) value indicates good stability between the ligand and the target protein (receptor) so that the bond formed are more powerful (Elgamal et al., 2021).

Based on Fig. 8, the of the compound that best contributes to its interaction with the receptor is the hydrogen bonding of the hydroxyl group (O–H). Furthermore, the nucleophilic water molecule interacts with the hydroxyl group attached to the aromatics. Essentially, this prevents the substrate from functioning by interfering with the hydrolysis reaction. Hydrogen bonding is an interaction that can stabilize ligand binding to receptors. Another interaction that occurs in the C. latifolia compound with the amino acid residue of the elastase is the van der Waals interaction which can also increase the conformational stability (Williams et al., 2012). The four best compounds from C. latifolia are phenolic derivatives with a planar-shaped aromatic ring that allows for Van der Waals and π-interactions. Electron-rich aromatic systems and electron-deficient anions can form π-anion interactions. The π-anion interaction plays a vital role in protein stability and ligand binding, and this interaction is energetically beneficial (Elgamal et al., 2021; Nursamsiar et al., 2020).

An overview of the in silico studies indicate that most of the phenolic group compounds identified in the extracts are well accommodated at the binding site of the target enzyme with a relatively large free binding energy averaging −6 kcal/mol. Findings the in silico molecular docking supported the in vitro results that provide anti-elastase effects, especially in extracts of the roots and stems of the C. latifolia plant.

5. Conclusion

Examination of the phytochemicals of the plant organ extract of C. latifolia identified 15 secondary metabolites, and the majority were phenolic compounds. Each extract of C. latifolia showed antioxidant and anti-elastase activities with moderate to powerful ability. Ethanol and ethyl acetate extracts of the root and stem (REE, SEE, REAE, and SEAE) gave the best antioxidant activity based on in the three methods applied. REE, SEE, REAE, and RHE extracts also provided very strong bioactivity against elastase inhibition. The anti-elastase effect of C. latifolia extracts is associated with its action as an antioxidant with a moderate correlation. The in vitro anti-elastase activity was correlated with the in silico activity against the elastase target protein (1B0F). The compounds of sugiol, aviprin, 4-O-caffeoylquinic acid-1, quercetin, and 5,7,3,5-tetrahydroxyflavanone gave the best interaction on the target protein with the most negative binding free energy of < -6 kcal/mol. It

has been suggested that the presence of identified phenolic compounds in C. latifolia extract contributes to its ability to act as a powerful antioxidant and anti-elastase in vitro and in silico studies. Through both in vitro and silico investigations, C. latifolia can be considered as an antiaging candidate.

CRediT authorship contribution statement

Syamsu Nur: Conceptualization, Investigation. **Heri Setiawan:** Validation. **Muhammad Hanafi:** Methodology, Investigation. **Berna Elya:** Conceptualization, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Berna Elya reports financial support, article publishing charges, equipment, drugs, or supplies, and statistical analysis were provided by the Indonesian Ministry of Higher Education and Culture. Muhammad Hanafi reports a relationship with the National Research and Innovation Agency Republic of Indonesia that includes: consulting or advisory.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103716.

References

Abarca-Vargas, R., Peña Malacara, C.F., Petricevich, V.L., 2016. Characterization of chemical compounds with antioxidant and cytotoxic activities in bougainvillea x buttiana holttum and standl, (Var. rose) extracts. Antioxidants. https://doi.org/10.3390/antiox5040045.

Abdul Karim, A., Azlan, A., Ismail, A., Hashim, P., Abd Gani, S.S., Zainudin, B.H., Abdullah, N.A., 2014. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. BMC Complement. Altern. Med. https://doi.org/10.1186/1472-6882-14-381.

Ahmad Wani, N., Tirumale, S., 2018. Evaluation of antioxidant properties of different extracts of Chaetomium cupreum SS02. Bull. Fac. Pharmacy, Cairo Univ. https://doi.org/10.1016/j.bfopcu.2018.08.001.

Aminjafari, A., Miroliaei, M., Angelova, V.T., Emamzadeh, R., Djukic, M.M., Djuric, A., Saso, L., 2016. Antioxidant activity and protective role on protein glycation of synthetic aminocoumarins. Electron. J. Biotechnol. https://doi.org/10.1016/j.ejbt.2016.08.004.

