



Thidiazuron-mediated callus culture in Ivy gourd (*Coccinia grandis* (L.) Voigt) as a promising source of antioxidant and anti-aging potential for phytocosmeceutical applications

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Abstract

Coccinia grandis (L.) Voigt (Ivy gourd), an edible plant in the family Cucurbitaceae, is widely consumed across tropical regions, particularly Southeast Asia. Rich in diverse phytochemicals, this species exhibits multiple pharmacological activities. To establish a sustainable platform for bioactive metabolite production, plant tissue culture represents a promising alternative to wild harvesting. The aim of this study was to establish a novel callus culture system of *C. grandis* and to evaluate the phytochemical profile, along with the antioxidant and anti-aging activities of its extracts. Here, we report for the first time the induction and optimization of *C. grandis* callus culture using thidiazuron (TDZ), a potent cytokinin-like regulator, in combination with 1-naphthaleneacetic acid (NAA). Among the tested conditions, Murashige and Skoog (MS) medium supplemented with 0.5 mg/L TDZ and 0.2 mg/L NAA resulted in the most robust callus formation, producing biomass yields of 162.87 g/L (fresh weight) and 8.39 g/L (dry weight). The callus extract displayed high levels of total phenolics (311.35 µg GAE/g DW) and flavonoids (545.06 µg QE/g DW). Antioxidant activity was significant in FRAP (605.16 µg TE/g DW) and ABTS (255.80 µg TE/g DW) assays. Notably, this is the first report of *C. grandis* callus extract demonstrating antiglycation (58.53% inhibition) and anti-aging potential, including inhibition of tyrosinase (41.06%), elastase (70.24%), and collagenase (72.48%). LC-ESI-QTOF-MS/MS analysis identified rutin as the major flavonoid in the extract. These findings provide valuable insights into the use of *C. grandis* callus cultures as a sustainable source of bioactive metabolites and highlight their potential for future phytopharmaceutical and cosmeceutical applications.

Key message

A novel thidiazuron-induced *C. grandis* callus culture was established, and its extract demonstrated significant antioxidant and anti-aging activities, highlighting its potential for phytocosmeceutical applications.

Keywords Aging · Antioxidant · Callus · *C. grandis* · Flavonoids · Ivy gourd

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Introduction

Modern medicine heavily relies on bioactive compounds derived from natural resources, including plants, microbes, marine organisms, and animals (Mathur and Hoskins 2017; Aneklaphakij et al. 2022; Li et al. 2022; Nasim et al. 2022). Among these, plants encompassing medicinal herbs, edible species, and those used in traditional remedies have long served as a cornerstone for drug discovery (Nasim et al. 2022). Given their rich phytochemical diversity, plants remain a primary focus in the search for novel therapeutic agents to combat emerging and re-emerging diseases such as Ebola, Monkey pox, severe acute respiratory syndrome,

etc (Polgreen and Polgreen 2016). Moreover, they provide bioactive constituents for functional foods, dietary supplements, and cosmeceuticals aimed at promoting health and well-being.

Most plant-derived drug discovery efforts traditionally begin with conventional extraction methods, where raw plant materials collected from nature are processed into powders and extracted using techniques such as maceration, percolation, infusion, or decoction (Sezer et al. 2024). However, these conventional approaches have significant limitations, including the continuous depletion of natural plant populations and the inability to control environmental variables such as temperature, water, soil, humidity, and light factors that critically influence the quantity and quality of phytochemicals (Mozafari et al. 2018; Zheng et al. 2022; Sabindo et al. 2024; Ogwu et al. 2025).

Plant cell and tissue culture techniques have emerged as powerful alternative strategies to overcome these challenges. These methods allow for the sustainable production of plant-specialized metabolites without relying on harvesting wild plant material and enable the generation of high-quality extracts suitable for pharmaceutical formulations, cosmetics, and bioactivity testing, as well as for isolating pure active compounds (Hasnain et al. 2022; Zuzarte et al. 2024). Various in vitro techniques, including whole plant culture, callus culture, cell suspension culture, and hairy root culture, have been developed to facilitate the controlled production of plant-derived bioactives (Espinosa-Leal et al. 2018). Among these, callus culture derived from undifferentiated plant stem cells with totipotency serves as a fundamental platform for further applications, such as regenerating specific plant tissues, establishing cell suspension cultures, or generating genetically modified plants (Espinosa-Leal et al. 2018; Bojko et al. 2024). The induction of callus requires optimization of plant growth regulators (phytohormones), a critical step that determines the success of subsequent culture processes (Dar et al. 2021; Rybin et al. 2024). Today, plant tissue culture is increasingly recognized as a sustainable source of raw materials for the production of a wide range of phytochemicals (Chandran et al. 2020; Krasteva et al. 2021). This approach has found extensive applications in both the cosmetic and pharmaceutical industries (Chandran et al. 2020; Gardiki et al. 2025).

