narra j

Original Article

Phytochemical profiling and enzyme inhibitory activity of *Sterculia populifolia* DC stem bark extract and fractions against elastase and tyrosinase

Nur Khairi[1*], Nursamsiar Nursamsiar[2], Novi F. Utami[3], Marwati Marwati[4], Syamsu Nur[1], Maulita Indrisari[5] and Sukriani Kursia[1,6]

[1]Department of Pharmaceutical and Technology, Universitas Almarisah Madani, Makassar, Indonesia; [2]Department of Pharmaceutical Chemistry, Universitas Almarisah Madani, Makassar, Indonesia; [3]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Pakuan, Bogor, Indonesia; [4]Department of Pharmaceutical Biology, Universitas Almarisah Madani, Makassar, Indonesia; [5]Department of Pharmacotherapy, Faculty of Medicine, Universitas Palangka Raya, Palangkaraya, Indonesia; [6]Department of Medical Technology, Faculty of Associated Medical Science, Khon Kaen University, Khon Kaen, Thailand

*Corresponding author: nurkhairijalil@gmail.com

Abstract

The demand for natural ingredients in cosmetic and medical applications is steadily increasing, particularly for anti-aging and skin-lightening products. *Sterculia populifolia* DC, a member of the *Sterculia* genus, is known to contain diverse bioactive compounds such as flavonoids, phenolics, and terpenoids, which may offer pharmacological benefits. The aim of this study was to evaluate the anti-aging potential of *S. populifolia* stem bark extract and its solvent-partitioned fractions through enzyme inhibition assays coupled with phytochemical profiling. The stem bark was extracted using 96% ethanol via maceration, followed by sequential liquid-liquid partitioning with *n*-hexane, ethyl acetate, *n*-butanol, and water. Phytochemical constituents were characterized using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The inhibitory activities of the extract and fractions against tyrosinase and elastase enzymes were evaluated using spectrophotometric assays, with kojic acid and quercetin as positive controls, respectively. IC_{50} values were calculated to quantify enzyme inhibition potency. LC-MS/MS analysis revealed key bioactive compounds, including 4-[(E)-(3,5-diamino-1H-pyrazol-4-yl)diazenyl]phenol, isofraxidin, and (22E)-ergosta-4,6,8(14),22-tetraen-3-one. Among the tested samples, the ethanol extract exhibited the most potent activity, with an IC_{50} of 93.35 µg/mL for elastase inhibition and 133.15 µg/mL for tyrosinase inhibition—classified as strong and moderate activity, respectively. Collectively, these findings demonstrate that *S. populifolia* stem bark extract possesses promising anti-aging and depigmenting properties, supporting its potential development as a natural bioactive ingredient in cosmetic and skincare formulations.

Keywords: *Sterculia populifolia* DC, antiaging, anti-pigmentation, tyrosinase inhibitor, elastase inhibitor

Introduction

Phytochemical components present in various *Sterculia* species have been the focus of extensive research due to their diverse biological activities and potential applications in traditional medicine and modern pharmacology. The genus *Sterculia*, which is part of the Malvaceae family, is known to have a rich phytochemical profile, including flavonoids, tannins, alkaloids,

https://narraj.org/

Received: November 15, 2024 - Accepted: August 18, 2025 - Published online: September 17, 2025

terpenoids, and phenolic compounds [1]. These compounds, generated through primary and secondary metabolic pathways, have been identified to play a role in various pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and antimicrobial effects [2-4].

One of the species in the *Sterculia* genus, *S. populifolia* DC, demonstrates great potential due to its rich bioactive content. However, this species remains largely underexplored, particularly in cosmetic and dermatological applications. With the increasing interest in nature-based cosmetic products, research on natural compounds that can inhibit the activity of enzymes associated with skin health, such as elastase and tyrosinase, is becoming highly relevant [5]. Targeting these enzymes is critical not only for preventing premature skin aging but also for addressing hyperpigmentation issues.

Research on the inhibition of elastase and tyrosinase enzymes not only has wide applications in the cosmetic industry but also in the medical field. Targeting these enzymes can help mitigate skin aging and pigmentation disorders, which are often difficult to treat with conventional therapies [6,7]. Moreover, growing awareness of the potential risks associated with synthetic chemicals in cosmetics has increased interest in plant extracts as safe and natural alternatives. [8,9]. Although several species within the genus *Sterculia* have been investigated for their bioactive contents and biological activities, the stem bark of *S. populifolia* DC has not been examined in the context of elastase and tyrosinase inhibition. This represents a clear research gap, highlighting the potential of exploring this species for cosmetic and dermatological applications. Unlike previous studies on plant-based enzyme inhibitors that primarily focused on well-characterized species or well-established compounds, this study investigates *S. populifolia* DC, a relatively understudied species, to provide novel insights into its phytochemical profile and its dual potential as an elastase and tyrosinase inhibitor. By addressing this gap, the research contributes to the growing body of knowledge on natural enzyme inhibitors. It identifies a promising candidate for the development of safe and effective cosmetic formulations. Thus, the aim of this study was to characterize the phytochemical profile of *S. populifolia* DC extracts and fractions and evaluate their effectiveness in inhibiting elastase and tyrosinase activities.

Methods

Sample preparation and extraction process

Stem bark of *S. populifolia* (5 kg) was collected from Kupang, East Nusa Tenggara, Indonesia. The material was thoroughly cleaned with running water to remove debris and then rinsed with distilled water. Cleaned samples were air-dried and subsequently placed in a drying cabinet at 50°C until a constant weight was achieved. The dried bark was then ground into a fine powder using a mechanical grinder to obtain simplicia.

Extraction was carried out by maceration using 96% ethanol with a solvent-to-sample ratio of 7:1 (v/w). A total of 600 g of the powdered simplicia was soaked in 4,200 mL of ethanol for 72 hours at room temperature, with intermittent stirring every 6–8 hours to enhance solvent penetration. After the maceration period, the mixture was filtered using filter paper to separate the ethanol extract (macerate) from the solid residue (marc). The marc was remacerated under the same conditions until the filtrate became nearly colorless, indicating exhaustion of extractable compounds.

