TRENDS IN SCIENCES 2025; 22(11): 10833
https://doi.org/10.48048/tis.2025.10833 RESEARCH ARTICLE

Potential of *Syzygium cumini* L. Seed as Cosmetic Bioactive Compounds: Matrix Metalloproteinases Inhibition, Antioxidant Activities, Cytotoxicity, and Safety Assessment

Jirasit Inthorn[1], Saranya Sornmanee[1] and Wannisa Keawbankrud[2,*]

[1]Health and Cosmetic Product Development Program, Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand
[2]Health Science and Aesthetic Program, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand

([*]Corresponding author's e-mail: Wannisa.k@mail.rmutk.ac.th)

Received: 3 June 2025, Revised: 15 June 2025, Accepted: 30 June 2025, Published: 5 August 2025

Abstract

This study systematically evaluated the extraction efficiency, phytochemical content, antioxidant activity, matrix metalloproteinase inhibition, cytotoxicity, stability, and safety of *Syzygium cumini* L. seed extracts using various solvents. Extraction yields were highest with 50 %v/v ethanol (19.19% ± 0.44%), while the 70 %v/v ethanol extract exhibited the greatest total phenolic (488.51 ± 3.60 mg GAE/g) and flavonoid (88.45 ± 1.61 mg QE/g) contents. Antioxidant assays revealed that the water extract (SC100W) had the strongest DPPH and ABTS radical scavenging activities. In contrast, the 70 %v/v ethanol extract (SC70ET) demonstrated superior ferric reducing antioxidant power, correlating with higher phenolic content. Matrix metalloproteinase inhibition assays identified the 50 %v/v ethanol extract (SC50ET) as the most effective, showing the lowest IC_{50} for strong collagenase, elastase, and tyrosinase inhibition. All *Syzygium cumini* L. seed extracts showed over 90% cell viability within a concentration range of 1 - 250 µg/mL, demonstrating that it is safe for skincare application. The active ingredients and efficacy have not significantly decreased, even when stored at high temperatures under accelerated conditions. Microbiological and heavy metal analyses confirmed the extract's safety for use as an active ingredient in cosmetic products. Collectively, these results highlight the influence of extraction solvent on bioactivity and identify optimal conditions for maximizing cosmeceutical properties of *Syzygium cumini* L. seed extracts.

Keywords: *Syzygium cumini* L., Antioxidant activity, Matrix metalloproteinase inhibition, Cytotoxicity

Introduction

Java plum (*Syzygium cumini* L.), commonly known as Jamun, is a tropical evergreen tree belonging to the family Myrtaceae. Native to South and Southeast Asia, including India, Myanmar, Malaysia, Indonesia, and the Philippines, it has spread widely across the tropics and subtropics, including Thailand, where it is cultivated both edible and medicinal uses [1]. In Thailand, *S. cumini* is found in various regions and is valued for its resilience in diverse environmental conditions. The fruit and seeds are rich in nutrients such as carbohydrates, proteins, vitamins, minerals (notably iron), and bioactive phytochemicals including anthocyanins, flavonoids (quercetin, myricetin, kaempferol), phenolic acids (gallic, caffeic, ellagic acids), and tannins. These compounds contribute to its nutritional value and are involved in various metabolic processes, notably its anti-diabetic and antioxidant activities [2,3].

Extensive research has highlighted the potent antioxidant properties of *Syzygium cumini* L., attributed to its high content of phenolic compounds, anthocyanins, and flavonoids [4,5]. These antioxidants

effectively scavenge free radicals, reduce oxidative stress, and may play a role in anti-aging by inhibiting matrix metalloproteinases (MMPs) such as MMP-1, which are involved in skin aging and tissue remodeling [6]. Studies on leaf and seed extracts have demonstrated significant free radical-scavenging activity, with methanolic seed extracts showing particularly high antioxidant capacity, sometimes surpassing standard antioxidants like ascorbic acid [4,7]. Toxicological evaluations indicate that *S. cumini* extracts generally exhibit low cytotoxicity and negligible acute toxicity in *in vitro* and *in vivo* models, supporting their safety for further pharmaceutical and cosmeceutical applications [8].

Given the abundance of bioactive compounds in *Syzygium cumini* L., researchers are increasingly interested in utilizing these seeds, often a by-product of juice production, for the extraction of cosmetic bioactive compounds. Consequently, water and ethanol, both safe and widely accepted in the cosmetic industry, were commonly chosen as solvents for basic plant extraction. In this context, seeds sourced from the Wabellas community enterprise in Wang Wa sub-district, Taphan Hin district, Phichit province, Thailand, were subjected to maceration extraction. The resulting crude extracts were investigated for their potential in cosmetic applications, focusing on activities such as matrix metalloproteinase inhibition, antioxidant effects, fibroblast cytotoxicity, thermal stability, and safety assessments. This approach not only adds value to agricultural by-products but also supports the development of natural, multifunctional ingredients for the cosmetic industry.

Plant materials

The study utilized seeds of *Syzygium cumini* L., sourced as a by-product from a local juice factory. These materials were generously provided by the Wabellas community enterprise, located in Wang Wa sub-district, Taphan Hin district, Phichit province, Thailand.

Chemicals

Folin-Ciocalteu reagent, gallic acid, quercetin, kojic acid, L-ascorbic acid, epigallocatechin gallate (EGCG), 3,4-dihydroxyphenylalanine (L-DOPA), tyrosinase from mushroom (E.C. 1.14.18.1, Sigma no. T3824), collagenase from *Clostridium histolyticum* (E.C. 3.4.24.3, Sigma no. C0130), elastase from porcine pancreas (E.C. 3.4.21.36, Sigma no. E1250), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ), N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (AAAPVN) were purchased from Merck KGaA (Darmstadt, Germany). Ethanol was purchased from RCI Labscan Ltd. (Bangkok, Thailand). Sodium carbonate, aluminum chloride, and sodium acetate were purchased from KemAus (Cloisters Cherrybrook, Australia). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was acquired from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Crystal violet was purchased from Riedel-de Haen (Munich, Germany).

Methods

Preparation of Syzygium cumini L. seed extract

Syzygium cumini L. seeds were first oven-dried at 60 °C for 48 h to remove moisture, then ground into a fine powder (60-mesh) using a commercial grinder (Powder Grinder PG500, Spring Green Evolution Co. Ltd., Bangkok, Thailand). In brief, the powdered material was subjected to maceration in distilled water (SC100W), 30 %v/v ethanol (SC30ET), 50 %v/v ethanol (SC50ET), and 70 %v/v ethanol (SC70ET), with continuous agitation at 150 rpm for 24 h on an orbital shaker (Stuart SSL1, Fisher Scientific, Leicestershire, England). After that, the mixture was filtered through Whatman No. 1 filter paper, and the liquid extract was concentrated under reduced pressure using a rotary evaporator (BUCHI Rotavapor R-300, Fisher Scientific, Leicestershire, England) to eliminate ethanol. The resulting crude extract was weighed to determine the extraction yield and stored at −20 °C for further phytochemical analysis [9].

Total phenolic content (TPC)

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method. Briefly, the sample solutions were aliquoted (20 µL) into a 96-well plate, followed by the addition of 100 µL of 0.25 N Folin-Ciocalteu reagent and 80 µL of 7.5 %w/v sodium carbonate solution. Then, the mixture was incubated in

the dark at ambient temperature for 2 h, and absorbance was measured at 765 nm using a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany). Gallic acid was used as a standard (5 - 100 µg/mL) to generate a calibration curve, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) [10]. The total phenolic content was calculated according to the following equation:

$$\text{TPC (mg GAE/g extract)} = (C \times V)/m, \quad (1)$$

where C is the concentration of gallic acid obtained from the standard curve (mg/mL), V is the volume of extract used in the assay (mL), and m is the weight of the extract used (g).

Total flavonoid content (TFC)

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric assay [11]. Briefly, the sample solutions were aliquoted (30 µL) into a 96-well plate. Subsequently, 50 µL of 5 %w/v sodium nitrite and 35 µL of 10 %w/v aluminum chloride were added to each well. After that, the mixtures were incubated in the dark at ambient temperature for 5 min, and 85 µL of 1 N sodium hydroxide was added. The mixtures were incubated in the dark at ambient temperature for 6 min, after which absorbance was measured at 520 nm using a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany). Quercetin was used as a standard (5 - 100 µg/mL) to generate a calibration curve, and results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract) [12]. The total flavonoid content was calculated according to the following equation:

$$\text{TFC (mg QE/g extract)} = (C \times V)/m \quad (2)$$

where C is the concentration of quercetin obtained from the standard curve (mg/mL), V is the volume of extract used in the assay (mL), and m is the weight of the extract used (g).

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity was evaluated using the DPPH radical scavenging assay. Briefly, 20 µL of each sample was pipetted into a 96-well plate. Subsequently, 180 µL of 0.2 mM DPPH solution was added. After that, the mixture was incubated in the dark at ambient temperature for 30 min, and the absorbance was measured at 520 nm using a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany) [13]. The percentage of DPPH radical scavenging was calculated according to the following equation:

$$\text{DPPH radical scavenging (\%)} = [(A - B)/A] \times 100 \quad (3)$$

where A refers to the absorbance of the reaction without the extracts, and B refers to the absorbance of the reaction with the extracts. Ascorbic acid was used as a standard. The inhibition was expressed as the half-maximal inhibitory concentration (IC_{50}), which was determined using GraphPad Prism Version 10 (GraphPad Software, San Diego, USA).

