Original Article

Antibacterial, antioxidant, and SucAla$_3$-based anti-aging activities of Johar flower extract (*Cassia siamea* Lamk.)

Kartini Hasballah[1*], Murniana Murniana[2], Muhammad Diah[3,4], Renzavaldy Rusly[5,6], Yosi Fadlia[2], Usratul Amna[2] and Muhammad NRR. Husni[2]

[1]Department of Pharmacology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia; [3]Department of Internal Medicine, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [4]Division of Cardiology, Department of Internal Medicine, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia; [5]Department of Surgery, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [6]Division of Urology, Department of Surgery, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia

*Corresponding author: kartini.hasballah.usk.ac.id

Abstract

Cassia siamea flowers are recognized for their diverse secondary metabolites, including flavonoids, triterpenoids, and steroids, which have been linked to various therapeutic properties. Although previous studies have primarily focused on the pharmacological activities of *C. siamea* leaves, bark, or crude extracts, evidence regarding the bioactivity of its flower extracts—particularly those obtained through sequential solvent partitioning—remains limited. The aim of this study was to investigate the antibacterial, antioxidant, and anti-aging potential of *C. siamea* flower extracts obtained through sequential solvent extraction. The extracts were prepared by maceration with methanol, followed by partitioning with n-hexane and ethyl acetate. Antibacterial activity was assessed using the agar well diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Anti-aging properties were evaluated through elastase inhibition assays, with the reduction of SucAla3 formation used as an indicator. Phytochemical profiling of the extracts was conducted using chromatography–mass spectrometry (GC–MS). The findings revealed that the ethyl acetate extract exhibited the highest antibacterial activity, with inhibition zones of 8.08±0.84 mm and 7.38±0.33 mm against *S. aureus* and *E. coli*, respectively. Antioxidant analysis showed the methanol extract to be the most effective (IC_{50}=66.76 µg/mL), followed by the methanol partition (IC_{50}=75.97 µg/mL). The methanol extract demonstrated significant elastase inhibition, achieving 97.53% activity (IC_{50}=13.89 µg/mL). GC–MS analysis identified two compounds as the major phytocomponents of the methanolic extract, namely octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester and tetradecanoic acid. In conclusion, *C. siamea* flower extracts have therapeutic potentials, particularly as antibacterial, antioxidant, and anti-aging agents.

Keywords: *Cassia* sp., phytochemicals, antibacterial activity, antioxidant activity, anti-aging potential

Introduction

Medicinal plants have long been utilized in traditional medicine, involving various plant parts, including roots, leaves, bark, fruit, flowers, and seeds [1,2]. Among these, the Johar plant (*Cassia siamea* Lamk) is commonly used in traditional remedies for conditions such as malaria, skin

Received: November 26, 2025 - Accepted: December 27, 2025 - Published online: December 31, 2025

diseases, diabetes, fever, wounds, and as a general tonic [3-6]. Its flowers, rich in flavonoids and carotenoids, are particularly noted for their therapeutic applications [7]. The bioactivity of *C. siamea* flowers is attributed to their secondary metabolites, including flavonoids, alkaloids, terpenoids, steroids, triterpenoids, saponins, and tannins, which exhibit antibacterial, antioxidant, and anti-inflammatory properties [8,9]. Previous studies have demonstrated the antibacterial efficacy of *C. siamea* extracts against *Staphylococcus aureus* and *Escherichia coli* [10-12]. Additionally, phenolic compounds in methanol extracts of *C. siamea* stems have been shown to inhibit *Bacillus subtilis* and *E. coli* [13]. Other isolated compounds, such as luteolin, quercetin, β-sitosterol, and apigenin, exhibit antioxidant, anticancer, and antibacterial activities [8,14,15].

Free radicals, highly reactive and unstable due to unpaired electrons, are primary contributors to oxidative stress, which damages cellular components such as lipids, proteins, and DNA, ultimately leading to pathological conditions such as diabetes, cardiovascular disorders, neurodegenerative diseases, premature aging, and cancer [16,17]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) exacerbate this damage by triggering oxidative cascades that impair cellular function and physiological integrity. It is well established that antioxidants play a crucial role in neutralizing ROS and RNS, thereby mitigating oxidative damage, slowing aging processes, and preventing degenerative diseases [17]. Flavonoids, alkaloids, terpenoids, steroids, triterpenoids, saponins, and tannins contained in *C. siamea* flowers have been known to possess antioxidant properties [18]. Flavonoids, such as quercetin and luteolin, scavenge free radicals by donating hydrogen atoms or electrons to stabilize ROS and RNS, thereby preventing cellular damage [19]. Additionally, flavonoids can chelate metal ions, inhibiting the Fenton reaction that generates hydroxyl radicals [19]. Triterpenoids, such as β-amyrin and lupeol, exhibit antioxidant properties by modulating signaling pathways like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby reducing oxidative stress and inflammation [20]. In addition, steroids and saponins contribute to cell membrane stabilization, protecting against ROS-induced lipid peroxidation [21,22].

However, despite the established biochemical knowledge, the relative contribution, comparative potency, and functional relevance of these phytochemical classes within *C. siamea* flower extracts remain insufficiently explored, particularly when assessed across different solvent fractions. Moreover, most available evidence is derived from isolated compounds or non-floral plant parts, and direct links between phytochemical profiles and combined antioxidant, antibacterial, and elastase-inhibitory activities of flower extracts are limited. Therefore, the aim of this study was to evaluate the antibacterial, antioxidant, and anti-aging activities of *C. siamea* flower extracts and to identify fractions with greater biological relevance, rather than to establish definitive therapeutic or clinical efficacy.

Methods

Biological specimens

Flower samples of *C. siamea* were collected from Limpok Village, Darussalam District, Aceh Besar, Aceh, Indonesia, and identified in the Herbarium of the Biology Department, Universitas Syiah Kuala, Banda Aceh, Indonesia. The cultures of *S. aureus* and *E. coli* bacteria were used to determine the antibacterial activities of the extract.

Maceration and partition

The extraction of *C. siamea* flowers was conducted following established protocols with minor modifications [23,24]. Briefly, fresh flowers (5 kg) were thoroughly rinsed with distilled water to remove impurities, cut into small pieces, and dried to eliminate water content. The dried flowers were ground into a fine powder and macerated with methanol as the solvent. The methanol extract was concentrated using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) to obtain the crude methanol extract. This extract was further partitioned with n-hexane, and the n-hexane layer was concentrated to produce the n-hexane extract. The remaining methanol layer was subsequently fractionated with ethyl acetate, yielding an ethyl acetate extract after

concentration. The residual methanol layer was concentrated to obtain the partitioned methanol extract.