All filtrates were pooled and concentrated under reduced pressure using a rotary evaporator at 50°C to obtain a viscous crude extract. The concentrate was then transferred to a drying oven and maintained at 50°C until a semi-solid extract was obtained. The final yield of the extract was calculated as a percentage of the initial dried weight of the dried powder. The dried extract was stored in an airtight container at 4°C, protected from light, until further use. These extraction steps followed the recommendations reported previously [10].

Fractionation

Liquid–liquid partitioning was employed to fractionate the ethanol extract of *S. populifolia* stem bark using a separating funnel. The extract was reconstituted in distilled water, and the solvent-to-extract ratio was maintained at 10:1 (v/w). Sequential partitioning was carried out using solvents of increasing polarity: n-hexane (non-polar), ethyl acetate (semi-polar), and n-butanol

(polar), leaving behind the aqueous (residual) fraction. Each solvent was added to the separating funnel and mixed thoroughly with the aqueous phase to allow efficient partitioning of the bioactive constituents. After phase separation, the organic (upper) layer was collected for each solvent, and the process was repeated multiple times until the respective solvent layers became colorless, indicating exhaustive extraction. The collected fractions—n-hexane, ethyl acetate, n-butanol, and aqueous—were concentrated under reduced pressure using a rotary evaporator at 50°C to yield dried residues. These dried fractions were then stored in airtight containers at 4°C until further biological evaluation, following previously described protocols [11].

Phytochemical screening

Preliminary phytochemical screening was conducted to identify the presence of major secondary metabolites in the *S. populifolia* stem bark extract. Standard qualitative tests were performed as follows for each phytochemical group. Flavonoids were detected using the cyanidin reaction, wherein a small amount of extract was mixed with magnesium powder and a few drops of concentrated hydrochloric acid. The development of a reddish or pink color indicated the presence of flavonoids. Phenolic compounds were assessed using ferric chloride ($FeCl_3$) reagent. A few drops of 1% $FeCl_3$ solution were added to the extract; the appearance of a dark green or blue-black color signified the presence of phenolics. Saponins were identified via the foam test, where the extract was vigorously shaken with distilled water until the formation of a stable and persistent froth indicated saponin content. Steroids, alkaloids, and triterpenoids were evaluated using the Liebermann–Burchard test, where the extract was treated with a mixture of anhydrous acetic acid and concentrated sulfuric acid. A color change (e.g., green, blue, or violet) indicated the presence of these compounds. All tests were performed following established protocols as previously described in the literature [12].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

Bioactive compounds in the *S. populifolia* stem bark extract were identified using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS), as described in a previous study [13]. The analysis was performed using an ACQUITY UPLC® H-Class System (Waters, USA) connected to a Xevo G2-S QToF mass spectrometer (Waters, USA). Chromatographic separation employed a binary mobile phase: (A) water containing 5 mM ammonium formate, and (B) acetonitrile with 0.05% formic acid. A step-gradient elution was carried out over 23 minutes at a flow rate of 0.2 mL/min. The injection volume was 5 µL, and all samples were pre-filtered through a 0.2 µm syringe filter prior to analysis. Mass spectrometric detection was performed in positive ion mode using electrospray ionization (ESI). Data acquisition and processing were conducted using MassLynx software, version 4.1 (Waters, USA), following the protocol outlined in the reference [13].

Tyrosinase enzyme inhibition assay

The anti-aging potential of the extract and its fractions was evaluated through a tyrosinase inhibition assay using a spectrophotometric method, following the protocol described in previous research [14]. The assay utilized *L*-3,4-dihydroxyphenylalanine (L-DOPA) as the enzymatic substrate and kojic acid as the positive control. Samples were prepared at various concentrations ranging from 12.5 to 400 µg/mL. For each reaction, 30 µL of the sample solution was mixed with 125 µL of phosphate buffer (0.1 M, pH 6.8) and 5 µL of mushroom tyrosinase enzyme (2500 units/mL) in a 96-well microplate. The mixture was incubated at 37°C for 30 minutes. After incubation, 40 µL of L-DOPA (2.5 mM) was added to initiate the reaction. Absorbance was measured at 515 nm using a microplate reader (Glomax GM 3000, Promega, USA). Blanks (solvent only) and kojic acid-treated wells served as negative and positive controls, respectively. The percentage of tyrosinase inhibition was calculated based on the absorbance values, and the inhibitory activity across concentrations was modeled using the linear regression equation ($y=a+bx$), where y represents the percentage inhibition, x is the sample concentration, and a and b are the intercept and slope of the regression line, respectively.

Elastase inhibition assay

The anti-aging potential of the *S. populifolia* extract and its fractions was evaluated based on their elastase inhibitory activity using an enzymatic colorimetric method, as previously described [15,16]. The assay utilized *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (SANA) as the substrate, which produces a yellow chromophore upon enzymatic cleavage by elastase. The absorbance of the resulting product was measured at 405 nm using a microplate reader. In a 96-well microplate, 30 µL of each test sample (extract or fraction) prepared at concentrations ranging from 10 to 1000 µg/mL was mixed with 15 µL of elastase solution (4 units/mL) in Tris-HCl buffer (pH 8.0). The mixture was incubated at 25°C for ten minutes. Following this, 30 µL of the SANA substrate solution (1.3 mM) was added, and the plate was further incubated at 25°C for 50 minutes in a Memmert IN55 incubator (Memmert, Schwabach, Germany). After incubation, the absorbance was read at 405 nm. Quercetin was used as the positive control. All experiments were performed in triplicate (n=3). The elastase inhibitory activity of each sample was quantified by determining the IC_{50} value, defined as the concentration required to inhibit 50% of enzyme activity. According to previous literature [17] IC_{50} values below 100 µg/mL indicate strong inhibitory activity, 100–450 µg/mL indicate weak activity, and 450–700 µg/mL indicate poor activity [17].