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The antioxidant activity was evaluated using the ABTS radical scavenging assay. In brief, 7 mM ABTS solution was mixed with 2.45 mM potassium persulfate solution in a 1:1.5 ratio and incubated in the dark for 12 h to generate $ABTS^{\bullet+}$ radicals. The resulting ABTS mixture was diluted with distilled water to achieve an absorbance of 0.70 ± 0.02 at 750 nm. For the assay, 20 µL of extract was added to a 96-well plate, followed by the addition of 180 µL of the prepared ABTS solution. After that, the mixture was incubated at ambient temperature in the dark for 6 min, and the absorbance was measured at 750 nm [14]. Ascorbic acid was used as the standard. The percentage of ABTS radical scavenging was calculated according to the following equation:

$$\text{ABTS radical scavenging (\%)} = [(A - B)/A] \times 100 \quad (4)$$

where A refers to the absorbance of the reaction without the extracts, and B refers to the absorbance of the reaction with the extracts. Antioxidant activity was expressed as the half-maximal inhibitory concentration (IC_{50}), which was determined using GraphPad Prism Version 10 (GraphPad Software, San Diego, USA).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of the extracts was evaluated using the FRAP assay with some modifications from a previous study [15,16]. Briefly, the FRAP reagent was freshly prepared by mixing 300 mM acetate buffer, 10 mM TPTZ solution in 40 mM HCl, and 20 mM ferric chloride solution at a ratio of 10:1:1 (v/v), followed by warming at 37v °C in a water bath. Subsequently, 25 µL of extract was added to 175 µL of FRAP solution in a 96-well microplate. The mixture was incubated at ambient temperature in the dark for 30 min, and the absorbance was measured at 595 nm using a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany). Ascorbic acid (5 - 20 mmol) solutions were used to construct the standard curve. The FRAP values were expressed in millimolar ascorbic acid equivalent per gram of extract (mmol AAE/g extract). The ferric reducing antioxidant power was calculated according to the following equation:

$$\text{FRAP (mM } Fe^{2+} \text{ equivalents)} = (B \times D)/V \quad (5)$$

where B is the amount of Fe^{2+} (mmol) determined from the standard calibration curve, D is the dilution factor, and V is the volume of the sample used in the assay.

Tyrosinase inhibitory determination

The tyrosinase inhibitory assay was conducted by a modified method from a previous study [17]. In brief, the tyrosinase enzyme solution was freshly prepared at a concentration of 500 units/mL in 0.1 M phosphate buffer, pH 6.8. Then, 20 µL of extract solution was mixed with 20 µL of tyrosinase solution, followed by the addition of 100 µL of phosphate buffer (pH 6.8). After that, the mixture was incubated in the dark at ambient temperature for 15 min. Subsequently, 60 µL of 2.5 mM L-DOPA solution in phosphate buffer was added and incubated for another 15 min under the same conditions. The absorbance of the resulting mixture was measured at 475 nm, and the percentage of tyrosinase inhibition was calculated using the following equation:

$$\text{Tyrosinase inhibition (\%)} = [(A - B)/A] \times 100 \quad (6)$$

where A refers to the controlled absorbance of the mixture that consists of PBS, tyrosinase, and L-DOPA, and B refers to the absorbance of the reaction of the mixture that consists of extract, tyrosinase, and L-DOPA.The tyrosinase inhibitory activity of extracts was expressed as the half-maximal inhibitory concentration (IC_{50}), which was calculated by using the program GraphPad Prism Version 10 (GraphPad Software, San Diego, USA).

Collagenase inhibitory determination

The inhibition of collagenase activity was determined by measuring the product from the reaction of collagenase and FLAGPA, using the spectrophotometric method [18]. Briefly, collagenase solution was prepared at a concentration of 5 units/mL in 50 mM tricine buffer pH 7.4. Subsequently, 20 µL of the extract was incubated with 20 µL of collagenase solution for 15 min. After that, 120 µL of 2 mM FLAGPA solution in 50 mM tris-HCl buffer (pH 7.5) was added. The absorbance of the mixture was immediately measured and tracked continuously for 20 min at a wavelength of 340 nm using a multimode detector (CLARIOstar®, BMG Labtech, Offenburg, Germany). The inhibition of collagenase was calculated according to the following equation:

$$\text{Collagenase inhibition (\%)} = [(A - B)/A] \times 100 \quad (7)$$

where A refers to the controlled reaction rate of the mixtures containing collagenase, tricine buffer, and FLAGPA solution, and B refers to the reaction rate of the mixtures containing the sample, collagenase, tricine buffer, and FLAGPA solution. EGCG was used as a positive collagenase inhibitor. The collagenase inhibition activity was expressed as the half-maximal inhibitory concentration (IC_{50}), which was calculated by using the program GraphPad Prism Version 10 (GraphPad Software, San Diego, USA).

Elastase inhibitory determination

The inhibition of elastase activity was determined by measuring the reaction of elastase and AAAVPN, using the spectrophotometric method [19]. Briefly, an elastase solution was prepared at a concentration of 0.042 units/mL in 200 mM Tris-HCl buffer, pH 8.0. After that, 40 µL of extract was incubated with 40 µL of elastase solution for 15 min. Subsequently, 120 µL of 1.33 mM AAAVPN in tris-HCI buffer (pH 8.0) was

added. The absorbance of the mixture was immediately measured and tracked continuously for 20 min at a wavelength of 410 nm using a multimode detector (CLARIOstar®, BMG Labtech, Offenburg, Germany). The inhibition of elastase was calculated by using the following equation:

$$\text{Elastase inhibition (\%)} = [(A - B)/A] \times 100 \quad (8)$$

A refers to the controlled reaction rate of the mixtures containing elastase, tris-HCl buffer, and AAAVPN solution, and B refers to the reaction rate of the mixtures containing the sample, elastase, tris-HCl buffer, and AAAVPN solution. EGCG was used as a positive elastase inhibitor. The elastase inhibition activity was expressed as the half-maximal inhibitory concentration (IC_{50}), which was calculated by using the program GraphPad Prism Version 10 (GraphPad Software, San Diego, USA).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

The cytotoxicity of extracts was evaluated on normal human dermal fibroblast (NHDF) cells using the MTT assay adapted from the method of Ala *et al.* [20]. NHDF cells were trypsinized at 1×10^4 cells/well in 96-well plates and cultured in DMEM-F12 medium supplemented with 10% FBS and 1% penicillin/streptomycin under standard conditions (37 °C, 5% CO_2) for 24 h. After that, the medium was removed, and cells were treated with medium containing extract at concentrations ranging from 1 to 500 µg/mL, alongside a control, which consisted of medium without any extracts, for 24 h. After 24 h of treatment, media were replaced with PBS-washed wells, followed by incubating with MTT solution for 2 h, at 37 °C, in the dark. The resulting formazan crystals were solubilized with DMSO, and absorbance was measured at 570 nm by using a microplate reader (CLARIOstar®, BMG LABTECH, Ortenberg, Germany). Cell viability percentages were calculated using the following equation:

$$\text{Cell viability (\%)} = (A/B) \times 100 \quad (9)$$

where A is the absorbance of the reaction with the extract at 570 nm, and B is the absorbance of the control without the extract.

Crystal violet staining

The crystal violet stain assay was used to confirm the cytotoxicity of the crude extracts, followed by morphology observation [21,22]. The NHDF cells were cultured and treated with different concentrations of the crude extract compared with the control for 24 h, similar to the MTT assay. After the treatment duration, the supernatant was removed, the cells were washed with PBS pH 7.4 and fixed with 4 %w/v paraformaldehyde for 1 h at room temperature. After fixation, the cells were stained with 0.5 %w/v crystal violet solution and incubated for 30 min. Then, the cells were rinsed with tap water to remove excess stain and air dried. Cell morphology was visualized under a light microscope (EVOS® XL Core Imaging System, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Syzygium cumini L. liquid extract formulation

Syzygium cumini L. seed extract obtained with 50 %v/v ethanol (SC50ET) was selected for its superior matrix metalloproteinase inhibition and potent bioactivity relevant to skin aging and cosmeceutical applications. Notably, SC50ET demonstrated high safety, maintaining over 90% cell viability. Based on preliminary efficacy and safety assessments, a concentration of 5 %w/w SC50ET was chosen to achieve optimal cosmetic performance. Since the study focused on evaluating the stability of the liquid extract based on its antioxidant activity, no additional antioxidants were added. For formulation, SC50ET was dissolved in distilled water at 5 %w/w, with PEG-40 hydrogenated castor oil (10 %w/w) as a solubilizer, glycerin (20 %w/w) as a humectant, and DMDM hydantoin (1 %w/w) as a preservative. This combination ensures optimal solubility, stability, and safety of the active ingredient within the cosmetic product.

Stability of Syzygium cumini L. liquid extract evaluation

The formulation was stored under varying temperature conditions (4, 25, and 50 °C) and evaluated over 8 weeks at intervals of 0, 2, 4, 6, and 8 weeks to

assess the bioactive stability and antioxidant properties. Total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and free radical scavenging activities via DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assays were quantified at each time point under all storage conditions. The results from all antioxidant activities of the liquid extract were expressed as milligrams of ascorbic acid equivalent antioxidant capacity per milliliter of extract (mg AAEAC/mL), providing a comprehensive analysis of temperature and time dependent degradation effects on bioactive compounds and antioxidant capacity.