Phytochemical screening

Phytochemical screening of *C. siamea* extract involved various tests to detect the presence of bioactive compounds, and details of the phytochemical screenings have been reported elsewhere [25,26]. Briefly, alkaloids were detected by treating the extract with concentrated ammonia, chloroform, and 5% HCl, followed by Mayer's, Wagner's, and Dragendorff's reagents, with the formation of white, brown, and red/orange precipitatesindicating positive results. Phenolic compounds were identified by the addition of 10% $FeCl_3$, which produced a blackish-green color indicating positivity. Tannins were confirmed by mixing the filtrate with ethanol, distilled water, and $FeCl_3$, resulting in blackish-green, brown, or dark blue coloration. Flavonoids were identified by adding 10% lead acetate, forming a brown precipitate. Saponins were detected by shaking the extract with distilled water, where stable foam formation indicated a positive result. Steroids and terpenoids were identified using the Liebermann-Burchard reagent, with green/blue and red/purple color changes indicating the presence of steroids and terpenoids, respectively.

Antibacterial test

Antibacterial activity was assessed using the agar well diffusion method, following procedures adapted from previous studies [27,28]. Nutrient agar (NA) was prepared, sterilized, and inoculated with standardized bacterial suspensions of *S. aureus* and *E. coli* adjusted to 1.5×10^8 CFU/mL to obtain a uniform bacterial lawn. After the agar solidified, wells of 6 mm diameter were aseptically created using a sterile cork borer. Each well was filled with 30 µL of plant extract at concentrations of 25% and 50% (w/v). Methanol, ethyl acetate, and n-hexane were used as negative controls corresponding to their respective extracts, while amoxicillin (for *S. aureus*) and chloramphenicol (for *E. coli*) served as positive control antibiotics. Plates were incubated at 37°C for 24 h, after which antibacterial activity was evaluated by measuring the total diameter of inhibition zones (mm), including the well diameter, using a digital caliper. All treatments were performed in triplicate. Antibacterial activity was classified as very strong (>20 mm), strong (11–20 mm), moderate (5–10 mm), or weak (<5 mm) based on the average inhibition diameter.

Antioxidant assay

The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. A 0.4 mM DPPH solution was freshly prepared by dissolving 7.9 mg DPPH in methanol. Extract concentrations (6.25, 12.5, 25, 50, and 100 ppm) and vitamin C standards (3, 6, 9, 12, and 15 ppm) were prepared from stock solutions and homogenized. Each extract concentration was mixed with 1 mL of DPPH solution and methanol to a final volume of 5 mL, covered with aluminum foil, and incubated at 37°C for 30 minutes. Absorbance spectra were scanned over 450–550 nm to identify the maximum absorption wavelength (λ_{max}) of DPPH, and antioxidant activity was quantified using absorbance values recorded at λ_{max}. Antioxidant activity was expressed as % inhibition, and half maximal inhibitory concentration (IC_{50}) values were determined from the concentration-inhibition curves.

Anti-aging activity assay

The anti-aging activity of *C. siamea* flower extracts was evaluated in vitro using a $SucAla_3$-based elastase inhibition assay employing porcine pancreatic elastase (PPE) and N-succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) as the chromogenic substrate. Reaction mixtures consisted of 100 mM Tris–HCl buffer (pH 8.0), PPE (0.08 U/mL), and SANA (4.4 mM). The enzymatic reaction was initiated by substrate addition and incubated for 15 minutes at 25°C, after which the release of p-nitroaniline was monitored by measuring absorbance at 405 nm using a microplate ELISA reader (Awareness Technology Inc., Palm City, USA). Methanol-macerated extract and partitioned fractions (ethyl acetate and n-hexane) were tested at final concentrations of 12.5, 25, and 50 µg/mL, prepared in dimethyl sulfoxide (DMSO). Oleanolic acid was used as the positive control inhibitor, while blank and control reactions were included to correct for non-enzymatic and solvent effects. Elastase inhibitory activity was expressed as percentage inhibition relative to the

control, calculated from corrected absorbance values. All experiments were performed in triplicate, and results were reported as mean inhibition values.

Phytochemical profiling by gas chromatography–mass spectrometry (GC–MS)

Phytochemical profiling of all extracts was performed using GC–MS based on the analytical conditions. Analyses were conducted using a Thermo Scientific ISQ 7000 GC–MS system (Thermo Fisher Scientific, Waltham, USA) equipped with a capillary column (5% phenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was set as follows: initial temperature 60°C for 2 min, increased at 10°C/min to 280°C, and held for 10 min. The injector temperature was maintained at 250°C, operating in split mode. Mass spectrometric detection was performed under electron ionization (EI) at 70 eV, with a scan range of m/z 40–600. Compounds were identified based on National Institute of Standards and Technology (NIST) library similarity scores, while the percentage peak area (% area) was used to indicate their relative abundance in the extract.

Statistical analysis

The statistical analysis was carried out on RStudio version 2024.04.2 (RStudio, Boston, USA). Continuous data were expressed as average ± standard deviation. The normality of the data distribution was tested through Shapiro-Wilk approach. Statistical comparison between groups was performed using analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical significance was set at $p<0.01$.

Results

Yields and appearance of the extracts

The extraction of *C. siamea* flowers was performed through methanol maceration to isolate secondary metabolites across a polarity gradient. Methanol, with both methyl (-CH_3) and hydroxyl (-OH) functional groups, acted as a universal solvent. Extraction continued until the methanol's color remained unchanged, ensuring exhaustive compound recovery. Rotary evaporation yielded 371.78 g of concentrated methanol extract with a 9.65% yield. Partitioning with n-hexane isolated non-polar metabolites, yielding 210.49 g, while re-extraction with ethyl acetate isolated semi-polar compounds, yielding 28.07 g. The n-hexane fraction exhibited the highest yield (56.61%), indicating a high abundance of non-polar secondary metabolites in *C. siamea* flowers. The separation process adhered to the principle of "like dissolves like," where compounds dissolve in solvents of similar polarity. Solvent polarity, determined by dielectric constant values, followed the order: methanol > ethyl acetate > n-hexane, facilitating targeted metabolite extraction.

Phytochemical screening

Phytochemical screening was conducted to identify the presence of secondary metabolite compounds. Based on the basic framework of compounds, secondary metabolite compounds are grouped into five groups: alkaloids, flavonoids, saponins, terpenoids, and steroids. The results of phytochemical tests on *C. siamea* flower extract are presented in **Table 1**.

Table 1. Phytochemical classes identified in *Cassia siamea* flower extracts through qualitative screening

Phytochemical test	Methanol extract	Methanol partition	n-Hexane extract	Ethyl acetate extract
Alkaloid	+	+	+	+
Saponin	+++	+++	-	-
Flavonoid	+	+	+	+
Phenolic	+++	+++	++	++
Steroid	+	+	++	-
Terpenoid	-	-	-	+
Tannin	+	+	+	+

+++: very positive; ++: positive; +: slightly positive; -: negative

Antibacterial activity

The results of the antibacterial test on *C. siamea* extracts are presented in **Figure 1**. Ethyl acetate extract demonstrated the highest activity, with inhibition zones of 6.20 mm and 8.08 mm against *S. aureus* at 25% and 50% concentrations, respectively. When the extract was tested on *E. coli*, the inhibition zones were 6.20±0.01 mm and 8.083±0.34 mm with concentrations of 25% and 50%, respectively. The positive controls for *E. coli* and *S. aureus* had inhibition zones up to 33.57±0.04 mm and 31.86±0.04 mm, respectively.