Statistical analysis

The inhibitory activities against tyrosinase and elastase were expressed as mean ± standard deviation (SD). Statistical comparisons among groups were conducted using one-way analysis of variance (ANOVA). When significant differences were observed, post-hoc analysis was performed using the least significant difference (LSD) test to compare the extract and fraction groups against the respective positive control groups. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Positive control groups were included in each assay: kojic acid for tyrosinase inhibition and quercetin for elastase inhibition. Each experimental condition was tested in three independent biological replicates, with technical triplicates within each replicate to ensure accuracy and reliability. Sample size determination was based on power analysis, targeting a statistical power of at least 80% and an alpha level of 0.05. The analysis indicated that a minimum of five samples per group was required to detect significant differences. Reproducibility was further confirmed by performing assays on separate days under consistent experimental conditions.

Results

Extract characteristics and yield

The extract obtained was brown and obtained in a dry form, while all fractions obtained were brown with concentrated consistency. The characteristics and yield of the extract and its fractions are presented in **Table 1**. Among the fractions, the n-hexane fraction exhibited the highest yield (16.09%), followed by the aqueous (13.22%) and ethyl acetate fractions (10.93%).

Table 1. Yields of *S. populifolia* stem bark extract and its fractions

Solvent	Simplia weight (gr)	Dry Extract weight (gr)	Weight of extract for fractionation (gr)	Fraction weight (gr)	Yield (%)
96% ethanol	600	9.33	-	-	1.55
n-hexane	-	-	5	0.8047	16.09
Ethyl acetate	-	-	5	0.5465	10.93
n-butanol	-	-	5	0.2267	4.53
Aqueous	-	-	5	0.6611	13.22

Phytochemical screening

Phytochemical screening of *S. populifolia* stem bark revealed that the ethanol extract and all fractions tested positive for alkaloids, flavonoids, tannins, steroids, and terpenoids. In contrast, the ethyl acetate, n-butanol, and aqueous fractions were positive only for alkaloids, flavonoids, and tannins. The results of phytochemical screening are presented in **Table 2**.

Table 2. Phytochemical screening results of *S. populifolia* stem bark extract and its fractions

Metabolite content	Reagent	Ethanol extract	n-Hexane fraction	Ethyl acetate fraction	n-Butanol fraction	Aqueous fraction
Alkaloids	Mayer	+	+	+	+	-
	Wagner	+	+	+	+	+
	Dragendorff	+	+	+	+	+
Flavonoids	Concentrated Mg+HCl powder	+	+	+	+	+
Saponins	Hot water	-	-	-	-	-
Tannis	$FeCl_3$	+	+	+	+	+
Steroid and terpenoid	Lieberman-Burchard	+	+	-	-	-

(+): present; (-): absent

Metabolite identification LC-MS/MS

Phytochemical profiling of the *S. populifolia* stem bark ethanol extract and its fractions was performed using LC-MS/MS. Metabolite identification was primarily based on liquid chromatography (LC) retention times and the interpretation of high-resolution mass spectra. Base peak chromatograms (BPC) of the extract and its respective fractions, detected at 203 nm using LC-ESI$^+$-MS, are presented in **Figure 1**. Peak identification in the LC-MS chromatograms was carried out using publicly available mass spectral databases—such as the MassBank of Europe and the National Library of Medicine—as well as relevant literature sources [18,19]. The identification comparisons are presented in **Table 3**.