Microbial and heavy metal contamination test

The *Syzygium cumini* L. liquid extract formulation was evaluated according to the criteria of Thai Industrial Standard in the issue of cosmetics: General specification (TIS 152 - 2555), as outlined in the Ministry of Industry Notification, which specifies general requirements for cosmetic products [23]. The total aerobic colony count was assessed using the methodology described in USP41/NF36:2018, Chapter 61. Furthermore, the detection of specific pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Clostridium spp.*, was conducted in accordance with USP41/NF36:2018 [24]. The quantification of heavy metals, e.g., total arsenic, cadmium, lead, and mercury compounds, was performed using Atomic Absorption Spectroscopy (AAS), with analyses carried out by the Institute of Chemical Technology (Chemlab Services, Thailand).

Statistical analysis

The results are presented as mean ± standard deviation (SD). All experimental data were analyzed using Analysis of Variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons between the treatment and control groups. A p-value < 0.05 was considered statistically significant. Statistical analyses were conducted using GraphPad Prism version 10 (version 10.0, San Diego, CA, USA).

Results and discussion

The result of *Syzygium cumini* L. extraction

The extraction results of *Syzygium cumini* L. seeds using distilled water, 30, 50, and 70 %v/v ethanol revealed that the highest extraction yield was obtained with 50 %v/v ethanol (SC50ET) and 30 %v/v ethanol (SC30ET), yielding 19.19% ± 0.44% and 18.40% ± 0.39%, respectively, as shown in **Table 1**. This aligns with previous studies that have demonstrated the efficiency of hydroalcoholic solvents, particularly ethanol at intermediate concentrations, for extracting bioactive compounds from plant materials. The findings indicate that ethanol at 50 %v/v concentration provides optimal extraction efficiency for bioactive constituents, likely due to its ability to dissolve both polar and non-polar metabolites, which enhances the release of chemical compositions from the plant matrix. Analysis of variance confirmed that the type of solvent significantly influenced extraction yield ($p < 0.05$). The Tukey test further indicated significant differences between the extraction yields of 50 %v/v ethanol (19.19% ± 0.44%) and 70 %v/v ethanol (16.15% ± 0.51%), while the distilled water extraction (SC100W) demonstrated the lowest yield (13.41% ± 0.73%). These results are consistent with reports that hydroalcoholic mixtures are more effective than water alone for extracting a broad spectrum of phytochemicals from *Syzygium cumini* L. [25-27].

Total phenolic and flavonoid content

The analysis of total phenolic and flavonoid content in *Syzygium cumini* L. seed extracts demonstrated that the 70 %v/v ethanol extract (SC70ET) contained the highest phenolic (488.51 ± 3.60 mg GAE/g extract) and flavonoid (88.45 ± 1.61 mg QE/g extract) contents, followed by the 50 and 30 %v/v ethanol extracts, respectively. In contrast, the distilled water extract (SC100W) exhibited the lowest phenolic and flavonoid contents, as shown in **Table 1**. These results were consistent with previous research, which found that ethanol-based solvents yield higher amounts of phenolic and flavonoid compounds from *Syzygium cumini* L. compared to water-based solvents [26,28]. The higher extraction efficiency of ethanol was attributed to the polarity of phenolic compounds, such as phenolic acids and flavonoids, which possess benzene rings in their structures, making them more soluble in

highly polar organic solvents like ethanol than in water. Thus, ethanol extraction yields a higher amount of phenolic and flavonoid content compared to water extraction, supporting the use of ethanol as an effective solvent for recovering antioxidant compounds from *Syzygium cumini* L. [25]. Although the 50% and 30% ethanol extracts (SC50ET and SC30ET) of *Syzygium cumini* L. seeds provided the highest overall extraction yields, the 70% ethanol extract (SC70ET) exhibited the highest total phenolic and flavonoid contents. This apparent discrepancy arises because extraction yield reflects the total mass of all solubilized compounds, including sugars, proteins, and other non-phenolic substances, while phenolic and flavonoid content specifically measures the concentration of these bioactive compounds. Higher ethanol concentrations, such as 70%, are more effective at selectively extracting phenolic and flavonoid compounds due to their intermediate polarity, which better matches the solubility characteristics of these compounds compared to more polar solvents or lower ethanol concentrations. As a result, even though SC70ET yields less total crude extract, it is richer in the desired bioactive constituents. This distinction underscores the importance of optimizing solvent composition not just for maximum yield, but for the targeted recovery of functional phytochemicals [29-31].

Table 1 Total phenolic and flavonoid contents of *Syzygium cumini* L. seed extracts obtained using different solvents.

***Syzygium cumini* L.**	**% Yield**	**Total Phenolic content (mg GAE/ g extract)**	**Total Flavonoid content (mg QE/ g extract)**
SC100W	13.41 ± 0.73^{c}	288.51 ± 1.65^{d}	49.50 ± 1.20^{c}
SC30ET	18.40 ± 0.39^{a}	383.55 ± 4.60^{c}	70.79 ± 1.35^{b}
SC50ET	19.19 ± 0.44^{a}	448.84 ± 2.18^{b}	86.71 ± 0.34^{a}
SC70ET	16.15 ± 0.51^{b}	488.51 ± 3.60^{a}	88.45 ± 1.61^{a}

Note: Data are expressed as mean ± standard deviation; GAE = gallic acid equivalent, QE = quercetin equivalent. Different letters in the same column indicate significant differences ($p < 0.05$), n = 3.

The result analysis of antioxidant activities

The DPPH assay results shown in **Table 2** demonstrated that SC70ET (70% ethanol extract) exhibited the highest radical scavenging activity with an IC_{50} value of 13.26 ± 0.27 µg/mL, significantly outperforming the water extract (SC100W) which showed the highest IC_{50} value of 17.28 ± 0.30 µg/mL, indicating the lowest antioxidant activity among all extracts. The other ethanol extracts (SC30ET and SC50ET) showed intermediate IC_{50} values of 14.45 ± 0.26 µg/mL and 13.33 ± 0.25 µg/mL, respectively. The DPPH radical scavenging mechanism involved multiple pathways, including hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT) Do *et al.* [32], Chen *et al.* [33], and sequential proton-loss electron transfer (SPLET). Research indicates that the DPPH assay primarily operates through HAT and SET-PT mechanisms, where antioxidants donate hydrogen atoms or electrons to the stable DPPH radical, converting it from a purple-colored radical to a yellow-colored DPPH-H compound [34,35]. The superior performance of ethanol extraction may be attributed to its ability to extract more phenolic compounds with optimal polarity that are particularly effective in hydrogen donation, as the DPPH reaction is influenced by the number and position of hydroxyl groups on phenolic compounds [36].

Moreover, the ABTS assay results shown in **Table 2** reveal that SC70ET demonstrated the highest antioxidant activity (IC_{50} = 36.73 ± 0.30 µg/mL), followed by SC50ET (39.10 ± 1.54 µg/mL) and SC30ET (41.63 ± 0.56 µg/mL), while SC100W showed the lowest activity (IC_{50} = 53.03 ± 0.59 µg/mL). The ABTS assay operates through a more complex mechanism than DPPH, involving the scavenging of $ABTS^{\bullet+}$ radical cations generated by potassium persulfate [37]. Research has demonstrated that the ABTS assay preferentially reacts via the SPLET

mechanism in aqueous solutions, and some antioxidants can form coupling adducts with ABTS, leading to secondary reactions that contribute to the total antioxidant capacity [32]. The ABTS assay can accommodate both hydrophilic and lipophilic antioxidants and is less dependent on steric hindrance compared to DPPH. The consistently higher IC_{50} values observed in the ABTS assay compared to DPPH suggest that the *Syzygium cumini* L. seed extracts contain compounds that are more effective at hydrogen atom donation (DPPH-preferred) than electron transfer (ABTS-preferred) [38].

Additionally, antioxidant capacities were demonstrated by FRAP values as represented in **Table 2**, which showed a distinct trend where SC70ET demonstrated the highest reducing power (4.17 ± 0.08 mmol AAE/g extract), followed closely by SC50ET (4.11 ± 0.01 mmol AAE/g extract), while SC100W showed significantly lower activity (3.00 ± 0.08 mmol AAE/g extract). The FRAP assay measured the ferric reducing ability through the reduction of Fe^{3+}-TPTZ complex to the intensely blue-colored Fe^{2+}-TPTZ complex at low pH, operating primarily through the SPLET mechanism [39]. This electron transfer-based mechanism was fundamentally different from the radical scavenging approaches of DPPH and ABTS assays, as it measured the ability of antioxidants to donate electrons for metal ion reduction rather than neutralizing free radicals [40]. The superior performance of ethanol extracts in the FRAP assay suggested that these extracts contain compounds with enhanced electron-donating capacity, possibly due to the extraction of less polar phenolic compounds that were more effective at reducing metal ions [40,41]. The mechanistic differences between assays explain why antioxidant rankings varied across different evaluation methods. While DPPH and ABTS assays primarily measure hydrogen atom transfer and single electron transfer mechanisms, respectively. FRAP specifically evaluates reducing power through metal ion reduction, which may favor different classes of antioxidant compounds. This mechanistic diversity accounts for the observed variations in extract performance across assays, as different phenolic compounds exhibit varying efficiencies in different antioxidant mechanisms. The correlation analysis revealed that FRAP and ABTS assays showed a strong positive correlation ($R^2 = 0.806$), indicating that extracts with higher reducing power generally demonstrated better radical scavenging activity in the ABTS system [38]. This strong correlation suggests that both assays may be influenced by similar antioxidant compounds, particularly those capable of both electron donation and radical neutralization. In contrast, DPPH correlations with other assays were notably weaker, likely due to its specific mechanism requiring direct hydrogen donation and its limitation to detecting antioxidants that can reduce the DPPH radical in methanol solution. In conclusion, based on the comprehensive analysis of all 3 assays, SC70ET demonstrated the most consistent and superior antioxidant activities across multiple parameters. While SC30ET showed optimal performance in the ABTS assay, SC70ET exhibited the highest FRAP activity combined with excellent antioxidant performance in both DPPH and ABTS assays. The superior performance of ethanol extracts could be attributed to the enhanced extraction of phenolic compounds and flavonoids, which were more effectively solubilized in ethanol-water mixtures, leading to improved antioxidant capacity through multiple mechanisms, including hydrogen donation, electron transfer, and metal chelation [33].