Amiri, H., 2014. Chemical composition and antioxidant activity of essential oil and methanolic extracts of ferula microcolea (Boiss.) Boiss (Apiaceae). Int. J. Food Prop. https://doi.org/10.1080/10942912.2012.665403.

Anggraini, N.B., Elya, B., Iskandarsyah, 2020. Anti-elastase, antioxidant, total phenolic and total flavonoid content of macassar kernels (Rhus javanica L) from pananjung pangandaran nature tourism park- Indonesia. J. Nat. Remedies. https://doi.org/10.18311/jnr/2020/24240

Azadfar, M., Gao, A.H., Chen, S., 2015. Structural characterization of lignin: A potential source of antioxidants guaiacol and 4-vinylguaiacol. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2014.12.049.

Bajpai, V.K., Sharma, A., Kang, S.C., Baek, K.H., 2014. Antioxidant, lipid peroxidation inhibition and free radical scavenging efficacy of a diterpenoid compound sugiol isolated from Metasequoia glyptostroboides. Asian Pac. J. Trop. Med. https://doi.org/10.1016/S1995-7645(13)60183-2.

Bogacz-Radomska, L., Harasym, J., 2018. β-Carotene-properties and production methods. Food Qual. Saf. https://doi.org/10.1093/fqsafe/fyy004.

Caillet, S., Yu, H., Lessard, S., Lamoureux, G., Ajdukovic, D., Lacroix, M., 2007. Fenton reaction applied for screening natural antioxidants. Food Chem. https://doi.org/10.1016/j.foodchem.2005.10.009.

Chao, K.P., Hua, K.F., Hsu, H.Y., Su, Y.C., Chang, S.T., 2005. Anti-inflammatory activity of sugiol, a diterpene isolated from Calocedrus formosana bark. Planta Med. https://doi.org/10.1055/s-2005-864094.

Chen, Y., Yu, H., Wu, H., Pan, Y., Wang, K., Jin, Y., Zhang, C., 2015. Characterization and quantification by LC-MS/MS of the chemical components of the heating products of the flavonoids extract in Pollen typhae for transformation rule exploration. Molecules. https://doi.org/10.3390/molecules201018352.

Chormey, D.S., Bakirdere, S., 2018. Principles and Recent Advancements in Microextraction Techniques, in: Comprehensive Analytical Chemistry. https://doi.org/10.1016/bs.coac.2018.03.011.

Cregge, R.J., Durham, S.L., Farr, R.A., Gallion, S.L., Hare, C.M., Hoffman, R.V., Janusz, M.J., Kim, H.O., Koehl, J.R., Mehdi, S., Metz, W.A., Peet, N.P., Pelton, J.T., Schreuder, H.A., Sunder, S., Tardif, C., 1998. Inhibition of human neutrophil elastase. 4. Design, synthesis, X-ray crystallographic analysis, and structure-activity relationships for a series of P2-modified, orally active peptidyl pentafluoroethyl ketones. J. Med. Chem. https://doi.org/10.1021/jm970812e.

Desmiaty, Y., Hanafi, M., Saputri, F.C., Elya, B., Rifai, E.A., Syahdi, R.R., 2021. Two triterpenoids from Rubus fraxinifolius leaves and their tyrosinase and elastase inhibitory activities. Sci. Rep. https://doi.org/10.1038/s41598-021-99970-x.

Dorfner, R., Ferge, T., Kettrup, A., Zimmermann, R., Yeretzian, C., 2003. Real-time monitoring of 4-vinylguaiacol, guaiacol, and phenol during coffee roasting by resonant laser ionization time-of-flight mass spectrometry. J. Agric. Food Chem. https://doi.org/10.1021/jf0341767.

Dravie, E.E., Kortei, N.K., Essuman, E.K., Tettey, C.O., Boakye, A.A., Hunkpe, G., 2020. Antioxidant, phytochemical and physicochemical properties of sesame seed (Sesamum indicum L.). Sci. African. https://doi.org/10.1016/j.sciaf.2020.e00349.

Elgamal, A.M., El Raey, M.A., Gaara, A., Abdelfattah, M.A.O., Sobeh, M., 2021. Phytochemical profiling and anti-aging activities of Euphorbia retusa extract: In silico and in vitro studies. Arab. J. Chem. https://doi.org/10.1016/j.arabjc.2021.103159.