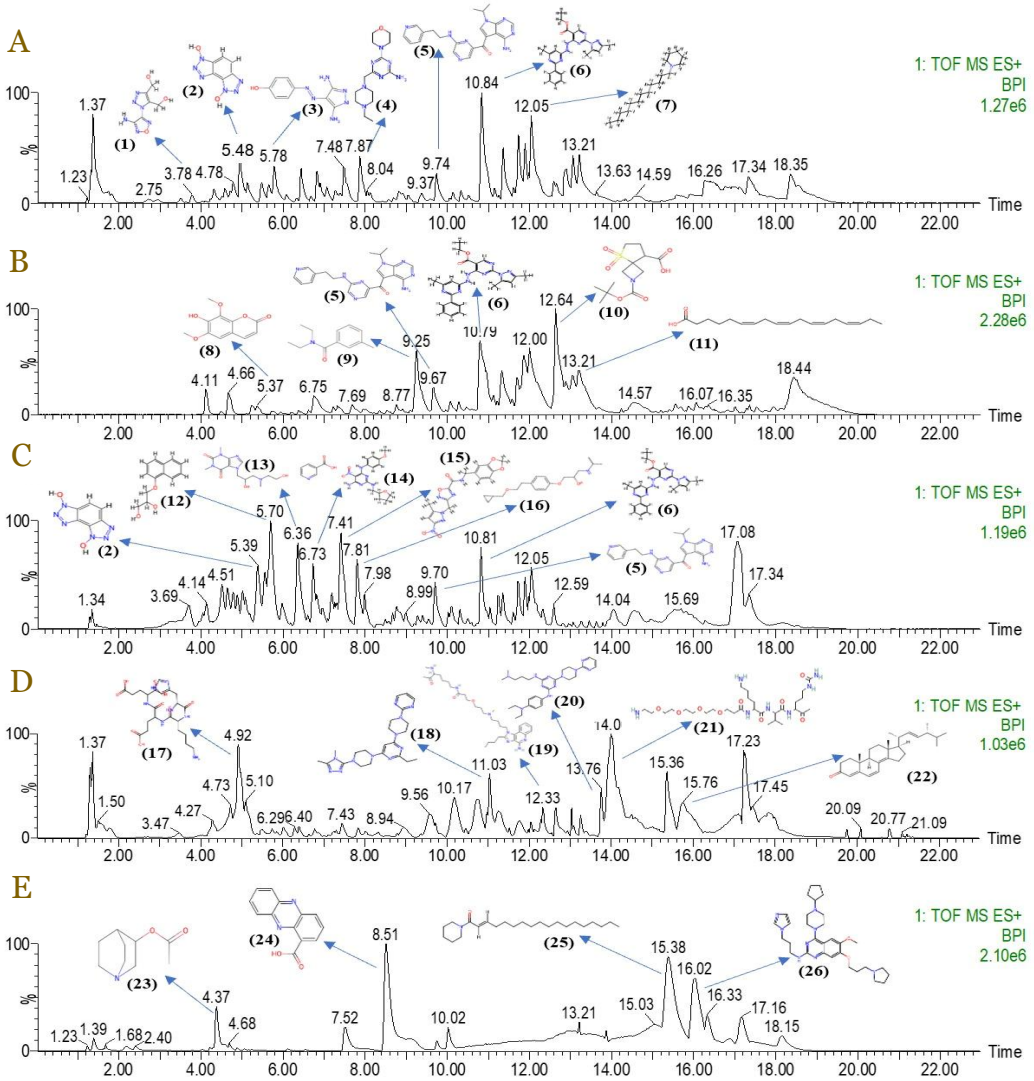

Figure 1. LC/MS.MS profile of *S. populifolia* stem bark extract and fractions: (A) ethanolic extract (EE); (B) n-hexane fractions (HF); (C) ethyl acetate fraction (EAF); (D) n-butanol fraction (BuF); and (E) aqueous fraction (AF).

Table 3. Identified phytocompounds from LC/MS.MS analysis

No	Retention time (min)	Observed MS (m/z)	Molecular ion	Compound name	Molecule formula	Extract/fractions
1	3.78	213.07	M+H	[1-(4-Amino-1,2,5-oxadiazol-3-yl)-1H-1,2,3-triazole-4,5-diyl]dimethanol	$C_6H_8N_6O_3$	EE
2	5.39	193.04	M+H	[1,2,3]Triazolo[4,5-e] benzotriazole-1,6-diol	$C_6H_4N_6O_2$	EE, EAF
3	5.78	219.10	M+H	4-[(E)-(3,5-Diamino-1H-pyrazol-4-yl)diazenyl]phenol	$C_9H_{10}N_6O$	EE
4	7.87	308.22	M+H	4-[(4-Ethyl-1-piperazinyl)methyl]-6-(4-morpholinyl)-1,3,5-triazin-2-amine	$C_6H_8N_6O_3$	EE
5	9.74	403.20	M+H	(4-Amino-7-isopropyl-7H-pyrrolo[2,3-d]pyrimidin-5-yl)(6-{[2-(3-pyridinyl)ethyl]amino}-2-pyrazinyl)methanone	$C_{21}H_{22}N_8O$	EE, HF, EAF
6	10.84	445.21	M+H	Ethyl 2-(3,5-dimethyl-1H-pyrazol-1-yl)-4-[2-(6-methyl-2-phenyl-4-pyrimidinyl)hydrazino]-5-pyrimidinecarboxylate	$C_{23}H_{24}N_8O_2$	EE, HF, EAF
7	12.05	254.28	M+H	N-Dodecylpiperidine	$C_{17}H_{35}N$	EE
8	5.37	223.06	M+H	Isofraxidin	$C_{11}H_{10}O_5$	HF
9	9.28	192/13	M+H	DEET	$C_{12}H_{17}NO$	HF
10	12.64	306.10	M+H	2-Boc-5-thia-2-azaspiro[3.4]octane-8-carboxylic acid, 5,5-dioxide	$C_{12}H_{19}NO_6S$	HF
11	13.21	277.21	M+H	Stearidonic acid	$C_{18}H_{28}O_3$	HF
12	5.70	219.10	M+H	Propanolol glycol	$C_{13}H_{14}O_3$	EAF
13	6.36	435.20	M+H	Xantinol nicotinate	$C_{19}H_{26}N_6O_6$	EAF
14	6.73	361.16	M+H	N~4~-(4-methoxyphenyl)-5-nitro-N~2~-(tetrahydrofuran-2-ylmethyl)pyrimidine-2,4,6-triamine	$C_{16}H_{20}N_6O_4$	EAF
15	7.41	387.10	M+H	N-(1,3-Benzodioxol-5-ylmethyl)-3-[(5-methyl-3-nitro-1H-pyrazol-1-yl)methyl] -1,2,4-oxadiazole-5-carboxamide	$C_{16}H_{14}N_6O_6$	EAF
16	7.81	308.22	M+H	Levobetaxolol	$C_{18}H_{29}NO_3$	EAF
17	4.92	542.26	M+H	his-lys-glu-glu	$C_{22}H_{35}N_7O_9$	BuF
18	11.03	450.28	M+H	4-[4-(4,5-Dimethyl-4H-1,2,4-triazol-3-yl)-1-piperazinyl]-2-ethyl-6-[4-(2-pyrimidinyl)-1-piperazinyl]pyrimidine	$C_{22}H_{31}N_{11}$	BuF
19	12.33	596.43	M+H	3-[3-[4-(4-amino-2-butylimidazo[4,5-c]quinolin-1-yl)butyl-methylamino]propoxy]-N-[5-(methylamino)-6-oxoheptyl]propenamide	$C_{33}H_{53}N_7O_3$	BuF
20	13.76	506.34	M+H	N-[4-(Diethylamino)phenyl]-N′-[3-(dimethylamino) propyl]-6-[4-(2-pyrimidinyl)-1-piperazinyl]-1,3,5-triazine-2,4-diamine	$C_{26}H_{39}N_{11}$	BuF
21	14.00	678.47	M+H	6-amino-2-[3-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]propanoylamino]-N-[1-[[6-(carbamoylamino)-2-oxohexan-3-yl]amino]-3-methyl-1-oxobutan-2-yl] hexanamide; ethane	$C_{31}H_{63}N_7O_9$	BuF
22	15.76	393.31	M+H	(22E)-Ergosta-4,6,8(14),22-tetraen-3-one	$C_{28}H_{40}O$	BuF
23	4.37	170.11	M+H	Aceclidine	$C_{13}H_{26}O_3S_2$	AF
24	8.51	225.06	M+H	Tubermycin B	$C_{13}H_8ON_2O_2$	AF
25	15.03	350.34	M+H	Pipercitine	$C_{23}H_{43}NO$	AF
26	16.33	563.38	M+H	4-(4-Cyclopentyl-1-piperazinyl)-N-[3-(1H-imidazol-1-yl)propyl]-6-methoxy-7-[3-(1-pyrrolidinyl)propoxy]-2-quinazolinamine	$C_{31}H_{46}N_8O_2$	AF