Table 2 Antioxidant activities of *Syzygium cumini* L. seed extracts using different solvents, as determined by DPPH, ABTS, and FRAP assays.

***Syzygium cumini* L.**	**DPPH (IC_{50} µg/mL)**	**ABTS (IC_{50} µg/mL)**	**FRAP (mmol AAE/ g extract)**
L-ascorbic acid	11.13 ± 0.06^{a}	37.53 ± 0.86^{ab}	-
SC100W	17.28 ± 0.30^{d}	53.03 ± 0.59^{d}	3.00 ± 0.08^{c}
SC30ET	14.45 ± 0.26^{c}	41.63 ± 0.56^{c}	3.41 ± 0.03^{b}

Syzygium cumini L.	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)	FRAP (mmol AAE/ g extract)
SC50ET	13.33 ± 0.25^{b}	39.10 ± 1.54^{b}	4.11 ± 0.01^{a}
SC70ET	13.26 ± 0.27^{b}	36.73 ± 0.30^{a}	4.17 ± 0.08^{a}

Note: Data were expressed as mean ± standard deviation; IC_{50} = half-maximal inhibitory concentration, AAE = Ascorbic acid equivalent. Different letters in the same column indicated significant differences between groups ($p < 0.05$)

Tyrosinase inhibition activity of *Syzygium cumini* L. extract

The tyrosinase inhibitory activities are presented in **Table 3,** demonstrating significant differences in inhibitory efficacy among the various *Syzygium cumini* L. extracts. SC100W exhibited the lowest activity with an IC_{50} value of 3.89 ± 0.33 mg/mL, while SC30ET and SC50ET showed superior inhibitory activities with IC_{50} values of 1.78 ± 0.14 mg/mL and 1.84 ± 0.07 mg/mL, respectively. The positive control, kojic acid, demonstrated exceptional activity at an IC_{50} value of 0.04 ± 0.01 mg/mL, establishing the benchmark for comparison. The study by Junlatat *et al.* [42] investigated the effects of ethanolic extracts from *Syzygium cumini* L. leaves and branches on tyrosinase inhibition and melanogenesis in B16-F10 murine melanoma cells. Results showed *Syzygium cumini* L. leaves (SLE) exhibited superior antioxidant activity and melanin suppression compared to *Syzygium cumini* L. branches (SBE), with both extracts containing high phenolic content correlated to these effects. RT-PCR analysis revealed dose-dependent inhibition of tyrosinase, TRP-1, and TRP-2 gene expression by both extracts, while demonstrating no cytotoxicity at the tested concentrations. In a related study, Lema *et al.* [43] evaluated the anti-aging potential of *Syzygium cumini* L. leaf ethanol extract, focusing Limaon *in vitro* tyrosinase inhibition. The extract showed < 50% tyrosinase inhibitory activity compared to kojic acid (positive control). The inhibition mechanisms of tyrosinase involve multiple pathways, including competitive inhibition where inhibitors bind to the free enzyme, preventing substrate binding, and mixed-type inhibition where compounds can bind to both the enzyme and enzyme-substrate complex [43,44]. Copper chelation represents another significant mechanism, as tyrosinase is a metalloenzyme requiring copper ions for catalytic activity. The differential activities observed among extracts likely result from varying concentrations of phenolic compounds and their specific binding affinities to the enzyme's active site [45,46].

Matrix metalloproteinases inhibition analysis of *Syzygium cumini* L. extract

The collagenase inhibition activities were revealed in **Table 3**, which showed that SC50ET demonstrated the most potent inhibitory activity among the tested extracts with an IC_{50} value of 0.41 ± 0.01 mg/mL, significantly outperforming other extracts. EGCG served as an effective positive control with an IC_{50} of 0.03 ± 0.01 mg/mL. A study by Ashmawy *et al.* [48] demonstrated that essential oil from *Syzygium cumini* L. seeds, analyzed via GC/MS and multivariate methods (PCA/HCA), exhibited potent collagenase inhibition alongside anti-elastase and anti-hyaluronidase activities, outperforming other plant parts. Moreover, including α-pinene, β-pinene, and caryophyllene oxide, were linked to these anti-aging enzyme inhibitory effects. Concurrently, methanol seed extracts showed significant suppression of gelatinase-B (MMP-9), a collagen-degrading enzyme, in high glucose-stimulated cardiac cells *in vitro*. Molecular docking analyses by Atale *et al.* [49] revealed that polyphenols (e.g., gallic acid, ellagic acid) in the extract competitively bind to MMP-9's active site, explaining its mechanism. Comparative studies confirmed seeds' superior collagenase inhibition over leaves and bark, with PCA/HCA highlighting distinct chemical profiles in seed-derived essential oils. These findings position *Syzygium cumini* L. seeds as a promising source of bioactive compounds for skincare and therapeutic applications targeting age-related tissue degradation. The inhibition mechanisms of bacterial collagenases involve zinc-binding groups (ZBGs) that interact with the catalytic zinc ion in the enzyme's active site. Diphosphonate and hydroxamate compounds have been

identified as potent collagenase inhibitors, with their efficacy depending on their ability to form stable complexes with the zinc ion. The superior performance of SC50ET suggested that optimal extraction conditions that preserve bioactive compounds capable of effective zinc chelation and active site binding. Structure-activity relationships indicate that the presence of hydroxyl groups and aromatic systems enhances binding affinity to collagenase enzymes [50].

Moreover, the elastase inhibition results shown in **Table 3** indicate that extracts showed a relatively narrow range of IC_{50} values, with SC50ET exhibiting the highest activity, ranging from 1.60 ± 0.06 mg/mL (SC50ET) to 1.73 ± 0.01 mg/mL (SC100W). EGCG demonstrated excellent control activity at 0.03 ± 0.01 mg/mL. Elastase inhibition mechanisms primarily involve competitive binding to the enzyme's active site, where inhibitors act as substrate mimics [47,48]. Natural polypeptides and phenolic compounds can form hydrogen bonds with key amino acid residues in the enzyme's binding pocket [47]. The relatively narrow range of IC_{50} values among extracts suggests that elastase inhibition may be less sensitive to extraction conditions compared to tyrosinase and collagenase activities. This consistency implies that the structural features of bioactive constituents – particularly quinoline derivatives and phenolic compounds – play a dominant role in elastase inhibition. These compounds have shown mixed-type inhibition patterns, indicating multiple binding sites on the elastase enzyme [51,52].

In summary, based on the comprehensive matrix metalloproteinase inhibition analysis, SC50ET extract demonstrated the optimal overall performance across all 3 enzymatic assays. This extract exhibited the lowest IC_{50} value for collagenase inhibition (0.41 ± 0.01 mg/mL), tyrosinase inhibition (1.84 ± 0.07 mg/mL), and superior elastase inhibition (1.60 ± 0.06 mg/mL). The balanced inhibitory profile of SC50ET suggests that 50 %v/v ethanol extraction conditions effectively preserve and concentrate the bioactive compounds responsible for matrix metalloproteinase inhibition, making it the most promising candidate for cosmetic applications requiring broad-spectrum enzymatic inhibition.

Table 3 Inhibitory effects of *Syzygium cumini* L. seed extracts on tyrosinase, collagenase, and elastase activities using different extraction solvents.

***Syzygium cumini* L. extract**	**Tyrosinase inhibition (IC_{50} mg/mL)**	**Collagenase inhibition (IC_{50} mg/mL)**	**Elastase inhibition (IC_{50} mg/mL)**
Kojic acid	0.04 ± 0.01^{a}	-	-
EGCG	-	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}
SC100W	3.89 ± 0.33^{d}	1.20 ± 0.27^{c}	1.73 ± 0.01^{d}
SC30ET	1.78 ± 0.14^{b}	0.93 ± 0.19^{c}	$1.61 \pm 0.05^{b,c}$
SC50ET	1.84 ± 0.07^{b}	$0.41 \pm 0.01^{a,b}$	1.60 ± 0.06^{b}
SC70ET	2.36 ± 0.14^{c}	$0.77 \pm 0.16^{b,c}$	$1.70 \pm 0.02^{c,d}$

Note: Data were expressed as mean ± standard deviation (SD). Different lowercase letters within the same column indicated significant differences between groups ($p < 0.05$), n = 3.