Coccinia grandis (L.) Voigt, commonly known as ivy gourd or scarlet gourd, is a member of the Cucurbitaceae family and is widely consumed as a daily edible plant, particularly its leaves (Lee and Joo 2022; Hossain et al. 2024). Native to tropical regions especially Southeast Asia and subtropical areas, *C. grandis* has attracted considerable attention for its rich phytochemical composition (Lee and Joo 2022; Hossain et al. 2024; Lee et al. 2025). Its bioactive constituents include flavonoids, phenolics, triterpenoids, alkaloids, sterols, and

glycosides, which have been associated with a wide range of pharmacological activities (Lee and Joo 2022; Hossain et al. 2024; Lee et al. 2025). Numerous studies on various parts of *C. grandis*, including the leaves, fruits, and stems, have reported diverse pharmacological properties, such as antidiabetic, antioxidant, spasmolytic, analgesic, antinociceptive, antipyretic, anticonvulsant, antitussive, anti-ulcerogenic, hepatoprotective, wound-healing, anti-dyslipidemic, antimicrobial, and immunomodulatory effects (Sakharkar and Chauhan 2017; Al-Madhagy et al. 2019; Namchaiw et al. 2021; Hossain et al. 2024).

In the context of plant cell and tissue culture, only a few studies have explored the potential of *C. grandis* in this field. This represents a significant research gap and an opportunity to develop an optimized culture system for *C. grandis* as a sustainable source of raw materials for pharmaceutical and cosmetic applications, leveraging its diverse bioactivities highlighted earlier. Recent efforts on *C. grandis* callus cultures have utilized media supplemented with phytohormones such as kinetin and benzylaminopurine (BAP) as cytokinins, and 2,4-dichlorophenoxyacetic acid (2,4-D) indole-3-acetic acid (IAA) and 1-naphthaleneacetic acid (NAA) as auxins, which have demonstrated antimicrobial, antioxidant, hepato-restorative and α -amylase inhibitory activities (Abul Kashem and Mahbubur Rahman 2018; Jayalakshmi et al. 2019; Singh et al. 2023; Yeramilli et al. 2024). However, limitations in the efficacy of these phytohormones suggest the need to explore alternative plant growth regulators. Thidiazuron (TDZ), a potent cytokinin-like regulator, has not yet been investigated in *C. grandis* but is well known for its superior effectiveness in inducing shoot multiplication and callus formation in numerous plant species, including jojoba (*Simmondsia chinensis* (Link) C.K.Schneid.), strawberry (*Fragaria vesca* L.), *Paeonia lactiflora* Pall (El-Ashry et al. 2017; Dewir et al. 2018; Chung and Ouyang 2020; Song et al. 2023; Wang et al. 2025). In addition to its role in morphogenesis, TDZ has been reported to enhance the production of bioactive compounds and biological activities in callus cultures, such as increasing antimicrobial properties in *Linum usitatissimum* L (Anjum and Abbasi 2016). The mechanism of TDZ-induced callogenesis has been hypothesized to involve either competitive inhibition of cytokinin-degrading enzymes or induction of specific gene expression and transcriptional regulators and subsequent auxin or cytokinin production such as trehalose-6-phosphate phosphatase encoding gene (TPP), 1-aminocyclopropane-1-carboxylic acid synthase gene (ACS), and transcription factor-type response regulator genes (ARR1) responsible for callus initiation of alfafa (*Medicago sativa* L. cv. Jinnan) (Zhang et al. 2006; Ali et al. 2022).