AF: aqueous fraction; BuF: n-butanol fraction; EAF: ethyl acetate fraction; EE: ethanolic extract; HF: n-hexane fractions

Antiaging activity on tyrosinase enzyme inhibition of *S. populifolia* stem bark extract and fractions

The tyrosinase inhibitory activity of *S. populifolia* extract and fractions is presented in **Table 4**. The results showed that the ethanol extract and n-butanol fraction exhibited weak activity, with IC_{50} values of 133.141±3.25 µg/mL and 229.60±3.75 µg/mL, respectively. In contrast, the n-hexane fraction (IC_{50}=461.21±4.23 µg/mL), ethyl acetate fraction (IC_{50}=587.99±13.38 µg/mL), and aqueous fraction (IC_{50}=641.17±2.97 µg/mL) demonstrated poor activity. All extracts and fractions were significantly less effective than the positive control, kojic acid, which showed strong inhibition with an IC_{50} of 13.06±0.54 µg/mL.

Table 4. Tyrosinase inhibitory activity of *Sterculia populifolia* DC stem bark extract and its fractions

Sample	Concentration (µg/mL)						IC50 value (µg/mL)
	12.5	25	50	100	200	400	
Ethanol extract	2.84±3.52	5.75±2.75	17.48±4.96	40.15±3.25	82.63 ±4.32	162.84± 3.51	133.15±3.25[b]
N-hexan fraction	18.78±4.36	25.18±5.58	35.65 ±23.36	38.4 ±19.69	40.65 ±2.03	43.33 ±2.53	461.21±4.23[d]
Ethyl acetate fraction	14.75±3.23	19.37±9.45	22.75±14.94	25.43 ±8.99	32.19±23.26	37.84±22.67	587.99±13.38[e]
N-butanol fraction	18.46±3,47	20.28±3.11	26.98±2.74	36.27 ±5.42	48.34±3.87	70.82±2.46	229.60±3.75[c]
Aqueous fraction	31.71±2.31	33.10±1.18	35.84±2.12	36.32±4.11	39.38±2.62	42.87±3.73	641.17±2.97[e]
Kojic acid							13.06±0.54[a]

All data were shown as mean±SD; each treatment was repeated three times.
[a,b,c,d,e] indicate significant difference with Tukey analysis (p<0.05, n=3).
Kojic acid was used as a positive control with concentrations of 0.62, 1.25, 2.5, 5, 10, and 20 µg/mL

Antiaging activity on elastase enzyme inhibition of *S. populifolia* stem bark extract and fractions

The results showed that ethanol extract of *S. populifolia* stem bark (IC_{50}=93.35±3.79µg/mL) and quercetin (IC_{50}=17.33±0.79 µg/mL) demonstrated strong elastase inhibitory activity, with a significant difference between them (**Table 5**). The n-butanol fraction exhibited weak activity (IC_{50}=173.53±2.57 µg/mL), while the n-hexane fraction (IC_{50}=886.63±3.40 µg/mL) and ethyl acetate (IC_{50}=1087.49±3.02 µg/mL) fractions showed poor activity.

Table 5. Elastase inhibitory activity of *S. populifolia* DC stem bark extract and its fractions

Sample	Concentration (µg/mL)						IC50 value (µg/mL)
	6.25	12.5	25	50	100	200	
Extract	41.87±3.2	51.35±5.6	64.75±3.8	68.15±3.25	75.4±4.73	79.9±3.21	93.35±3.7[b]
N-hexane fraction	3.63±0.96	4.45±1.21	5.95±1.16	8.27±2.23	11.27±1.84	13.74±1.89	886.63±3.40[e]
Ethyl acetate fraction	1.11± 0.30	3.29±0.22	4.84±1.03	6.58±1.13	9.53±1.18	10.40±2.06	1087.49±3.02[f]
N-butanol fraction	9.86±1.52	14.10±3.28	23.72±4.71	30.79±0.91	42.65±3.68	50.37±0.15	173.53±2.57[c]
Aqueous fraction	2.56±0.23	4.89±0.97	5.52±1.19	6.48±1.30	12.05±2.40	29.37±3.69	368.04±7.69[d]
Quarcetin							17.33±0.79[a]

All data were shown as mean±SD; each treatment was repeated three times.
[a,b,c,d,e,f] indicate significant difference with Tukey analysis (p<0.05, n=3)
Quercetin was used as a positive control with concentrations of 0.62, 1.25, 2.5, 5, 10, and 20 µg/mL

Discussion

The analysis of *S. populifolia* DC stem bark extract and its fractions demonstrated promising anti-aging potential, particularly through the inhibition of tyrosinase and elastase enzymes. The dry extract exhibited a brown color, and the extraction process yielded a consistent and concentrated product across all fractions [20]. Among the fractions, the *n*-hexane fraction produced the highest yield (16.09%), highlighting the effectiveness of this non-polar solvent in extracting lipophilic constituents from the sample matrix [1]. This finding aligns with previous reports suggesting that

solvent selection plays a critical role in optimizing extraction efficiency and is a key factor in pharmaceutical and phytochemical research [21].

Phytochemical screening revealed that the *S. populifolia* stem bark extract contains key secondary metabolites, including alkaloids, phenolics, and steroids, all of which are associated with significant pharmacological activity. The presence of alkaloids and phenolics in both the extract and its fractions suggests strong antioxidant potential and enzyme inhibitory activity, making them relevant for anti-aging applications. Alkaloids are known for a broad spectrum of biological effects, including anti-inflammatory and antimicrobial properties, thereby supporting the therapeutic potential of *S. populifolia* in anti-aging interventions [22,23]. Phenolic compounds further reinforce the antioxidant function by inhibiting cellular oxidative stress, a key contributor to premature aging and degenerative changes [24,25].