Syzygium cumini L. seed extracts effect on cell viability

The cytotoxicity test results of *Syzygium cumini* L. seed extracts (**Figure 1**) demonstrated excellent biocompatibility across a broad concentration range. Human dermal fibroblasts maintained over 90% cell viability at concentrations from 1 to 250 µg/mL across all extract types (SC100W, SC30ET, SC50ET, and SC70ET), indicating the high safety profile of the extracts for skin cells. However, when the concentration was increased to 500 µg/mL, cell viability decreased to 30.2% - 53.6%. Importantly, this reduction in cell viability at 500 µg/mL tested concentration does not necessarily indicate cytotoxicity concerns for topical applications, as the concentrations typically employed in cosmetic formulations are substantially lower than those evaluated *in vitro*. The MTT assay findings align with morphological analyses via crystal violet staining (**Figure 2**), where higher cell viability corresponded to normal cell growth, robust adhesion to the culture

surface, and darker staining intensity. Morphological observations confirmed preserved cell shape and typical spatial organization, particularly in control and low-concentration groups, with progressive changes in cell density and morphology becoming apparent only at the higher concentrations of 250 - 500 µg/mL.

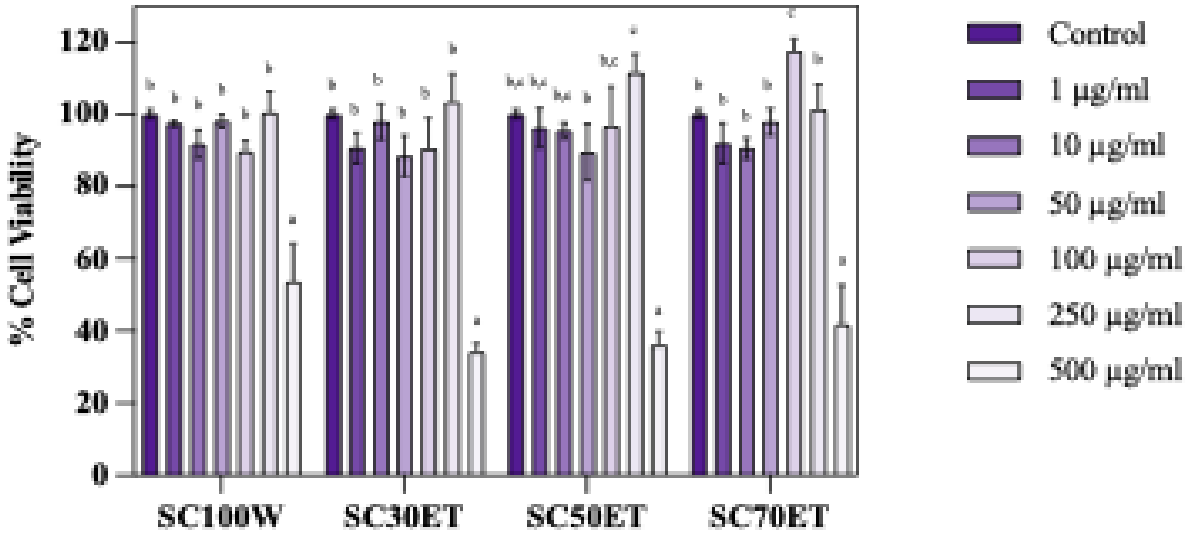

Figure 1 Cytotoxicity of *Syzygium cumini* L. seed extracts on human dermal fibroblasts. Percentages of HNDF cell viability after 24 h treated with different concentrations of extract, water (SC100W), 30% v/v ethanol (SC30ET), 50 %v/v ethanol (SC50ET), and 70 %v/v ethanol (SC70ET). Values were expressed as mean ± SD. Different letters indicated significant differences between groups ($p < 0.05$).

The results corroborated previous studies indicating that extracts from the seeds of *Syzygium cumini* L. at concentrations ranging from 6.25 to 100 µg/mL can maintain the viability of human skin cells, including fibroblasts and keratinocytes [53]. Additionally, the results from Prasathkumar *et al.* [54] showed that the extract (5 - 75 µg/mL) kept 99.33% ± 0.26% of mouse fibroblasts viable. More than that, previous studies on *Syzygium cumini* L. toxicity in animal models and biological systems further support these results, reporting that hydroalcoholic and methanol extracts from its leaves and fruits exhibit high safety when administered orally to mice and rats, with lethal dose (LD_{50}) values exceeding 3,000 - 5,000 mg/kg. Long-term administration revealed no adverse effects on behavior, body weight, hematological/biochemical parameters, or histology of vital organs [55].

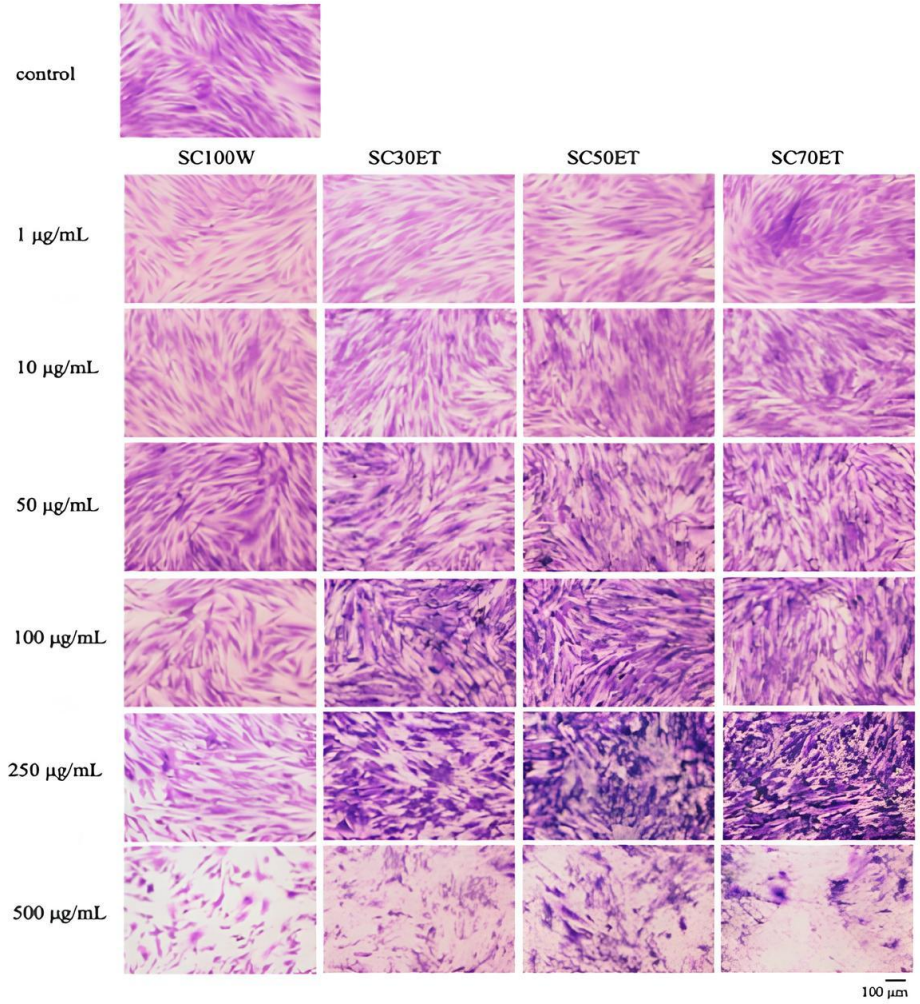

Figure 2 Cell morphology and density of human dermal fibroblast (NHDF) cells after 24 h exposure to *Syzygium cumini* L. seed extracts; water extract (SC100W), 30 %v/v ethanol extract (SC30ET), 50 %v/v ethanol extract (SC50ET), and 70 %v/v ethanol extract (SC70ET) at concentrations (1 to 500 µg/mL) compared to untreated control cells. Cells stained with crystal violet and visualized at 200× magnification, with a scale bar of 100 µm.

Evaluation of Syzygium cumini L. liquid extract stability

The stability evaluation of *Syzygium cumini* L. liquid extract over 8 weeks at 3e storage temperatures (4, 25, and 50 °C). The result from the stability study was interpreted by 1 mL of liquid extract, which was prepared at a concentration of 5 %w/w of SC50ET. The result revealed a consistent decline in total phenolic content across all conditions. At 4 °C, the total phenolic content decreased from 28.77 ± 0.43 mg GAE/mL initially to 21.43 ± 0.37 mg GAE/mL at week 8, representing the most substantial reduction among the tested temperatures. Storage at 25 °C resulted in a more moderate decrease, with values dropping to 26.13 ± 0.25 mg GAE/mL, while storage at 50 °C led to a final concentration of 25.59 ± 0.23 mg GAE/mL. The data indicate that 25 °C provided the best retention of total phenolic content, as the final value at this temperature was higher than that observed at 50 °C. Generally, high temperature conditions can degrade polyphenols. This highlights that moderate temperature storage (25 °C) was more effective in preserving phenolic compounds than both refrigeration (4 °C) and elevated temperature (50 °C) storage over the 8-week period. Interestingly, higher temperature storage appeared to provide better phenolic compound stability compared to refrigerated conditions. Additionally, total flavonoid content displayed variable stability patterns depending on storage temperature. The initial concentration of 4.49 ± 0.14 mg QE/mL decreased most significantly at 25 °C, reaching 3.25 ± 0.01 mg QE/mL by week 8. Storage at 4 °C resulted in intermediate