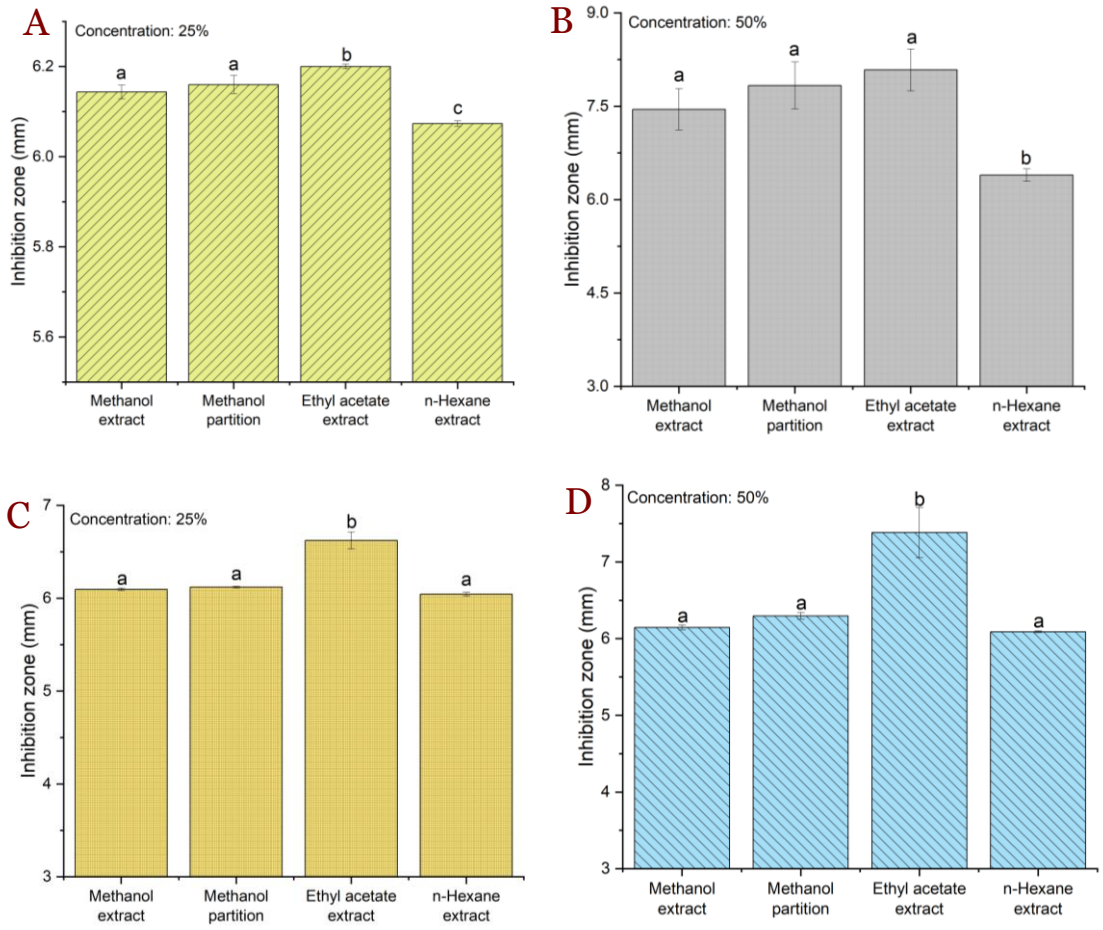

Figure 1. Antibacterial activity of *Cassia siamea* flower extracts against *Escherichia coli* and *Staphylococcus aureus*. Inhibition zone against *E. coli* by *C. siamea* extract with concentrations of 25% (A) and 50% (B). Inhibition zone against *S. aureus* by *C. siamea* extract with concentrations of 25% (C) and 50% (D). Different letter annotations indicate statistically significant differences at $p<0.01$, as determined by Tukey's post-hoc test.

Statistically, at 25% concentration, ethyl acetate extract was found to be higher compared to methanol extract ($p=0.003$) and n-hexane extract ($p<0.001$). No significant difference between methanol partition and methanol extract ($p=0.439$), indicating similar activities. When the concentration was increased to 50%, no statistical difference was observed among methanol extract, methanol partition, and ethyl acetate extract. The n-hexane extract exhibited the lowest inhibition as compared to methanol extract ($p=0.013$), methanol partition ($p=0.002$), and ethyl acetate extract ($p<0.001$). At 25% or 50% concentration, inhibition against *S. aureus* was more pronounced in ethyl acetate extract as compared to methanol extract, methanol partition, and n-hexane extract (each with $p<0.001$). The photographed images of antibacterial activities observed in this study are presented in **Figure 2**.

Antioxidant activity

The *C. siamea* extracts exhibited DPPH radical scavenging activity, indicating antioxidant potential, with detailed results presented in **Figure 3**. The inhibition appeared to be

concentration dependent. In methanol extract, significant inhibition was achieved when the concentration was increased from 25 µg/mL to 50 µg/mL (p<0.001). Increasing the concentration from 12.5 µg/mL to 25 µg/mL of the methanol partition significantly enhanced the scavenging activity on DPPH (p<0.001). In n-hexane and ethyl acetate extract, the significant inhibition was firstly achieved by 25 µg/mL (p=0.003) and 12.5 µg/mL (p=0.009), respectively. At the lowest concentration of 3 µg/mL, ascorbic acid inhibited 12.33% of DPPH radical activity, while at 15 µg/mL, inhibition reached 87.46%. Based on the regression equation, the IC_{50} value of the methanolic *C. siamea* extract was 66.76 µg/mL, compared with 7.26 µg/mL for the positive control, vitamin C (**Figure 4**).