The LC-MS/MS-based characterization of secondary metabolites provides valuable insight into the compound profiles of *S. populifolia* stem bark and sheds light on the potential mechanisms underlying its pharmacological activities, particularly the inhibition of elastase and tyrosinase. Among the identified metabolites, alkaloids were the predominant compound group consistently present across all extracts and fractions. Additionally, minor constituents from the phenolic and steroid groups were also detected. Phenolic compounds such as 4-[(E)-(3,5-diamino-1H-pyrazol-4-yl)diazenyl]phenol (compound 3) were identified in the ethanol extract, while Isofraxidin (compound 8) was detected in the *n*-hexane fraction. A steroid compound, (22E)-Ergosta-4,6,8(14),22-tetraen-3-one (compound 22), was identified in the *n*-butanol fraction of *S. populifolia* stem bark [26,27]. The abundance of alkaloid compounds suggests their potential contribution to the observed depigmentation effect, likely through tyrosinase inhibition. Mechanistically, this may involve competition at the active site of melanogenesis-related enzymes, analogous to the kynurenine pathway, where structurally similar alkaloids interfere with amino acid substrates [28]. Phenolic compounds, on the other hand, are known to inhibit elastase and tyrosinase through multiple pathways, including metal ion chelation and direct enzyme binding, thereby reinforcing their role in anti-aging mechanisms.

The tyrosinase inhibitory activity observed in the extract and fractions of *S. populifolia* stem bark highlights its potential as an anti-pigmentation or anti-aging agent. Based on IC_{50} values, the ethanol extract (133.15 µg/mL) demonstrated moderate inhibitory activity, while the *n*-butanol fraction showed weak activity (229.60 µg/mL). Other fractions—including the *n*-hexane, ethyl acetate, and aqueous fractions—exhibited very weak inhibition. This enzymatic inhibition is likely attributed to the presence of phenolic compounds, which are known to interact with the active site of tyrosinase and modulate its function [29]. Among all samples, the ethanol extract exhibited the lowest IC_{50} value, indicating the most potent inhibitory effect against tyrosinase. As commonly interpreted in enzyme kinetics, lower IC_{50} values correspond to higher inhibitory potency [30]. LC-MS/MS analysis revealed the presence of 4-[(E)-(3,5-diamino-1H-pyrazol-4-yl)diazenyl]phenol in the ethanol extract—a phenolic compound structurally bound to a pyrazole alkaloid moiety. This hybrid structure likely enhances both hydrogen bonding and metal-chelating interactions with the enzyme. Pyrazole derivatives have been previously reported to act as effective tyrosinase inhibitors due to their nitrogen-containing heterocyclic ring (N-heterocycle), which contributes to Cu^{2+} ion chelation at the tyrosinase active site [31]. The nitrogen ring (=N–) in the pyrazole structure facilitates interaction with the enzyme's copper ion, thereby inactivating enzymatic activity. This dual functionality—arising from both the phenolic and pyrazole components—likely contributes to the superior tyrosinase inhibition observed in the ethanol extract. Supporting evidence from Lee *et al*. (2020) further confirms that phenolic compounds possess strong modulatory effects on tyrosinase activity through direct interaction with catalytic residues and metal ions [32].

In addition to tyrosinase inhibition, the ethanol extract of *S. populifolia* stem bark also demonstrated significant inhibitory activity against elastase, with an IC_{50} value of 93.35 µg/mL, indicating strong anti-elastase potential. Elastase is a serine protease responsible for the degradation of elastin in skin tissue, and its inhibition is considered a key therapeutic target in anti-aging skincare [7]. For comparison, quercetin—used as a positive control—exhibited greater elastase inhibitory activity, consistent with its well-established role as an anti-aging and anti-inflammatory compound in cosmetic formulations [33]. Among the tested fractions, the *n*-

butanol fraction displayed weak elastase inhibitory activity, while the *n*-hexane and ethyl acetate fractions showed very weak activity. This may be attributed to the lower concentrations or absence of key bioactive compounds in these fractions. The elastase inhibition observed for the ethanol extract closely correlates with its tyrosinase inhibition activity, suggesting a shared or overlapping mechanism of action. This dual inhibitory activity is likely influenced by the presence of phenolic pyrazole compounds in the extract. These compounds are hypothesized to contribute to enzyme inhibition through interactions with the active sites of both tyrosinase and elastase. Mechanistically, a similarity may exist in the mode of inhibition, as elastase—like tyrosinase—is a hydrolase enzyme and belongs to the metalloenzyme family [34]. Such structural similarities could facilitate parallel binding and inactivation pathways involving metal ion coordination and hydrogen bonding.

Previous research suggested that antioxidant-rich plant extracts, particularly those containing polyphenols and flavonoids, possess significant anti-aging potential due to their ability to inhibit matrix-degrading enzymes such as elastase and tyrosinase [35]. These findings align with the present study, where flavonoid content is believed to contribute to the observed enzyme inhibitory activity. Furthermore, two other studies support the use of natural extracts for tyrosinase inhibition as a strategy to prevent hyperpigmentation, one of the hallmarks of skin aging [36,37]. These outcomes are consistent with the chemical constituents identified in the ethanol extract of *S. populifolia*, including flavonoids and phenolic alkaloids.

Based on in vitro findings, the ethanol extract of *S. populifolia* stem bark exhibits moderate tyrosinase inhibition and strong elastase inhibition, indicating its promising potential for further development as an anti-aging agent. Although the IC_{50} values are relatively high compared to synthetic standards, they remain within a usable range for natural product development. Several strategies may be employed to enhance the extract's bioactivity, such as optimizing the extraction process to maximize the yield of active compounds or combining it with other bioactive natural extracts to achieve synergistic effects. Furthermore, the clinical relevance of this study lies in the potential development of *S. populifolia* stem bark extract as an active ingredient in skincare formulations, particularly those targeting anti-aging pathways via elastase and tyrosinase inhibition. With its demonstrated antioxidant and enzyme-inhibitory properties, *S. populifolia* represents a natural alternative to synthetic agents, which are often associated with adverse effects such as skin irritation [38,39].