losses (3.43 ± 0.02 mg QE/mL), while 50 °C storage showed the best flavonoid retention (3.67 ± 0.09 mg QE/mL). Recent studies indicate that elevated temperatures may suppress the enzymatic activity of polyphenol oxidase, which is responsible for the oxidation and degradation of phenolic and flavonoid compounds. As a result, increased temperatures during storage both improve the stability of these valuable phytochemicals and help preserve their bioactive properties [56]. Moreover, DPPH radical scavenging activity demonstrated relatively stable performance across all storage conditions. The activity decreased modestly from 28.03 ± 0.18 mg AAEAC/mL initially to approximately 26 - 27 mg AAEAC/mL after 8 weeks, with 50 °C storage showing the smallest reduction (27.11 ± 0.05 mg AAEAC/mL). The 25 °C storage condition resulted in the greatest activity loss (26.03 ± 0.07 mg AAEAC/mL), while 4 °C storage showed intermediate stability. In addition, ABTS radical scavenging activity exhibited the most pronounced degradation among all measured parameters. The *Syzygium cumini* L. liquid extract started at 57.95 ± 2.24 mg AAEAC/mL and declined significantly over the 8-week storage period at all tested temperatures. The most pronounced decrease was observed at 25 °C, where the activity dropped to 46.53 ± 0.82 mg AAEAC/mL. In comparison, storage at 4 °C resulted in a final value of 48.73 ± 0.62 mg AAEAC/mL, indicating better retention of antioxidant activity. Notably, storage at 50 °C exhibited the highest retention among the tested conditions, with the ABTS activity remaining at 51.74 ± 0.31 mg AAEAC/mL at week 8. Furthermore, ferric reducing antioxidant power showed the most stable profile among all tested parameters. Ferric reducing antioxidant power demonstrated remarkable stability throughout the storage period. The initial activity of 220.82 ± 1.68 mg AAEAC/mL extract showed minimal degradation across all storage conditions, with final values at week 8 ranging from 210.56 ± 2.03 mg AAEAC/mL extract (25 °C) to 214.15 ± 0.83 mg AAEAC/mL extract (50 °C). Notably, statistical analysis revealed no significant differences between storage temperatures at the final time point ($p > 0.05$), indicating that FRAP activity was maintained consistently regardless of the thermal stress conditions applied, as shown in **Table 4**. The relatively small decreases suggest this antioxidant mechanism is particularly robust under various storage conditions. Conclusion, the stability study revealed that *Syzygium cumini* L. liquid extract demonstrates time- and temperature-dependent degradation patterns, with statistically significant differences observed for most parameters ($p < 0.05$). Generally, phenolic compounds are relatively stable at moderate temperatures, but prolonged exposure to high temperatures or other conditions can lead to degradation and a decrease in their antioxidant activity. The findings of this study align with those of previous research. After storing grape stem extract solutions at 40 °C for 2 months, approximately 80% of the phenolic compounds were retained [57]. Contrary to conventional expectations, storage at 50 °C generally provided superior retention of bioactive compounds and antioxidant activities compared to refrigerated storage at 4 °C. In comparison, room temperature storage at 25 °C typically resulted in the greatest losses. According to the experimental results, it appeared that storing the aqueous extract at high temperatures showed higher amounts of phenolic compounds than at low temperatures. The explanation regarding high-temperature conditions is the solubility of the bioactive compound. The mechanism may be that increasing storage temperature enhances both diffusion coefficients and the solubility of polyphenol content [58]. The exceptions were total flavonoid content, ABTS radical scavenging activity, and ferric reducing antioxidant power, which showed no statistically significant differences between extract amounts under different storage conditions. These findings suggest that the extract possesses inherent thermal stability properties that may be leveraged for optimal preservation of its bioactive components. In conclusion, it is notable that after 8 weeks, the percentage retention of total phenolic content ranged from 74.5% at 4 °C to 90.8% at 25 °C, while DPPH radical scavenging activity remained above 92% across all temperatures, indicating high antioxidant stability. These findings highlight that most bioactive compounds and antioxidant activities retained over 70 - 97% of their initial values, demonstrating substantial stability of *Syzygium cumini* L. liquid extract under various storage conditions.

Table 4 Stability of SC50ET extract, changes in bioactive compound content and antioxidant activities under different storage temperatures over 8 weeks.

Parameter	Temperature °C	Time (week)				
		0	2	4	6	8
Total Phenolic Content (mg GAE/mL)	4	$28.77 \pm 0.43^{a,B}$	$20.57 \pm 0.26^{a,A}$	$20.42 \pm 0.64^{a,A}$	$20.10 \pm 0.64^{a,A}$	$21.43 \pm 0.57^{a,A}$
	25	$28.77 \pm 0.43^{a,D}$	$20.13 \pm 0.30^{a,A}$	$21.84 \pm 0.18^{b,B}$	$28.48 \pm 0.20^{b,D}$	$26.13 \pm 0.25^{b,C}$
	50	$28.77 \pm 0.43^{a,D}$	$25.18 \pm 0.43^{b,B}$	$23.75 \pm 0.13^{c,A}$	$27.50 \pm 0.23^{b,C}$	$25.59 \pm 0.23^{b,B}$
Total Flavonoid Content (mg QE/ mL)	4	$4.49 \pm 0.14^{a,D}$	$4.26 \pm 0.14^{b,C,D}$	$4.04 \pm 0.10^{b,B,C}$	$3.79 \pm 0.17^{b,A,B}$	$3.43 \pm 0.02^{b,A}$
	25	$4.49 \pm 0.14^{a,C}$	$3.78 \pm 0.19^{a,B}$	$3.55 \pm 0.08^{a,A,B}$	$3.19 \pm 0.20^{a,A}$	$3.25 \pm 0.01^{a,A}$
	50	$4.49 \pm 0.14^{a,C}$	$3.69 \pm 0.07^{a,A}$	$4.01 \pm 0.08^{b,B}$	$3.83 \pm 0.04^{bA,B}$	$3.67 \pm 0.09^{c,A}$
DPPH radical scavenging activity (mg AAEAC/mL)	4	$28.03 \pm 0.18^{a,B}$	$26.35 \pm 0.09^{a,A}$	$27.68 \pm 0.17^{b,B}$	$27.80 \pm 0.36^{b,B}$	$26.49 \pm 0.10^{b,A}$
	25	$28.03 \pm 0.18^{a,C}$	$26.22 \pm 0.07^{a,A}$	$27.12 \pm 0.20^{b,B}$	$26.23 \pm 0.45^{a,A}$	$26.03 \pm 0.07^{a,A}$
	50	$28.03 \pm 0.18^{a,C}$	$26.84 \pm 0.09^{b,B}$	$26.02 \pm 0.26^{a,A}$	$27.46 \pm 0.34^{b,B,C}$	$27.11 \pm 0.05^{c,B}$
ABTS radical scavenging activity (mg AAEAC/mL)	4	$57.95 \pm 2.24^{a,C}$	$47.13 \pm 0.31^{a,A}$	$52.94 \pm 0.31^{c,B}$	$53.34 \pm 0.54^{c,B}$	$48.73 \pm 0.62^{b,A}$
	25	$57.95 \pm 2.24^{b,C}$	$47.53 \pm 0.31^{a,A}$	$51.74 \pm 0.31^{b,B}$	$49.13 \pm 0.54^{a,A,B}$	$46.53 \pm 0.82^{a,A}$
	50	$57.95 \pm 2.24^{b,B}$	$50.13 \pm 0.31^{b,A}$	$49.53 \pm 0.31^{a,A}$	$51.94 \pm 0.31^{b,A}$	$51.74 \pm 0.31^{c,A}$
Ferric Reducing Antioxidant Power (mg AAEAC/ mL)	4	$220.82 \pm 1.68^{a,C}$	$215.90 \pm 2.34^{b,A}$	$207.59 \pm 3.02^{a,B}$	$218.36 \pm 1.36^{b,B}$	$214.05 \pm 0.97^{a,A}$
	25	$220.82 \pm 1.68^{a,C}$	$208.62 \pm 0.28^{a,b,A}$	$216.72 \pm 0.69^{b,B}$	$211.90 \pm 1.04^{aA,B}$	$210.56 \pm 2.03^{a,A}$
	50	$220.82 \pm 1.68^{a,B}$	$213.64 \pm 2.03^{a,A}$	$213.64 \pm 2.03^{b,A}$	$217.33 \pm 0.84^{b,A}$	$214.15 \pm 0.83^{a,A}$

Note: GAE; Gallic Acid Equivalent, QE; Quercetin Equivalent, AAEAC; Ascorbic Acid Equivalent Antioxidant Capacity. Different lowercase superscript letters within the same column indicate statistically significant differences between storage temperatures ($p < 0.05$), n = 3. Different uppercase superscript letters within the same row indicate statistically significant differences between time points ($p < 0.05$), n = 3.