Farzinebrahimi, R., Mat Taha, R., Rashid, K.A., Ali Ahmed, B., Danaee, M., Rozali, S.E., 2016. Preliminary Screening of Antioxidant and Antibacterial Activities and Establishment of an Efficient Callus Induction in Curculigo latifolia Dryand (Lemba). Evidence-based Complement. Altern. Med. https://doi.org/10.1155/2016/6429652.

Ganzon, J.G., Chen, L.G., Wang, C.C., 2018. 4-O-Caffeoylquinic acid as an antioxidant marker for mulberry leaves rich in phenolic compounds. J. Food Drug Anal. https://doi.org/10.1016/j.jfda.2017.11.011.

Gao, J.-J., Min, B.-S., Ahn, E.-M., Nakamura, N., Lee, H.-K., Hattori, M., 2010. ChemInform abstract: New Triterpene Aldehydes, Lucialdehydes A-C, from Ganoderma lucidum and their cytotoxicity against murine and human tumor cells. ChemInform. https://doi.org/10.1002/chin.200248182.

Hanafi, H., Irawan, C., Rochaeni, H., Sulistiawaty, L., Roziafanto, A.N., Supriyono, 2018. Phytochemical screening, LC-MS studies and antidiabetic potential of methanol extracts of seed shells of Archidendron bubalinum (Jack) I.C. Nielson (Julang Jaling) from Lampung, Indonesia. Pharmacogn. J. https://doi.org/10.5530/pj.2018.6s.15.

Hikmawanti, N.P.E., Fatmawati, S., Asri, A.W., 2021. The effect of ethanol concentrations as the extraction solvent on antioxidant activity of Katuk (Sauropus androgynus (L.) Merr.) leaves extracts, in: IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/755/1/012060.

Ishihara, K., Fukutake, M., Asano, T., Mizuhara, Y., Wakui, Y., Yanagisawa, T., Kamei, H., Ohmori, S., Kitada, M., 2001. Simultaneous determination of byak-angelicin and oxypeucedanin hydrate in rat plasma by column-switching high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl. https://doi.org/10.1016/S0378-4347(00)00569-7.

Jadoon, S., Karim, S., Asad, M.H.H. Bin, Akram, M.R., Kalsoom Khan, A., Malik, A., Chen, C., Murtaza, G., 2015. Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longitevity. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2015/709628.

Koleva, I.I., Van Beek, T.A., Linssen, J.P.H., De Groot, A., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem. Anal. https://doi.org/10.1002/pca.611.

Kontoyianni, M., McClellan, L.M., Sokol, G.S., 2004. Evaluation of docking performance: Comparative data on docking algorithms. J. Med. Chem. https://doi.org/10.1021/jm0302997.

Kulisic, T., Radonic, A., Katalinic, V., Milos, M., 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. https://doi.org/10.1016/j.foodchem.2003.07.024.

Lago, J.C., Puzzi, M.B., 2019. The effect of aging in primary human dermal fibroblasts. PLoS One. https://doi.org/10.1371/journal.pone.0219165.

Lapornik, B., Prošek, M., Wondra, A.G., 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. J. Food Eng. https://doi.org/10.1016/j.jfoodeng.2004.10.036.

Lee, S.Y., Kim, M.R., Choi, H.S., Moon, H.I., Chung, J.H., Lee, D.G., Woo, E.R., 2009. The effect of curculigoside on the expression of matrix metalloproteinase-1 in cultured human skin fibroblasts. Arch. Pharm. Res. https://doi.org/10.1007/s12272-009-2013-4.

Lestari, O.A., Palupi, N.S., Setiyono, A., Kusnandar, F., Yuliana, N.D., 2022. In vitro antioxidant potential and phytochemical profiling oMelastoma malabathricum leaf water extract. Food Sci. Technol. https://doi.org/10.1590/fst.92021.

Li, Y., Tang, W., Chen, J., Jia, R., Ma, L., Wang, S., Wang, J., Shen, X., Chu, Z., Zhu, C., Ding, X., 2016. Development of marker-free transgenic potato tubers enriched in Caffeoylquinic acids and flavonols. J. Agric. Food Chem. https://doi.org/10.1021/acs.jafc.6b00270.

Lim, Y.Y., Lim, T.T., Tee, J.J., 2007. Antioxidant properties of several tropical fruits: A comparative study. Food Chem. https://doi.org/10.1016/j.foodchem.2006.08.038.