Despite the promising findings on the antiaging potential of *S. populifolia* stem bark, this study has several limitations. First, the anti-aging activities were evaluated solely through in vitro enzymatic assays (tyrosinase and elastase inhibition), which may not fully represent the extract's effects in physiological or clinical settings. Second, the identified compounds were tentatively assigned based on LC-MS/MS data and database matching, without further structural confirmation via techniques such as NMR or MS/MS fragmentation analysis. Third, the study did not investigate the cytotoxicity, skin permeability, or stability of the extract and its fractions, which are critical parameters for cosmetic application. Additionally, molecular mechanisms, including signaling pathways involved in melanogenesis or extracellular matrix degradation, were not explored. These limitations highlight the need for further research, including in vivo studies, mechanistic assays, and formulation development, to validate the efficacy and safety of *S. populifolia* stem bark extract as an anti-aging agent.

Conclusion

LC-MS/MS analysis confirmed the presence of key bioactive compounds, including 4-[(E)-(3,5-diamino-1H-pyrazol-4-yl)diazenyl]phenol, isofraxidin, and (22E)-ergosta-4,6,8(14),22-tetraen-3-one, which may contribute to its observed inhibitory effects on elastase and tyrosinase enzymes. The ethanol extract exhibited the strongest overall activity among the samples, indicating its potential as a natural ingredient in anti-aging skincare formulations. These findings support further exploration of *S. populifolia* for cosmetic and healthcare applications. Future research should include in vivo studies, mechanistic assays, and formulation development to validate the efficacy and safety of *S. populifolia* stem bark extract as an anti-aging agent.

Ethics approval

Ethics approval was not required.

Acknowledgments

The authors would like to thank the *Kementerian Pendidikan, Kebudayaan, Riset dan Teknologi* Indonesia, LLDIKTI Region IX Indonesia, and *Lembaga Penelitian dan Pengabdian Kepada Masyarakat* Universitas Almarisah Madani Indonesia for providing financial support.

Competing interests

All the authors declare that they have no conflicts of interest

Funding

This research was funded by *Kementrian Pendidikan, Riset dan Teknologi* Indonesia, through a Fundamental Research Grant (617/LL9/PK.00.PG/2024; 2907/UNIVERAL/LPPM/VI/2024).

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study employed the artificial intelligence (AI) tool ChatGPT to support manuscript writing, including language refinement (improving grammar, sentence structure, and overall readability) and technical writing assistance (providing suggestions for structuring complex technical descriptions more effectively). All AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were made solely by the authors.

How to cite

Khairi N, Nursamsiar N, Utami NF, *et al*. Phytochemical profiling and enzyme inhibitory activity of *Sterculia populifolia* DC stem bark extract and fractions against elastase and tyrosinase. Narra J 2025; 5 (3): e1778 - http://doi.org/10.52225/narra.v5i3.1778.

References

1. Dai Y, Harinantenaina L, Brodie P, *et al.* Isolation and synthesis of two antiproliferative calamenene-type sesquiterpenoids from sterculia tavia from the madagascar rain forest. Bioorg Med Chem 2012;20(24):6940-6944.
2. Costa DA, Chaves MH, Silva WCS, *et al.* Constituintes químicos, fenóis totais e atividade antioxidante de *Sterculia striata* St. Hil. et Naudin. Acta Amazon 2010;40(1):207-212.
3. Zhang JJ, Li Y, Lin SJ, *et al.* Green extraction of natural antioxidants from the *Sterculia nobilis* fruit waste and analysis of phenolic profile. Molecules 2018;23(5):1059.
4. Rabbi F, Zada A, Adhikari A, *et al. Sterculia diversifolia* bears anti-cancer and immunomodulatory activities. Bangladesh J Pharmacol 2017;12(1):51.
5. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. J Enzyme Inhib Med Chem 2017;32(1):403-425.
6. Qarani W, Husna F, Yulia W, *et al.* Antioxidant and antiaging activities of Cinnamomum burmannii, Michelia champaca and their combinations. Narra J 2023;3(2).
7. Nur S, Aisyah A, Nursamsiar N, *et al.* Standardization and GC-MS analysis of Kersen (*Muntingia calabura* L.) fruit ethanol extract as an herbal raw material. Bull Pharm Sci Assiut Univ 2023;46(1):173-187.
8. Ndlovu G, Fouché G, Tselanyane M, *et al.* In vitro determination of the anti-aging potential of four southern African medicinal plants. BMC Complement Altern Med 2013;13(1).
9. Pintus F, Floris S, Fais A, *et al.* Hydroxy-3-phenylcoumarins as multitarget compounds for skin aging diseases: synthesis, molecular docking and tyrosinase, elastase, collagenase and hyaluronidase inhibition, and sun protection factor. Molecules 2022;27(20):6914.