Microbiological assessment and heavy metal contamination in the extract

The results presented in **Table 5** demonstrate that the liquid extract passed the microbiological and heavy metal safety requirements specified by the TIS 152 - 2555 standard for cosmetics. None of the tested heavy metals arsenic, cadmium, lead, or mercury were detected in the extract, indicating that their concentrations were below the detection limits and well within the permissible thresholds (< 5 mg/kg for arsenic, < 3 mg/kg for cadmium, < 20 mg/kg for lead, and < 1 mg/kg for mercury). This absence of detectable heavy metals suggests a low risk of toxicity from these contaminants in the extract. Microbiological analysis further confirms the safety and quality of the liquid extract. The total colony count was less than 10 CFU/mL, significantly lower than the standard limit of 1000 CFU/mL, indicating minimal microbial contamination. Additionally, specific pathogenic microorganisms, including *Clostridium spp.*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa*, were not detected in the sample. The absence of these pathogens was critical for ensuring product safety, as their presence could pose health risks to consumers. In summary, the liquid extract complies with the TIS 152 - 2555 standard for both heavy metal and microbiological safety. The extract contains no detectable levels of arsenic, cadmium, lead, or mercury, and shows no evidence of contamination by harmful microorganisms. These findings confirm that the extract is safe for use in cosmetic applications, as it meets all relevant regulatory requirements for heavy metal and microbial contamination.

Table 5 Microbial and heavy metal contamination analysis of the liquid extract according to TIS 152 - 2555 Standard.

Test list (Units)	liquid extract	TIS 152 - 2555 standard	Result
Arsenic (mg/kg)	Not detected	< 5 mg/kg	Passed
Cadmium (mg/kg)	Not detected	< 3 mg/kg	Passed
Lead (mg/kg)	Not detected	< 20 mg/kg	Passed
Mercury (mg/kg)	Not detected	< 1 mg/kg	Passed
Total colony count (CFU/mL)	< 10 CFU/mL	< 1000 CFU/mL	Passed
Clostridium spp. (in 1 g)	Not detected	Not detected	Passed
Staphylococcus aureus (in 1 g)	Not detected	Not detected	Passed
Candida albicans (in 1 g)	Not detected	Not detected	Passed
Pseudomonas aeruginosa (in 1 g)	Not detected	Not detected	Passed

Note: CFU = colony forming unit; not detected = analyte was below the detection limit; Passed = meets the specified safety standard.

Conclusions

The findings demonstrated that the extraction solvent significantly impacts the yield, bioactive compounds, and cosmeceutical bioactivity of *Syzygium cumini* L. seed extracts. The 50 %v/v ethanol extract achieved the highest extraction efficiency and matrix metalloproteinase inhibition, while the 70 %v/v ethanol extract exhibited the greatest phenolic content and antioxidant reducing power. Water extracts provided superior radical scavenging activity. *Syzygium cumini* L. seed extracts exhibited excellent biocompatibility with skin cells and can be considered as a cosmetic ingredient. Stability assessments revealed that higher storage temperatures better preserved key bioactive compounds and antioxidant activities. The extracts met stringent microbiological and heavy metal safety standards, confirming their suitability for cosmetic applications. Overall, 50 %v/v ethanol extraction offers the best balance of yield, bioactive compounds, cosmeceutical bioactivity, and stability, making it the most promising approach for the cosmeceutical active ingredient for cosmetic products.

Acknowledgements

The authors are grateful to the Faculty of Food and Agricultural Technology and the Regional Science Park, Pibulsongkram Rajabhat University, Phitsanulok province, Thailand, for a grant from the Industrial Research and Technology Capacity Development Program (IRTC).

Declaration of Generative AI in Scientific Writing

The authors declare that during the preparation of this manuscript, generative AI tools such as language models were utilized solely to assist in improving the clarity and fluency of English expression. All content, scientific analysis, and interpretations were conceptualized and validated by the authors, ensuring the integrity and originality of the research.

CRediT Author Statement

Jirasit Inthorn contributed substantially to the conceptualization, methodology, and supervision of the study. Saranya Sornmanee was responsible for performing the investigation, data curation, and initial drafting of the manuscript. Wannisa Keawbankrud oversaw the project administration and provided significant input in reviewing and editing the manuscript. All authors have read and approved the final version of the manuscript.

References

[1] SB Swami, NSJ Thakor, MM Patil and PM Haldankar. Jamun (*Syzygium cumini* (L.)): A review of its food and medicinal uses. *Food and Nutrition Sciences* 2012; **3(8)**, 1100-1117.

[2] M Qamar, S Akhtar, T Ismail, M Wahid, MW Abbas, MS Mubarak, Y Yuan, R T Barnard, ZM Ziora and T Esatbeyoglu. Phytochemical profile, biological properties, and food applications of the

medicinal plant *syzygium cumini*. *Foods* 2022; **11(3)**, 378.

[3] Y Tak, M Kaur, MC Jain, MK Samota, NK Meena, G Kaur, R Kumar, D Sharma, JM Lorenzo and R Amarowicz. Jamun seed: A review on bioactive constituents, nutritional value and health benefits. *Polish Journal of Food and Nutrition Sciences* 2022; **72(3)**, 211-228.

[4] S Ahmed, KS Ahmed, MS Hossain, MS Azam, M Rahman and MM Hoque. Proximate composition and antioxidant activity of *Syzygium cumini* fruit grown at different regions in Bangladesh. *Food Research* 2020; **4(5)**, 1693-1699.

[5] ZP Ruan, LL Zhang and YM Lin. Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules* 2008; **13(10)**, 2545-2556.

[6] T Grimm, A Schafer and P Hogger. Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (pycnogenol). *Free Radical Biology and Medicine* 2004; **36(6)**, 811-822.

[7] SM Kotakadi, MJ Bangarupeta, K Kandati, DPR Borelli, JA Sayyed, MI Shaik and JS Nannepaga. Biosynthesized MgONPs using *Syzygium cumini* seed extract: Characterization, *In vitro* anti-oxidant and anti-microbial activity. *Biotechnology Reports* 2024; **43**, e00846.

[8] M Srisayam, C Puengtang, A Srisopa and A Chodnakarin. Biological activities, phenolic and vitamin C contents from *Syzygium cumini* (L.) extract. *PSRU Journal of Science and Technology* 2022; **7(2)**, 103-113.

[9] AK Yadav, S Saraswat, P Sirohi, M Rani, S Srivastava, MP Singh and NK Singh. Antimicrobial action of methanolic seed extracts of *Syzygium cumini* Linn. on Bacillus subtilis. *AMB Express* 2017; **7(1)**, 196.

[10] R Srimoon and S Niyomwan. Development and validation of the folin-ciocalteu semi-micro method for total phenolic compounds determination in rangdaeng (*Ventilago denticulata* Willd.) extract. *Burapha Science Journal* 2021; **26(1)**, 378-398.

[11] M Chatatikun and A Chiabchalard. Phytochemical screening, total flavonoid and total phenolic contents and antioxidant activity of *moringa oleifera* leaf extracts. *Current Topics in Pharmacology* 2020; **18(1)**, 93-99.

[12] S Kamtekar, V Keer and V Patil. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *Journal of Applied Pharmaceutical Science* 2014; **4(9)**, 061-065.

[13] M Minarti, N Idiawati and MA Wibowo. Potential antioxidant activity methods DPPH, ABTS, FRAP, total phenol and total flavonoid of methanol extract and its fractions from *macaranga hypoleuca* leaves. *E3S Web of Conferences* 2024; **371**, 07005.

[14] R Re, N Pellegrini, A Proteggente, A Pannala, M Yang and C Rice-Evans. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 1999; **26(9-10)**, 1231-1237.

[15] O Bulut, IE Kose, C Sonmez and HA Oktem. Phenolic compounds, carotenoids, and antioxidant capacities of a thermo-tolerant *Scenedesmus* sp. (Chlorophyta) extracted with different solvents. *Journal of Applied Phycology* 2019; **31(3)**, 1675-1683.

[16] O Bulut, IE Kose, C Sonmez and HA Oktem. Antioxidant activity of *Micractinium* sp. (Chlorophyta) extracts against H_2O_2 induced oxidative stress in human breast adenocarcinoma cells. *Scientific Reports* 2024; **14(1)**, 27593.

[17] MM Younis, IM Ayoub, NM Mostafa, MAE Hassab, WM Eldehna, ST Al-Rashood and OA Eldahshan. GC/MS profiling, anti-collagenase, anti-elastase, anti-tyrosinase and anti-hyaluronidase activities of a *stenocarpus sinuatus* leaves extract. *Plants* 2022; **11(7)**, 918.

[18] N Laothaweerungsawat, J Sirithunyalug, W Chaiyana, C Saenjum, N Waranuch, C Chaiyasut, K Pattamapun and S Saokaew. Chemical compositions and anti-skin-ageing activities of *Origanum vulgare* L. essential oil from tropical and Mediterranean region. *Molecules* 2020; **25(5)**, 1101.

[19] W Wang, H Lin, W Shen, X Qin, J Gao, W Cao, H Zheng, Z Chen and Z Zhang. Optimization of a novel tyrosinase inhibitory peptide from atrina pectinata: Identification, molecular docking and enzyme kinetics. *Foods* 2023; **12(21)**, 3884.

[20] AA Ala, BB Olotu and CMD Ohia. Assessment of cytotoxicity of leaf extracts of *andrographis paniculata* and *aspilia africana* on murine cells *in vitro*. *Archives of Basic and Applied Medicine* 2018; **6(1)**, 61-65.

[21] R Asada, K Kageyama, H Tanaka, M Kimura, Y Saitoh and N Miwa. Carcinostatic effects of diverse ascorbate derivatives in comparison with aliphatic chain moiety structures: Promotion by combined hyperthermia and reduced cytotoxicity to normal cells. *Oncology Letters* 2012; **3(5)**, 1042-1046.