Lim, J.C., Park, J.H., Budesinsky, M., Kasal, A., Han, Y.H., Koo, B.S., Lee, S.I., Lee, D.U., 2005. Antimutagenic constituents from the thorns of gleditsia sinensis. Chem. Pharm. Bull. https://doi.org/10.1248/cpb.53.561.

Lukitaningsih, E., Nur, S., Qonithah, F., Zulbayu, A., Kuswahyuning, R., Rumiyati, R., 2020. In vitro anti-wrinkle and tyrosinase inhibitory activities of grapefruit peel and strawberry extracts. Maj. Obat Tradis. 25, 182–189. https://doi.org/10.22146/mot.59194.

Marco, G.J., 1968. A rapid method for evaluation of antioxidants. J. Am. Oil Chem. Soc. https://doi.org/10.1007/BF02668958.

Morales, G., Paredes, A., 2014. Antioxidant activities of Lampaya medicinalis extracts and their main chemical constituents. BMC Complement. Altern. Med. https://doi.org/10.1186/1472-6882-14-259.

Nickavar, B., Esbati, N., 2012. Evaluation of the Antioxidant Capacity and Phenolic Content of Three Thymus Species. JAMS J. Acupunct. Meridian Stud. https://doi.org/10.1016/j.jams.2012.03.003.

Nur, S., Mubarak, F., Jannah, C., Winarni, D.A., Rahman, D.A., Hamdayani, L.A., Sami, F.J., 2019. Total phenolic and flavonoid compounds, antioxidant and toxicity profile of extract and fractions of paku atai tuber (Angiopteris ferox Copel). Food Res. https://doi.org/10.26656/fr.2017.3(6).135.

Nur, S., Aisyah, A.N., Lukitaningsih, E., Rumiyati, Juhardi, R.I., Andirah, R., Hajar, A.S., 2021a. Evaluation of antioxidant and cytotoxic effect against cancer cells line of Angiopteris ferox Copel tuber and its compounds by LC-MS analysis. J. Appl. Pharm. Sci. https://doi.org/10.7324/JAPS.2021.110808.

Nur, S., Angelina, A.A., Aswad, M., Yulianty, R., Burhan, A., Nursamsiar, 2021b. In vitro anti-aging activity of Muntingia calabura L. fruit extract and its fractions. J. Pharm. Pharmacogn. Res.

Nursamsiar, Siregar, M., Awaluddin, A., Nurnahari, N., Nur, S., Febrina, E., Asnawi, A., 2020. Molecular docking and molecular dynamic simulation of the aglycone of curculigoside a and its derivatives as alpha glucosidase inhibitors. Rasayan J. Chem. https://doi.org/10.31788/RJC.2020.1315577

Nurul Islam, M., Downey, F., Ng, C., 2013. Comprehensive profiling of flavonoids in Scutellaria incana L. using LC-Q-TOF-MS. Acta Chromatogr. https://doi.org/10.1556/AChrom.25.2013.3.11.

Ooi, D.J., Chan, K.W., Sarega, N., Alitheen, N.B., Ithnin, H., Ismail, M., 2016. Bioprospecting the curculigoside-cinnamic acid-rich fraction from Molineria latifolia rhizome as a potential antioxidant therapeutic agent. Molecules. https://doi.org/10.3390/molecules21060682.

Ooi, D.J., Azmi, N.H., Imam, M.U., Alitheen, N.B., Ismail, M., 2018. Curculigoside and polyphenol-rich ethyl acetate fraction of Molineria latifolia rhizome improved glucose uptake via potential mTOR/AKT activated GLUT4 translocation. J. Food Drug Anal. https://doi.org/10.1016/j.jfda.2018.03.003.

Pientaweeratch, S., Panapisal, V., Tansirikongkol, A., 2016. Antioxidant, anti-collagenase and anti-elastase activities of Phyllanthus emblica, Manilkara zapota and silymarin: an in vitro comparative study for anti-aging applications. Pharm. Biol. https://doi.org/10.3109/13880209.2015.1133658.

Priyanto, J.A., Prastya, M.E., Primahana, G., Randy, A., Utami, D.T., 2023. Paederia foetida Linn Leaves-Derived Extract Showed Antioxidant and Cytotoxic Properties Against Breast Carcinoma Cell. HAYATI J. Biosci. https://doi.org/10.4308/hjb.30.2.271-280.