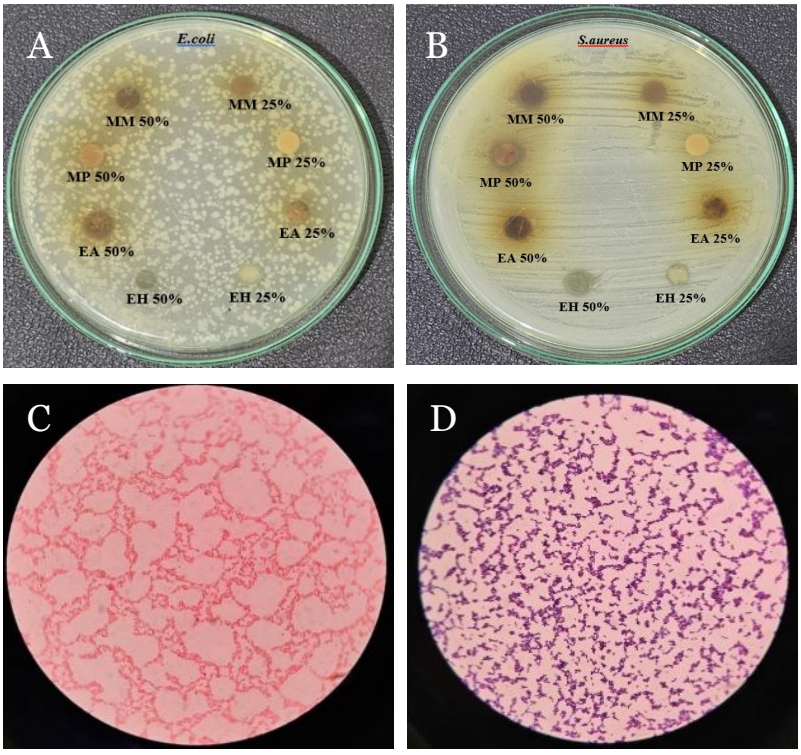

Figure 2. Inhibition zone formed by *Cassia siamea* flower extracts against *Escherichia coli* (A) and *Staphylococcus aureus* (B). Cell wall structure of Gram-positive – *S. aureus* (C) and Gram-negative – *E. coli* (D).

Anti-aging activity

The elastase inhibitory activity of *C. siamea* flower extracts was evaluated in vitro at concentrations of 50, 25, and 12.5 µg/mL to assess their anti-aging potential. Oleanolic acid, used as a positive control, demonstrated potent inhibition with an IC_{50} value of 5.78, achieving nearly 100% elastase inhibition (**Table 2**). A regression analysis of oleanolic acid revealed a strong linear correlation between concentration and inhibition percentage (R^2=0.9559), confirming its significant anti-aging potential. Among the extracts, the methanol extract exhibited the highest elastase inhibitory activity, with a percentage inhibition of 97.53% and an IC_{50} value of 13.89, closely approaching the efficacy of oleanolic acid (**Table 2**).

Table 2. Elastase inhibition activities of *Cassia siamea* flower extracts

Sample	Regression equation	Inhibition (%) at 50 µg/mL extract	IC_{50} (µg/mL)
Oleanolic acid	y=1.032x + 44.03	95.59	5.785
Methanol extract	y=1.1457x + 34.08	97.53	13.895
Methanol partition	y=1.194x + 23.383	94.94	22.292
Ethyl acetate extract	y=1.0377x + 24.129	96.69	24.931
n-Hexane extract	y=1.1343x + 6.2189	95.67	38.597

IC_{50} was estimated through linear regression with logit transformation

Hasballah et al. Narra X 2025; 3 (3): e236 - http://doi.org/10.52225/narrax.v3i3.236

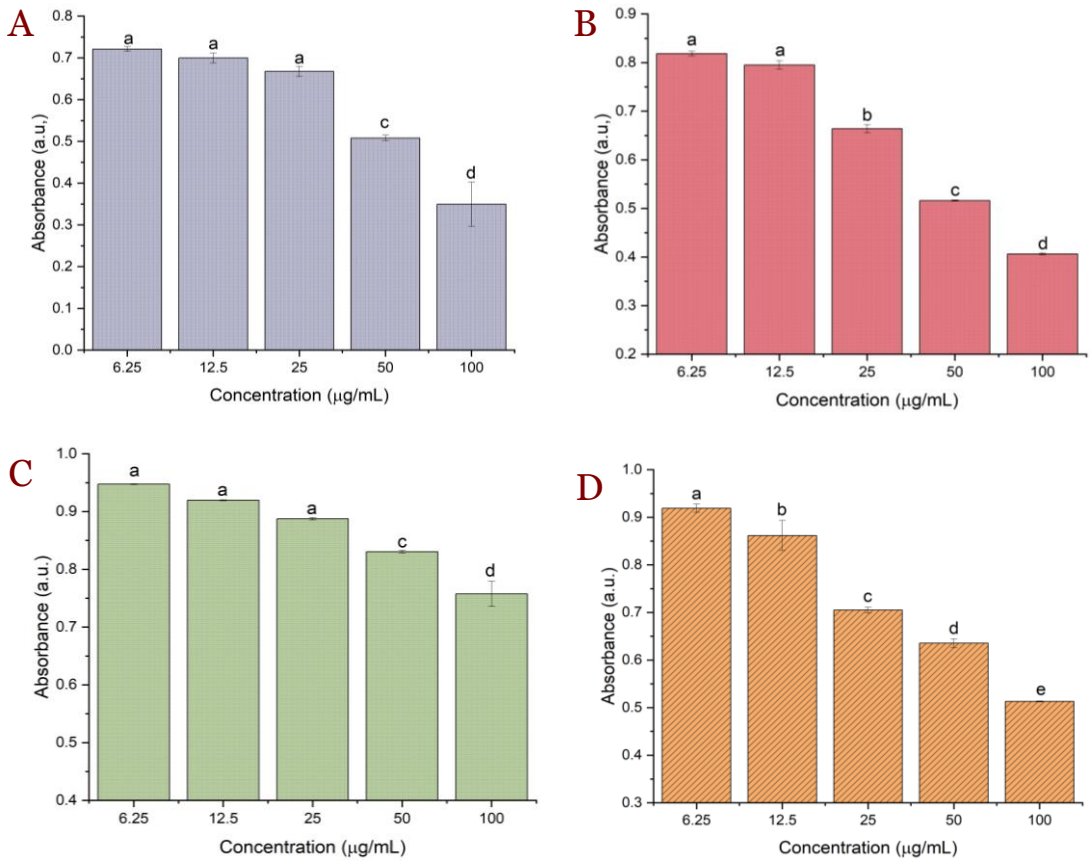

Figure 3. Antioxidant activity of *Cassia siamea* flower extracts assessed using 1,1-diphenyl-2-picryhydrazyl (DPPH) assay. DPPH scavenging activities of *C. siamea* as observed in its methanol extract (A), methanol partition (B), n-hexane (C), and ethyl acetate (D). Different letter annotations indicate statistically significant differences at $p<0.01$, as determined by Tukey's post-hoc test.