[22] S Garg, H Huifu, SC Kaul and R Wadhwa. Integration of conventional cell viability assays for reliable and reproducible read-outs: Experimental evidence. *BMC Research Notes* 2018; **11(1)**, 403.

[23] Thai Industrial Standards Institute Ministry of Industry. *Thai industrial standard for cosmetics: General requirements (TIS 152-2555)*. Ministry of Industry, Bangkok, Thailand, 2012.

[24] United States Pharmacopeial Convention. *United states pharmacopeia 41–national formulary 36 (USP41/NF36).* United States Pharmacopeial Convention, Maryland, 2018.

[25] CAF Artilha-Mesquita, AP Stafussa, PDSD Santos, OO Santos, SCD Costa and GS Madrona. Extraction of bioactive compounds from the fruits of jambolan (*Syzygium cumini* (L.)) using alternative solvents. *Plants* 2024; **13(15)**, 2065.

[26] M Faradilla, I Fidrianny and MI Iwo. Antioxidant and immunomodulatory activities of ethanol extracts from *Syzygium cumini* L. skeels and pogostemon cablin Benth. *Narra Journal* 2024; **4(3)**, e918.

[27] R Lodhi, M Yadav, S Jain and S Nayak. Phytochemical investigation and comparative study on percentage yield with different solvent of "*syzygium cumini*" leaf. *Journal of Emerging Technologies and Innovative Research* 2023; **10(8)**, f587-f597.

[28] SH Priya, N Prakasan and J Purushothaman. Antioxidant activity, phenolic-flavonoid content and high-performance liquid chromatography profiling of three different variants of *Syzygium cumini* seeds: A comparative study. *Journal of Intercultural Ethnopharmacology* 2017; **6(1)**, 107-114.

[29] M Platzer, S Kiese, T Herfellner, U Schweiggert-Weisz, O Miesbauer and P Eisner. Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. *Molecules* 2021; **26(5)**, 1244.

[30] C Vieito, E Fernandes, MV Velho and P Pires. The effect of different solvents on extraction yield, total phenolic content and antioxidant activity of extracts from pine bark (Pinus pinaster subsp. atlantica). *Chemical Engineering Transactions* 2018; **64**, 127-132.

[31] TV Tung, NH Duy, PLK My, NTT Thuy, TPM Nam, LT Hue and TQ Minh. Optimization of phenolic and flavonoid extraction from durian peel: Effects of solvent type, extraction parameters, and antioxidant activity evaluation. *Journal of Xi'an Shiyou University, Natural Science Edition* 2025; **21(1)**, 161-179.

[32] QD Do, AE Angkawijaya, PL Tran-Nguyen, LH Huynh, FE Soetaredjo, S Ismadji and H Ju. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis* 2014; **22(3)**, 296-302.

[33] J Chen, J Yang, L Ma, J Li, N Shahzad and CK Kim. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Scientific Reports* 2020; **10(1)**, 2611.

[34] S Baliyan, R Mukherjee, A Priyadarshini, A Vibhuti, A Gupta, RP Pandey and CM Chang. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of ficus religiosa. *Molecules* 2022; **27(4)**, 1326.

[35] SB Kedare and RP Singh. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology* 2011; **48(4)**, 412-422.

[36] S Rana, NMA Rayhana, SH Emon, T Islam, K Rathry, M Hasana, MI Mansura, BC Srijona, S Islamb, A Raya, A Rakib, A Islamc, KE Zahana, F Hossena and A Asraf. Antioxidant activity of Schiff base ligands using the DPPH scavenging assay: An updated review. *RSC Advances* 2024; **14(45)**, 33094-33123.

[37] IR Ilyasov, VL Beloborodov, IA Selivanova and RP Terekhov. ABTS/PP decolorization assay of antioxidant capacity reaction pathways. *International Journal of Molecular Sciences* 2020; **21(3)**, 1131.

[38] A Kiss, VA Papp, A Pal, J Prokisch, S Mirani, BE Toth and T Alshaal. Comparative study on antioxidant capacity of diverse food matrices: Applicability, suitability and inter-correlation of multiple assays to assess polyphenol and antioxidant status. *Antioxidants* 2025; **14(3)**, 317.

[39] RPP Fernandes, MA Trindade, FG Tonin, CG Lima, SM Pugine, PE Munekata, JM Lorenzo and MPD Melo. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. *Journal of Food Science and Technology* 2016; **53(1)**, 451-460.

[40] NS Rajurkar and SM Hande. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences* 2011; **73(2)**, 146-151.

[41] SJ Hwang and JH Lee. Comparison of antioxidant activities expressed as equivalents of standard antioxidant. *Food Science and Technology, Campinas* 2023; **43**, e121522.

[42] J Junlatat, N Fangkrathok and B Sripanidkulchai. Antioxidative and melanin production inhibitory effects of *Syzygium cumini* (L.) Skeels extracts. *Songklanakarin Journal of Science and Technology* 2018; **40(5)**, 1136-1143.

[43] RM Lima, HC Polonini, MAF Brandao, FJ Raposo, RC Dutra and NRB Raposo. *In vitro* assessment of anti-aging properties of *Syzygium cumini* (L.) leaves extract. *International Journal of Pharmaceutical Sciences and Research* 2022; **13(4)**, 10185-10191.

[44] B Deri, M Kanteev, M Goldfeder, D Lecina, V Guallar, N Adir and A Fishman. The unravelling of the complex pattern of tyrosinase inhibition. *Scientific Reports* 2016; **6**, 34993.

[45] S Zolghadri, A Bahrami, MTH Khan, J Munoz-Munoz, F Garcia-Molina, F Garcia-Canovas and AA Saboury. A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry* 2019; **34(1)**, 279-309.

[46] F Liang. Inhibition mechanism investigation of quercetagetin as a potential tyrosinase inhibitor. *Frontiers in Chemistry* 2024; **12**, 1411801.

[47] MN Masum, K Yamauchi and T Mitsunaga. Tyrosinase inhibitors from natural and synthetic sources as skin-lightening agents. *Reviews in Agricultural Science* 2019; **7**, 41-58.

[48] NS Ashmawy, HA Gad and HAS El-Nashar. Comparative study of essential oils from different organs of *Syzygium cumini* (Pamposia) based on GC/MS chemical profiling and *in vitro* antiaging activity. *Molecules* 2023; **28(23)**, 7861.

[49] N Atale, CB Mishra, S Kohli, RK Mongre, A Prakash, S Kumari, UCS Yadav, R Jeon and V Rani. Anti-inflammatory effects of *S. cumini* seed extract on gelatinase-B (MMP-9) regulation against hyperglycemic cardiomyocyte stress. *Oxidative Medicine and Cellular Longevity* 2021; **2021**, 8839479.

[50] Q Lei, SH Alotaibi and D Yang. Anti-small cell lung cancer and collagenase inhibition properties of hydroxysafflor yellow A. *Archives of Medical Science* 2021. https://doi.org/10.5114/aoms/138833

[51] S Ahmad, M Saleem, N Riaz, Y S Lee, R Diri, A Noor, D Almasri, A Bagalagel and MF Elsebai. The natural polypeptides as significant elastase inhibitors. *Frontiers in Pharmacology* 2020; **11**, 688.

[52] BD Vanjare, YES Eom, H Raza, M Hassan, KH Lee and SJ Kim. Elastase inhibitory activity of quinoline Analogues: Synthesis, kinetic mechanism, cytotoxicity, chemoinformatics and molecular docking studies. *Bioorganic & Medicinal Chemistry* 2022; **63**, 116745.

[53] RJRS Thanapaul, CK Nambur and K Giriraj. Development of multi-herbal formulation with enhanced antimicrobial, antioxidant, cytotoxic, and antiaging properties. *Journal of the Indian Chemical Society* 2024; **101(11)**, 101402.

[54] M Prasathkumar, S Anisha, A Khusro, MM Essa, SB Chidambaram, MW Qoronfleh, S Sadhasivam, MUK Sahibzada, S Alghamdi, M Almehmadi, O Abdulaziz, MU Khandaker, MRI Faruque and TB Emran. Anti-pathogenic, anti-diabetic, anti-

inflammatory, antioxidant, and wound healing efficacy of *datura metel* L. leaves. *Arabian Journal of Chemistry* 2022; **15(9)**, 104112.

[55] SN Silva, IC Abreu, GFC Silva, RM Ribeiro, AS Lopes, MSS Cartagenes, SMF Freire, ACR Borges and MOR Borges. The toxicity evaluation of *Syzygium cumini* leaves in rodents. *Revista Brasileira de Farmacognosia* 2012; **22(1)**, 102-108.

[56] T Alide, P Wangila and A Kiprop. Effect of cooking temperature and time on total phenolic content, total flavonoid content and total *in vitro* antioxidant activity of garlic. *BMC Research Notes* 2020; **13(1)**, 564.

[57] I Esparza, MJ Cimminelli, JA Moler, N Jimenez-Moreno and C Ancin-Azpilicueta. Stability of phenolic compounds in grape stem extracts. *Antioxidants* 2020; **9(8)**, 720.

[58] C Wan, Y Yu, S Zhou, W Liu, S Tian and S Cao. Antioxidant activity and free radical-scavenging capacity of Gynura divaricata leaf extracts at different temperatures. *Pharmacognosy Magazine* 2011; **7(25)**, 40-45.