Purohit, T., He, T., Qin, Z., Li, T., Fisher, G.J., Yan, Y., Voorhees, J.J., Quan, T., 2016. Smad3-dependent regulation of type I collagen in human dermal fibroblasts: Impact on human skin connective tissue aging. J. Dermatol. Sci. https://doi.org/10.1016/j.jdermsci.2016.04.004.

Putri, I.R., Handayani, R., Elya, B., 2019. Anti-elastase activity of Rumput Teki (Cyperus rotundus L.). rhizome extract. Pharmacogn. J. https://doi.org/10.5530/pj.2019.11.119.

Rabeta, M.S., Nur Faraniza, R., 2013. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of garcinia atrovirdis and cynometra cauliflora. Int. Food Res. J.

Saleem, H., Zengin, G., Sarfraz, M., Alafnan, A., Locatelli, M., Tartaglia, A., Ahmad, I., Mahomoodally, M.F., Khurshid, U., Ahemad, N., 2021. Phytochemical composition and in -vitro pharmacological evaluation of Emex australis Steinh: A natural source of enzyme inhibitors. South African J. Bot. https://doi.org/10.1016/j.sajb.2021.02.023.

Seghiri, R., Mekkiou, R., Boumaza, O., Benayache, S., Bermijo, J., Benayache, F., 2006. Phenolic compounds from Centaurea africana. Chem. Nat. Compd. https://doi.org/10.1007/s10600-006-0228-x.

Simamora, A., Santoso, A.W., Timotius, K.H., Rahayu, I., 2020. Antioxidant Activity, Enzyme Inhibition Potentials, and Phytochemical Profiling of Premna

serratifolia L. Leaf Extracts. Int. J. Food Sci. https://doi.org/10.1155/2020/3436940.

Simoneit, B.R.T., Otto, A., Oros, D.R., Kusumoto, N., 2019. Terpenoids of the swamp cypress subfamily (Taxodioideae), Cupressaceae, an overview by GC-MS. Molecules 24, 1–18. https://doi.org/10.3390/molecules24173036.

Spiegel, M., Kapusta, K., Kołodziejczyk, W., Saloni, J., Zbikowska, B., Hill, G.A., Sroka, Z., 2020. Antioxidant activity of selected phenolic acids-ferric reducing antioxidant power assay and QSAR analysis of the structural features. Molecules. https://doi.org/10.3390/molecules25133088.

Sun, J.M., Yang, J.S., Zhang, H., 2007. Two new flavanone glycosides of Jasminum lanceolarium and their anti-oxidant activities. Chem. Pharm. Bull. https://doi.org/10.1248/cpb.55.474.

Szewczyk, K., Miazga-Karska, M., Pietrzak, W., Komsta, Ł., Krzemińska, B., Grzywa-Celińska, A., 2020. Phenolic composition and skin-related properties of the aerial parts extract of different hemerocallis cultivars. Antioxidants. https://doi.org/10.3390/antiox9080690.

Umar, A.H., Ratnadewi, D., Rafi, M., Sulistyaningsih, Y.C., 2021. Untargeted metabolomics analysis using ftir and uhplc-q-orbitrap hrms of two curculigo species and evaluation of their antioxidant and α-glucosidase inhibitory activities. Metabolites. https://doi.org/10.3390/metabo11010042.

Varani, J., Warner, R.L., Gharaee-Kermani, M., Phan, S.H., Kang, S., Chung, J.H., Wang, Z.Q., Datta, S.C., Fisher, G.J., Voorhees, J.J., 2000. Vitamin A antagonizes decreased cell growth and elevated collagen- degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J. Invest. Dermatol. https://doi.org/10.1046/j.1523-1747.2000.00902.x.

Wang, Y., Wang, Y., Zhao, L., Xu, J., Nie, Y., Guo, Y., Tong, Y., Qin, L., Zhang, Q., 2012. Curculigoside isolated from Curculigo orchioides prevents hydrogen peroxide-induced dysfunction and oxidative damage in calvarial osteoblasts. Acta Biochim. Biophys. Sin. (Shanghai). https://doi.org/10.1093/abbs/gms014.