10. Salman Fareza M, Amalia Choironi N, Sutji Susilowati S, *et al.* LC-MS/MS Analysis and Cytotoxic Activity of Extract and Fractions of Calophyllum soulattri Stembark. 32. 2020.
11. Sulistyowati S, Elya B, Iswandana R, *et al.* Phytocompounds and in vitro antiaging activity of ethanolic extract and fractions of Rubus fraxinifolius Poir. leaves. J Pharm Pharmacogn Res 2023;11(4):595-610.
12. Candra A, Fahrimal Y, Yusni Y, *et al.* Phytochemistry and antifatigue activities of Carica papaya leaf from geothermal, coastal and urban areas, Indonesia. Narra J 2024;4(1).
13. Ismed F, Desti WN, Arifa N, *et al.* TLC-Bioautographic and LC-MS/MS Detection of Antimicrobial Compounds from Four Semipolar Extracts of *Cladonia* Species. 2021.
14. Hong Y, Jung E, Noh D, *et al.* Physiological effects of formulation containing tannase-converted green tea extract on skin care: physical stability, collagenase, elastase, and tyrosinase activities. Integr Med Res 2014;3(1):25-33.
15. Desmiaty Y, Saputri F, Hanafi M, *et al.* Anti-elastase, anti-tyrosinase and anti-oxidant of rubus fraxinifolius stem methanolic extract. Pharmacognosy Journal 2020;12(2):271-275.
16. Oh YS, Shin SY, Kim S, *et al.* Comparison of antiaging, anti-melanogenesis effects, and active components of Raspberry (*Rubus occidentalis* L.) extracts according to maturity. J Food Biochem 2020;44(11).
17. Widelski J, Gaweł-Bęben K, Czech K, *et al.* Extracts from European Propolises as Potent Tyrosinase Inhibitors. Molecules 2022;28(1):55.
18. Coppin JP, Xu Y, Chen H, *et al.* Determination of flavonoids by LC/MS and anti-inflammatory activity in Moringa oleifera. J Funct Foods 2013;5(4):1892-1899.
19. Saldanha L, Vilegas W, Dokkedal A. Characterization of Flavonoids and Phenolic Acids in Myrcia bella Cambess. Using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS Combined with NMR. Molecules 2013;18(7):8402-8416.
20. Costa C, Chaves M, Silva W, *et al.* Constituintes químicos, fenóis totais e atividade antioxidante de *Sterculia striata* st. hil. et naudin. Acta Amazon 2010;40(1):207-212.
21. Herrera-Meza S, Martínez A, Sánchez-Otero M, *et al.* Fatty acid composition and some physicochemical characteristics of *Sterculia apetala* seed oils. Grasas Y Aceites 2014;65(3).
29. Li D, Qian Z, Li S. Inhibition of three selected beverage extracts on α-glucosidase and rapid identification of their active compounds using hplc-dad-ms/ms and biochemical detection. J Agric Food Chem 2010;58(11):6608-6613.
23. Zhang J, Li Y, Lin S, *et al.* Green extraction of natural antioxidants from the S*terculia nobilis* fruit waste and analysis of phenolic profile. Molecules 2018;23(5):1059.
24. Sayuti M, Supriatna I, Hismayasari I, *et al.* Proximate, amino acid composition and antibacterial activity of woton (*Sterculia* sp.) leaves from Raja Ampat, West Papua, Indonesia. J Airaha 2019;8(1):1-7.
25. Susanto Y, Widodo A, Khaerati K, *et al.* Phytochemical analysis and cytotoxic activities of hantap leaves (*Sterculia coccinea* Jack) extract. Indones J Chem 2023;23(3):671.
32. Mosca F, Hidalgo G, Villasante J, *et al.* Continuous or batch solid-liquid extraction of antioxidant compounds from seeds of *Sterculia apetala* plant and kinetic release study. Molecules 2018;23(7):1759.
33. El-Nashar H, El-labbad E, Al-Azzawi M, *et al.* A new xanthone glycoside from *Mangifera indica* L.: Physicochemical properties and in vitro anti-skin aging activities. Molecules 2022;27(9):2609.
28. Apaza TL, Sánchez Sánchez-Corral J, Díaz-Guerra Martín C, *et al. Rubus urticifolius* compounds with antioxidant activity, and inhibition potential against tyrosinase, melanin, hyaluronidase, elastase, and collagenase. Pharmaceuticals 2024;17(7).
35. Abdillah S, Wijiyanti G, Setiawan M, *et al.* In vitro anti-tyrosinase and anti-elastase activity of collagen from sea cucumber (Holothuria leucospilota). Afr J Biotechnol 2017;16(15):771-776.
36. Nur S, Aswad M, Yulianty R, *et al.* The antioxidant and anti-ageing activity of lyophilisate kersen (*Muntingia calabura* L) fruit in vitro. Food Res 2023;7(2):23-30.
31. Zhang J, Yang W, He M, *et al.* Development of novel pyrazole-1,2,4-triazole derivatives as tyrosinase inhibitors: Design, preparation, mechanism of action and anti-browning application. Food Chem 2024;460:140722.
32. Lee J, Moon S, Hong Y, *et al.* Anti-elastase and anti-hyaluronidase activity of phosvitin isolated from hen egg yolk. Br Poult Sci 2020;61(1):17-21.
33. Horng C, Wu H, Chiang N, *et al.* Inhibitory effect of burdock leaves on elastase and tyrosinase activity. Exp Ther Med 2017;14(4):3247-3252.
34. Nur S, Hanafi M, Setiawan H, *et al.* Chemical characterization and biological activity of *Molineria latifolia* root extract as dermal antiaging: Isolation of natural compounds, in silico and in vitro study. Biocatal Agric Biotechnol 2024;56:103039.

35. Petruk G, Giudice R, Rigano M, *et al.* Antioxidants from plants protect against skin photoaging. Oxid Med Cell Longev 2018;2018(1).
36. Hofmann E, Webster J, Do T, *et al.* Hydroxylated chalcones with dual properties: Xanthine oxidase inhibitors and radical scavengers. Bioorg Med Chem 2016;24(4):578-587.
37. Maamoun H, Rabie G, Shaker I, *et al.* Biochemical properties of tyrosinase from *Aspergillus terreus* and *Penicillium copticola*; Undecanoic acid from *Aspergillus flavus*, an endophyte of moringa oleifera, is a novel potent tyrosinase inhibitor. Molecules 2021;26(5):1309.
38. Morisaki N, Moriwaki S, Sugiyama-Nakagiri Y, *et al.* Neprilysin is identical to skin fibroblast elastase. J Biol Chem 2010;285(51):39819-39827.
39. Desmiaty Y, Faizatun F, Noviani Y, *et al.* Review article: Potential of natural products in inhibiting premature skin aging. Int J Appl Pharm 2022;14(3):1-5.