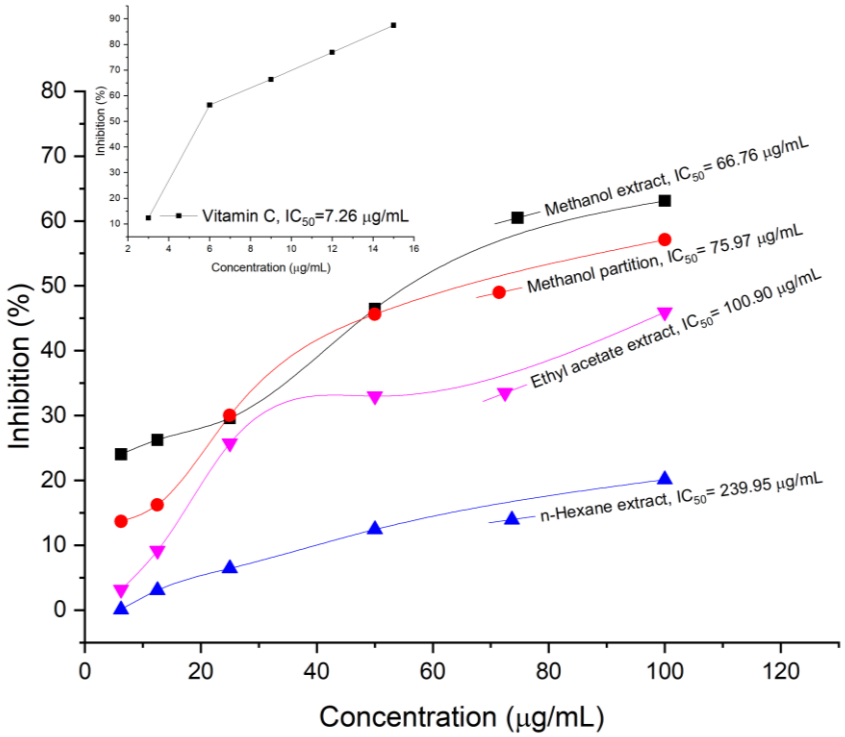

Figure 4. DPPH inhibition by *Cassia siamea* flower extracts as observed in its methanol extract, methanol partition, n-hexane, and ethyl acetate. Vitamin C served as the positive control, and the IC_{50} value was estimated through linear regression with logit transformation.

Phytocompounds identified by GC-MS

GC-MS analysis of *C. siamea* flower extracts revealed the presence of various secondary metabolites across different solvent fractions. The gas chromatogram results, including the detailed list of the identified compounds, are presented in **Table 3–6**. The methanol extract produced 36 compound peaks, including five secondary metabolites from the triterpenoid and steroid groups, such as 4-hydroxy-4a,8-dimethyl-3-methylene3,3a,4,4a,7a,8,9,9a-octahydroazuleno[6,5-b]furan-, Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, squalene, stigmasta-3,5-diene, and 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol (3ß,5Z,7E) (**Table 3**). The methanol partition extract showed four peaks, with the compound 3-O-methyl-d-glucose—identified as a flavonoid glycoside with diverse biological activities—being the most abundant at 61.37% (**Table 4**).

The ethyl acetate extract showed 17 peaks, containing six secondary metabolites from flavonoid, triterpenoid, and steroid groups, including 1H-2-benzopyran-1-one, 3,4-dihydro-3,8-dihydroxy-3-methyl-, (-)-, β-sitosterol, stigmasterol, ß-amyrin, and lupeol (**Table 5**). Similarly, the n-hexane extract exhibited 63 peaks, with notable secondary metabolites from the terpenoid and steroid groups, such as β-sitosterol, stigmasterol, ß-amyrin, and lupeol (**Table 6**). It should be noted that several detected peaks corresponded to siloxanes and phthalate derivatives, which are widely recognized as analytical artifacts or laboratory contaminants in GC–MS analyses. These compounds were therefore not considered genuine plant metabolites and were excluded from subsequent biological interpretation.

Table 3. Phytocompounds of *Cassia siamea* flower methanol extract identified by GC-MS and compared to the reference compound library

#Peak	Compound	Retention time (minute)	Area (%)	Similarity (%)
1	Acetone, 1-[4- (dimethylaminoethoxy)phenyl]	22.65	0.84	83,08
2	1-Dodecanamine, N,N-dimethyl-	23.08	2.95	97.43
3	Dodecanoic acid	24.21	1.75	94.97
4	Cyclooctasiloxane, hexadecamethyl-	26.32	0.97	94.29
5	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)- one, 9-[[[2- (dimethylamino)ethyl]amino]methyl]octahy dro-2,5a-dimethyl	27.55	1.69	95.36
6	Tetradecanoic acid	28.70	6.02	98.81
7	4-Hydroxy-4a,8-dimethyl-3-methylene3,3a,4,4a,7a,8,9,9a-octahydroazuleno[6,5-b]furan-2,5-dione	29.21	1.13	96.45
8	Cyclononasiloxane, octadecamethyl-	29.72	1.11	96.02
9	Phthalic acid, butyl tetradecyl ester	30.58	1.90	90.43
10	Hexadecanoic acid, methyl ester	31.68	2.78	98.59
11	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	32.97	24.85	99.77
12	l-(+)-Ascorbic acid 2,6-dihexadecanoate	33.58	0.55	96.67
13	l-(+)-Ascorbic acid 2,6-dihexadecanoate	35.06	3.81	99.78
14	9-Octadecenoic acid (Z)-, methyl ester	35.49	4.56	99.67
15	Methyl stearate	36.00	8.26	98.55
16	ß-Amyrin	36.37	1.89	96.57
17	trans-13-Octadecenoic acid	36.64	4.31	89.50
18	Octadecanoic acid	36.64	1.14	99.25
19	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	37.139	1.14	99.25
20	1H-2,8aMethanocyclopenta[a]cyclopropa[e]cyclodec en-11-one, 1a,2,5,5a,6,9,10,10a-octahydro5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)- 1,7,9-trimethyl-, [1S- (1a,1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	37.517	1.27	100.00
21	Pentacosane	38.442	2.38	94.78
22	Eicosanoic acid, methyl ester	38.932	0.84	92.79
23	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	39.289	1.23	97.56
24	Pentacosane	40.078	5.31	91.51
25	Pentacosane	41.639	2.09	94.48
26	Bis(2-ethylhexyl) phthalate	42.462	4.68	99.23
27	Tritetracontane	43.152	1.70	91.92
28	Octadecane,3-ethyl-5-(2-ethylbutyl)-	44.605	1.23	99.50
29	Z-5-Methyl-6-heneicosen-11-one	45.077	0.61	98.18
30	7-Methyl-Z-tetradecen-1-ol acetate	46.006	0.60	94.62
31	Squalene	46.448	2.17	96.51

#Peak	Compound	Retention time (minute)	Area (%)	Similarity (%)
32	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	47.366	0.70	99.49
33	7-Methyl-Z-tetradecen-1-ol acetate	47.795	0.56	96.51
34	E-8-Methyl-9-tetradecen-1-ol acetate	49.689	0.01	93.38
35	Stigmasta-3,5-diene	50.046	0.91	97.38
36	9,10-Secocholesta-5,7,10(19)-triene-3,24,25- triol, (3ß,5Z,7E)-	53.080	1.52	95.83

Table 4. Phytocompounds of *Cassia siamea* methanol partition identified by GC-MS and compared to the reference compound library

#Peak	Compound	Retention time (minute)	Area (%)	Similarity (%)
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy6-methyl	13.25	16.10	80.13
2	3-O-Methyl-d-glucose	31.17	32.37	98.20
3	3-O-Methyl-d-glucose	31.24	46.12	98.30
4	Estra-1,3,5(10)-trien-17ß-ol	32.49	5.41	96.79

Table 5. Phytocompounds of *Cassia siamea* flower ethyl acetate extract identified by GC-MS and compared to the reference compound library