Wang, Y., Zhang, H., Su, X., Yang, H., Sun, W., 2016. Flavonoids and Phenolic Compounds of Petrosimonia sibirica. Chem. Nat. Compd. https://doi.org/10.1007/s10600-016-1679-3.

Wang, Y., Li, J., Li, N., 2021. Phytochemistry and pharmacological activity of plants of genus Curculigo: An updated review since 2013. Molecules. https://doi.org/10.3390/molecules26113396.

Wang, Z.L., Wang, S., Kuang, Y., Hu, Z.M., Qiao, X., Ye, M., 2018. A comprehensive review on phytochemistry, pharmacology, and flavonoid biosynthesis of scutellaria baicalensis. Pharm. Biol. https://doi.org/10.1080/13880209.2018.1492620.

Wiley, J., Sons, Inc. SpectraBase; https://spectrabase.com/ad?sid=5tD8wBuqn2&r=https://spectrabase.com/spectrum/5tD8wBuqn2 (accessed 6/11/2023).

Williams, L.K., Li, C., Withers, S.G., Brayer, G.D., 2012. Order and disorder: Differential structural impacts of myricetin and ethyl caffeate on human amylase, an antidiabetic target. J. Med. Chem. https://doi.org/10.1021/jm301273u.

Woo, M.H., Do, J.C., Son, K.H., 1992. Five new spirostanol glycosides from the subterranean parts of smilax sieboldii. J. Nat. Prod. https://doi.org/10.1021/np50086a015.

Xia, W., Quan, T., Hammerberg, C., Voorhees, J.J., Fisher, G.J., 2015. A mouse model of skin aging: Fragmentation of dermal collagen fibrils and reduced fibroblast spreading due to expression of human matrix metalloproteinase-1. J. Dermatol. Sci. https://doi.org/10.1016/j.jdermsci.2015.01.009.

Yang, Y., Sun, X., Liu, J., Kang, L., Chen, S., Ma, B., Guo, B., 2016. Quantitative and qualitative analysis of flavonoids and phenolic acids in snow chrysanthemum (Coreopsis tinctoria Nutt.) by HPLC-DAD and UPLC-ESI-QTOF-MS. Molecules. https://doi.org/10.3390/molecules21101307.

Zabidi, N.A., Ishak, N.A., Hamid, M., Efliza Ashari, S., 2019. Subcritical water extraction of antioxidants from Curculigo latifolia root. J. Chem. https://doi.org/10.1155/2019/8047191.

Zahri, S., Razavi, S.M., Moatamed, Z., 2012. Antioxidant activity and cytotoxic effect of aviprin and aviprin-3″-O-D-glucopyranoside on LNCaP and HeLa cell lines. Nat. Prod. Res. https://doi.org/10.1080/14786419.2010.529442.

Zengin, G., Zheleva-Dimitrova, D., Gevrenova, R., Nedialkov, P., Mocan, A., Ćirić, A., Glamočlija, J., Soković, M., Aktumsek, A., Mahomoodally, M.F., 2018. Identification of phenolic components via LC–MS analysis and biological activities of two Centaurea species: C. drabifolia subsp. drabifolia and C. lycopifolia. J. Pharm. Biomed. Anal. https://doi.org/10.1016/j.jpba.2017.11.045.

Zhang, S., Duan, E., 2018. Fighting against skin aging: The way from bench to bedside. Cell Transplant. https://doi.org/10.1177/0963689717725755.

Zhang, Quanlong, Zhao, L., Shen, Y., He, Y., Cheng, G., Yin, M., Zhang, Qiaoyan, Qin, L., 2019. Curculigoside Protects against Excess-Iron-Induced Bone Loss by Attenuating Akt-FoxO1-Dependent Oxidative Damage to Mice and Osteoblastic MC3T3-E1 Cells. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2019/9281481.

Zhao, G., Yuan, F., Zhu, J., 2014. An LC-MS/MS method for determination of curculigoside with anti-osteoporotic activity in rat plasma and application to a pharmacokinetic study. Biomed. Chromatogr. https://doi.org/10.1002/bmc.3025.

Zhuang, B., Ramanauskaite, G., Koa, Z.Y., Wang, Z.G., 2021. Like dissolves like: A first-principles theory for predicting liquid miscibility and mixture dielectric constant. Sci. Adv. https://doi.org/10.1126/sciadv.abe7275.