#Peak	Compound	Retention time (minute)	Area (%)	Similarity (%)
1	3-O-Methyl-d-glucose	24.52	6.70	97.16
2	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	25.93	1.18	86.64
3	1H-2-Benzopyran-1-one, 3,4-dihydro-3,8-dihydroxy-3-methyl-, (-)-	26.20	9.50	88.50
4	Estra-1,3,5(10)-trien-17ß-ol	26.61	3.82	98.11
5	n-Hexadecanoic acid	26.81	22.84	96.13
6	2,4,4,6,6,8,8-Heptamethyl-2-nonene	27.08	1.36	82.36
7	Methyl 9-cis,11-trans-octadecadienoate	28.97	1.77	98.98
8	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl] -, methyl ester	29.08	1.17	99.63
9	(Z)-18-Octadec-9-enolide	29.82	13.90	99.77
10	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	29.92	11.75	95.85
11	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	30.31	2.94	98.62
12	9-Acetoxynonanal	32.85	1.11	83.80
13	Diisooctyl phthalate	36.33	1.43	97.35
14	Stigmasterol	45.34	3.56	93.52
15	β-Sitosterol	46.03	7.46	95.81
16	ß-Amyrin	46.25	3.66	92.30
17	Lupeol	46.82	5.85	87.68

Table 6. Phytocompounds of *Cassia siamea* flower n-hexane extract identified by GC-MS and compared to the reference compound library

#Peak	Compound	Retention time (minute)	Area (%)	Similarity (%)
1	Benzene, 1-ethyl-3-methyl-	4.40	0.23	95.74
2	Decane, 2,6,8-trimethyl-	6.88	0.21	93.74
3	Benzene, 1,2,4,5-tetramethyl-	7.29	0.49	96.62
4	Benzene, 1,2,4,5-tetramethyl-	8.06	0.19	90.86
5	1-Hexadecanol	14.28	0.45	95.29
6	1-Hexadecanol	19.00	1.03	95.96
7	Estra-1,3,5(10)-trien-17ß-ol	22.39	0.25	97.39
8	Tetradecanoic acid	23.08	0.35	95.55
9	1-Heneicosyl formate	23.27	0.63	96.53
10	Z-8-Methyl-9-tetradecenoic acid	24.70	0.23	98.19
11	Pentadecanoic acid	25.05	0.29	95.47
12	Hexadecanoic acid, methyl ester	26.02	3.54	99.47
13	Benzenepropanoic acid, 3,5-bis(1,1- dimethylethyl)-4-hydroxy-, methyl ester	26.27	1.51	96.24
14	n-Hexadecanoic acid	26.66	1.80	76.24
15	n-Hexadecanoic acid	26.97	3.22	94.45
16	n-Hexadecanoic acid	27.28	8.87	97.84
17	l-(+)-Ascorbic acid 2,6-dihexadecanoate	27.50	6.66	98.97

#Peak	Compound	Retention time (minute)	Area (%)	Similarity (%)
18	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	28.77	0.43	99.76
19	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	29.08	5.47	98.49
20	Phytol	29.38	0.54	99.68
21	Methyl stearate	29.66	1.38	95.59
22	9(E),11(E)-Conjugated linoleic acid	30.41	18.78	98.24
23	Octadecanoic acid	30.76	3.03	98.77
24	Tetradecane, 2,6,10-trimethyl-	32.50	1.26	99.33
26	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	33.75	0.48	95.09
27	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	33.30	0.49	96.31
28	Eicosanoic acid	33.75	0.88	94.99
29	Pentacosane	34.11	1.13	97.57
30	Z-5-Methyl-6-heneicosen-11-one	34.45	0.17	93.75
31	7-Methyl-Z-tetradecen-1-ol acetate	35.22	0.22	95.27
32	Pentacosane	35.66	1.02	99.10
33	Z-5-Methyl-6-heneicosen-11-one	36.83	0.22	96.48
34	7-Methyl-Z-tetradecen-1-ol acetate	36.16	0.26	97.02
35	Bis(2-ethylhexyl) phthalate	34.43	2.01	97.16
36	Docosanoic acid	38.78	0.54	93.98
37	Pentacosane	37.14	1.21	98.31
38	17-Pentatriacontene	38.62	0.27	90.32
39	17-Pentatriacontene	38.17	0.23	96.96
40	Tetratriacontane	38.58	1.33	95.09
41	17-Pentatriacontene	39.05	0.28	97.68
42	Tetrapentacontane, 1,54-dibromo-	39.56	0.31	97.74
43	Tetratriacontane	39.95	1.32	96.32
44	2,2,4-Trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexa	40.30	0.49	95.32
45	Tetrapentacontane, 1,54-dibromo-	40.94	0.40	97.73
46	Nonyl tetracosyl ether	41.31	2.40	97.15
47	17-Pentatriacontene	41.70	0.20	96.11
48	Tritetracontane	42.58	0.93	96.46
49	1-Heptatriacotanol	43.55	0.44	97.36
50	17-Pentatriacontene	43.91	1.24	98.16
51	dl-a-Tocopherol	44.16	0.24	97.49
52	Campesterol	45.01	1.69	98.63
53	Stigmasterol	45.28	3.15	93.15
54	β-Sitosterol	46.20	5.60	98.48
55	ß-Amyrin	46.37	2.33	99.56
56	Lup-20(29)-en-3-one	46.66	1.15	99.12
57	Lupeol	46.95	4.41	96.78
58	Cholest-22-ene-21-ol, 3,5-dehydro-6- methoxy-, pivalate	47.39	0.26	98.44
59	β-Sitostenone	47.61	0.80	99.55
60	Friedelan-3-one	48.32	0.35	94.62
61	Phytyl heptadecanoate	49.13	0.32	88.91
62	Phytyl linoleate	51.08	0.18	98.14
63	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	51.28	0.20	88.52

Discussion

This present study found that the ethyl acetate extract of *C. siamea* flower exhibited the highest antibacterial activity among the tested fractions, which may be attributed to its enrichment in alkaloids, flavonoids, terpenoids, steroids, and phenolic compounds. The larger inhibition zones observed against *S. aureus* compared with *E. coli* are consistent with well-established differences in bacterial cell wall architecture. Gram-negative bacteria possess an additional outer membrane composed of lipopolysaccharides and lipoproteins, which restricts the penetration of many bioactive compounds, whereas Gram-positive bacteria are characterized by a thicker but more permeable peptidoglycan layer [28,29]. Although the observed inhibition zones were modest, these findings indicate a selective antibacterial effect that is more pronounced against Gram-positive bacteria.

In the antioxidant assay, the methanol extract and methanol partition extract exhibited IC_{50} values of 66.76 µg/mL and 75.97 µg/mL, respectively, indicating moderate radical scavenging activity when compared with the positive control, ascorbic acid. The stronger antioxidant potency of ascorbic acid is expected and has been consistently reported in previous studies [32-34]. Nevertheless, the extracts demonstrated a clear dose-dependent increase in DPPH inhibition, in agreement with earlier reports on *C. siamea* and other phenolic-rich plant extracts [35,36]. The DPPH assay, which evaluates the ability of compounds to donate electrons or hydrogen atoms to neutralize stable free radicals, remains a widely accepted screening method for antioxidant capacity [30,31]. Thus, the results support the presence of redox-active constituents in *C. siamea* flower extracts, particularly in the methanolic fractions.

The anti-aging potential of the extracts was further evaluated using an elastase inhibition assay. The methanol extract showed the strongest elastase inhibitory activity, with a high percentage inhibition and an IC_{50} value approaching that of the positive control, oleanolic acid. In contrast, the ethyl acetate and methanol partition extracts displayed moderate activity, while the n-hexane extract showed the weakest inhibition. Elastase is a serine protease responsible for the degradation of elastin fibers in the extracellular matrix, a process closely associated with skin aging, wrinkle formation, and loss of elasticity [37]. The observed inhibition suggests that polar to semi-polar constituents in the methanol-based extracts may interact with the elastase active site or its surrounding regions, thereby reducing enzymatic activity. Previous studies have shown that flavonoids and triterpenoids can inhibit elastase through hydrogen bonding and hydrophobic interactions with key catalytic residues [38,39,40].

The bioactivity patterns observed in the antibacterial, antioxidant, and elastase inhibition assays are supported by the GC–MS profiling results. After exclusion of likely analytical contaminants, the extracts were found to contain flavonoids, triterpenoids, and phytosterols, including β-sitosterol, stigmasterol, β-amyrin, and lupeol, which have been previously reported in *C. siamea* extracts [5,41,42]. These compounds are frequently associated with antioxidant and enzyme-inhibitory activities. Phytosterols, such as β-sitosterol and stigmasterol, have been reported to contribute to redox homeostasis and cellular protection against oxidative stress, while triterpenoids, including β-amyrin and lupeol, have been linked to elastase inhibition and preservation of extracellular matrix integrity [43-47].

Overall, the results of the present study indicate that *C. siamea* flower extracts possess promising in-vitro bioactivities, providing valuable comparative information across solvent fractions and demonstrating a consistent pattern linking antibacterial, antioxidant, and elastase-inhibitory activities with phytochemical profiles. The strength of this study lies in its integrated screening approach, combining multiple bioassays with GC–MS-based phytochemical profiling to identify fractions with the highest biological relevance. However, these findings cannot be directly translated to therapeutic or anti-aging efficacy, as the study was limited to in-vitro assays, relied on zone-of-inhibition and enzyme inhibition endpoints, and employed tentative compound identification without structural confirmation or bioavailability assessment. In addition, minimum inhibitory concentrations, cytotoxicity, and in vivo relevance were not evaluated. Therefore, future studies should focus on bioactivity-guided fractionation, isolation, and structural confirmation of active compounds, mechanistic validation using cellular or molecular models, and safety evaluation, to substantiate the functional roles of *C. siamea* flower constituents and support their potential development in pharmaceutical or cosmetic applications.

Conclusions

The extraction of *C. siamea* flowers yielded four fractions, with the ethyl acetate extract showing the strongest antibacterial activity and the methanol extract demonstrating the highest antioxidant and elastase inhibitory potential. These findings suggest the potential of *C. siamea* flower extracts as effective antibacterial, antioxidant, and anti-aging agents, with promising applications in therapeutic development.

Ethics approval

Not required.

Acknowledgments

The authors acknowledge the support of Universitas Syiah Kuala and the Ministry of Education, Culture, Research, and Technology of Indonesia.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study is funded by the Universitas Syiah Kuala and the Ministry of Education, Culture, Research, and Technology of Indonesia through the Professor Research Grant Scheme with contract no. 551/UN11.2.1/PG.01.03/SPK/PTNBH/2024, as of June 6, 2024.

Underlying data

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

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

How to cite

Hasballah K, Murniana M, Diah M, *et al*. Antibacterial, antioxidant, and $SucAla_3$-based anti-aging activities of Johar flower extract (*Cassia siamea* Lamk.). Narra X 2025; 3 (3): e236 - http://doi.org/10.52225/narrax.v3i3.236.

References

1. Harahap D, Niaci S, Mardina V, *et al.* Antibacterial activities of seven ethnomedicinal plants from family Annonaceae. J Adv Pharm Technol Res 2022;13(3):148-153.
2. Rahman FI, Zulfa PO, Beočanin A, *et al.* Targeting phosphoglycerate dehydrogenase enzyme using ginger compounds to suppress thyroid cancer progression. Narra X 2024;2(1):e112.
3. Adedokun O, Akinlo M, Adedeji I, *et al.* Unfolding the cytotoxic potential of *Cassia siamea* L (Fabaceae) stem via a combination of cost-effective anticancer screening templates. Trop J Nat Prod Res 2024;8(1).
4. Ezeabara C, Umeka L, Anyanele W. Phytochemical and proximate studies of leaf, stem and root of *Cassia mimosoides* L. Int J Sci Res Multidiscip Stud 2023;9(8).
5. Kumar D, Jain A, Verma A. Phytochemical and pharmacological investigation of *Cassia siamea* Lamk: An insight. Nat Prod J 2017;7(4):255-266.
6. Tasiam E, Primaharinastiti R, Ekasari W. In vitro antimalarial activity and toxicity studies of *Cassia siamea* leaves from three different locations. Afr J Infect Dis 2020;14(2):23-29.
7. Zibaee E, Javadi B, Sobhani Z, *et al. Cassia* species: A review of traditional uses, phytochemistry and pharmacology. Pharmacol Res Mod Chin Med 2023;100325.
8. Khurm M, Wang X, Zhang H, *et al.* The genus *Cassia* L: Ethnopharmacological and phytochemical overview. Phytother Res 2021;35(5):2336-2385.
9. Abdellatif NA, Abdelhameed RF, Eltamany EE, *et al.* Review on phytochemical constituents of plants of genus *Cassia*. Rec Pharm Biomed Sci 2023;7(2):93-110.
10. Ogunniran AO, Dauda OS, Rotimi D, *et al.* Nutritional, phytochemical, and antimicrobial properties of *Senna siamea* leaves. Toxicol Rep 2024;13:101793.
11. Musa A, Bawa HW, Mohammed AH, *et al.* Green synthesis of silver nanoparticles and its antibacterial activity using flower extract of *Senna siamea*. Int J Nanosci Nanotechnol 2021;17(3):173-179.
12. Gideon MM, James T, Mohammed U, *et al.* Flower extract mediated green synthesis of silver nanoparticles from *Senna siamea* plant. Gombe State Polytech J Sci Technol 2024;1(1):53-57.

13. Usman N, Adeshina G, Tytler B, *et al.* Biofilm-inhibition activities of *Senna siamea* leaf fractions against *Escherichia coli*. Afr J Microbiol Res 2020;14(2):77-84.
14. Ganapathy AA, Priya VH, Kumaran A. Medicinal plants as a potential source of phosphodiesterase-5 inhibitors. J Ethnopharmacol 2021;267:113536.
15. Adewole KE. Nigerian antimalarial plants and their anticancer potential. J Integr Med 2020;18(2):92-113.
16. Forman HJ, Zhang H. Targeting oxidative stress in disease. Nat Rev Drug Discov 2021;20(9):689-709.
17. Santos DF, Simão S, Nóbrega C, *et al.* Oxidative stress and aging: Synergies for age-related diseases. FEBS Lett 2024;598(17):2074-2091.
18. Wongchum N, Dechakhamphu A. Ethanol extract of *Cassia siamea* increases lifespan in *Drosophila melanogaster*. Biochem Biophys Rep 2021;25:100925.
19. Zheng YZ, Deng G, Zhang YC. Multiple free radical scavenging reactions of flavonoids. Dyes Pigments 2022;198:109877.
20. Yusuf H, Novia H, Fahriani M. Cytotoxic activity of *Chromolaena odorata* extract on MCF7 and T47D cells. Narra J 2023;3(3):e326.
21. Chen I, Chen M, Chung T. Analysis of antioxidant property of the extract of saponin by experiment design methodology. IOP Conf Ser Earth Environ Sci 2020;594(1):012002.
22. Coulibaly AC, Kabré W, Traoré MN, *et al.* Phenolic content, antioxidant activity, 15-lipoxygenase and lipid peroxidase inhibitory effects of two medicinal plants from Burkina Faso: *Acacia macrostachya* Reich ex Benth (Mimosaceae) and *Lepidagathis anobrya* Nees (Acanthaceae). Int J Biochem Res Rev 2020;29(8):18-25.
23. Quranayati Q, Iqhrammullah M, Saidi N, *et al.* Extracts from *Phyllanthus emblica* L stem barks ameliorate blood glucose level and pancreatic and hepatic injuries in streptozotocin-induced diabetic rats. Arab J Chem 2023;16(9):105082.
24. Indriaty I, Ginting B, Hasballah K, Djufri D. A comparative study of total tannin contents and antimicrobial activities in methanol extracts of Rhizophoraceae species. Heca J Appl Sci 2023;1(2):62-70.
25. 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):e321.
26. Quranayati Q, Iqhrammullah M, Saidi N, *et al.* Cytotoxicity and phytochemical profiles of *Phyllanthus emblica* stem barks with in silico drug-likeliness: Focusing on antidiabetic potentials. J Adv Pharm Technol Res 2022;13(4):281-285.
27. Cane H, Musman M, Yahya M, *et al.* Antibacterial plants from Gayo Lues Highland. J Pharm Pharmacogn Res 2023;11(1):117-128.
28. Onuigbo MC, Ukegbu CY, Uzoigwe KC. Antibacterial activity of *Chrysophyllum albidium* seed oil extract on pathogenic *Staphylococcus aureus*. Narra X 2023;1(1):e77.
29. Tavares TD, Antunes JC, Padrão J, *et al.* Activity of specialized biomolecules against gram-positive and gram-negative bacteria. Antibiotics 2020;9(6):314.
30. Yang J, Chen J, Hao Y, Liu Y. Identification of the DPPH radical scavenging reaction adducts of ferulic acid and sinapic acid and their structure-antioxidant activity relationship. LWT-Food Sci Technol 2021;146:111411.
31. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. Processes 2023;11(8):2248.
32. Kasali FM, Ali MS, Tusiimire J, *et al.* Phytochemical constituents found in *Physalis peruviana* L leaf extracts and their ability to inhibit alpha-glucosidase and scavenge DPPH free radicals in vitro. Trends Phytochem Res 2022;6(1):3-10.
33. Himawan HC, Isa AF, Wiharja DS. Antioxidant activity of 70% ethanol extract combination of kemangi leaf (*Ocimum Americanum* Linn) and binahong leaf (*Anredera cordifolia* (Ten) Steenis) using DPPH. J Phys Conf Ser 2021;1764(1):012009.
34. Ihsanpuro SI, Gunawan S, Ibrahim R, Aparamarta HW. Extract with high 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibitory capability from pericarp and seed of mangosteen (*Garcinia mangostana* L) using microwave-assisted extraction (MAE) two-phase solvent technique. Arab J Chem 2022;15(12):104310.
35. Chang Y, Zheng C, Chinnathambi A, *et al.* Cytotoxicity, anti-acute leukemia, and antioxidant properties of gold nanoparticles green-synthesized using *Cannabis sativa* L leaf aqueous extract. Arab J Chem 2021;14(4):103060.
36. Arab Y, Sahin B, Ceylan O, *et al.* Assessment of in vitro activities and chemical profiling of *Senecio hoggariensis* growing in Algerian Sahara. Biodiversitas 2022;23(7).
37. Donarska B, Łączkowski KZ. Recent advances in the development of elastase inhibitors. Future Med Chem 2020;12(20):1809-1813.
38. Jiratchayamaethasakul C, Ding Y, Hwang O, *et al.* In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. Fish Aquat Sci 2020;23:1-9.

39. Deniz FSS, Orhan IE, Duman H. Profiling cosmeceutical effects of various herbal extracts through elastase, collagenase, tyrosinase inhibitory and antioxidant assays. Phytochem Lett 2021;45:171-183.
40. Nur S, Setiawan H, Hanafi M, Elya B. Phytochemical composition, antioxidant, in vitro and in silico studies of active compounds of *Curculigo latifolia* extracts as promising elastase inhibitor. Saudi J Biol Sci 2023;30(8):103716.
41. Veerachari U, Bopaiah A. Preliminary phytochemical evaluation of the leaf extract of five *Cassia* species. J Chem Pharm Res 2011;3(5):574-583.
42. Daskum A, Chessed G, Qadeer M, Ling L. Phytochemical screening, gas chromatography mass spectroscopy and in vitro antiplasmodial analysis of *Senna siamea* leaves as antimalarial, Yobe State, Nigeria. Niger J Parasitol 2020;41(1).
43. Bakrim S, Benkhaira N, Bourais I, *et al.* Health benefits and pharmacological properties of stigmasterol. Antioxidants 2022;11(10):1912.
44. Aboobucker SI, Suza WP. Why do plants convert sitosterol to stigmasterol? Front Plant Sci 2019;10:354.
45. Sultana N, Saeed Saify Z. Naturally occurring and synthetic agents as potential anti-inflammatory and immunomodulants. Antiinflamm Antiallergy Agents Med Chem 2012;11(1):3-19.
46. Maity N, Nema NK, Abedy MK, *et al.* Exploring *Tagetes erecta* Linn flower for the elastase, hyaluronidase and MMP-1 inhibitory activity. J Ethnopharmacol 2011;137(3):1300-1305.
47. Manjia JN, Njoya EM, Harishchander A, *et al.* Anti-elastase, anti-tyrosinase, and anti-inflammatory activities of three compounds isolated from *Psorospermum aurantiacum*: In silico and in vitro assays. Rev Bras Farmacogn 2024;1-13.