In this study, we present the first investigation into *C. grandis* callus induction using TDZ, aiming to establish an efficient culture system and evaluate the biological activities of the resulting callus extracts. Antioxidant potential was assessed using three assays (DPPH, FRAP, and ABTS), and

anti-aging activities were evaluated through antiglycation, α -glucosidase inhibition, and skin-related assays targeting tyrosinase, elastase, and collagenase inhibition. Furthermore, qualitative metabolite profiling was performed using LC-ESI-QTOF-MS/MS. The findings of this study provide valuable insights that could support the development of novel cosmeceutical products and other high-value applications in the future.

Materials and methods

Chemicals and reagents

Murashige and Skoog (MS) medium was obtained from PhytoTech Laboratories (Shawnee Mission, Kansas, USA). LC-MS grade solvents for LC-ESI-QTOF-MS/MS analysis were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical grade and sourced from Sigma-Aldrich (St. Louis, MO, USA).

Establishment of *C. grandis* callus cultures

C. grandis seeds were obtained from a commercial supplier in Chiang Khong, Chiang Rai Province, Thailand. Surface sterilization was performed by immersion in 2% (v/v) sodium hypochlorite for 5 min, followed by 70% (v/v) ethanol for 1 min, and rinsing five times with sterile distilled water. Sterilized seeds were cultured on half-strength Murashige and Skoog (Murashige and Skoog 1962) ($\frac{1}{2}$ MS) medium supplemented with 2 g/L phytagel and 30 g/L sucrose, and incubated in the dark at 25 °C. In vitro plants aged 30 days were used as explant sources for callus induction. Leaf segments ($\sim 0.5 \times 0.5$ cm) and stem segments (0.5 cm in length) were excised and placed on MS medium containing 2 g/L phytagel, 30 g/L sucrose, and thidiazuron (TDZ) at five concentrations (0.1, 0.5, 1.0, 5.0, and 10.0 mg/L) in combination with 1-naphthaleneacetic acid (NAA) at 0.2 mg/L. Each treatment consisted of five replicates with seven explants per replicate. Cultures were maintained at 25°C under a 16/8 h light/dark photoperiod. Calli were subcultured every 30 days on the same medium. After the second subculture, calli were harvested to determine fresh weight and dried at 50°C for 24 h to assess dry weight.

Extraction

Dried calli were ground into a fine powder using a mortar and pestle. Ultrasound-assisted extraction was performed by mixing the callus powder with 90% ethanol at a ratio of 100 mg/mL. The mixture was subjected to an ultrasonic

processor (GT Sonic-D3, Guangdong, China) for 15 min at 40 kHz and 40°C. Following extraction, the samples were centrifuged to obtain a clear supernatant. The extraction process was repeated twice, and the resulting supernatants were pooled. The combined extract was filtered through a 0.45 μ m syringe filter and stored at -20°C until further analysis. For the anti-tyrosinase, anti-elastase, and anti-collagenase assays, the extracts were first evaporated to dryness and subsequently re-dissolved in an equal volume of dimethyl sulfoxide (DMSO) prior to testing.

Determination of total phenolic content (TPC)

The total phenolic content was quantified using the Folin–Ciocalteu method following the protocol of Boonsongcheep and colleagues with minor modification (Boonsongcheep et al. 2019). Briefly, 20 μ L of extract was mixed with 100 μ L of 10% (v/v) Folin–Ciocalteu reagent and incubated at 25°C in the dark for 10 min. Subsequently, 80 μ L of 7% (v/v) sodium carbonate was added, and the mixture was incubated at 25°C in the dark for an additional 30 min. Absorbance was measured at 760 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Gallic acid (3.125–50 μ g/mL) was used to generate a calibration curve ($R^2=0.999$). The TPC of each extract was expressed as micrograms of gallic acid equivalents per gram of dry weight (μ g GAE/g DW).

Determination of total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method, following a previously published protocol with minor modifications (Tungmunthum et al. 2022). Briefly, the reaction mixture consisted of 10% (w/v) aluminum chloride, 10 μ L of 1 M potassium acetate, 160 μ L of deionized water, and 20 μ L of extract. The mixture was incubated at 25°C in the dark for 30 min. Absorbance was measured at 415 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Quercetin (3.125–100 μ g/mL) was used to generate a calibration curve ($R^2=0.999$). TFC was expressed as micrograms of quercetin equivalents per gram of dry weight (μ g QE/g DW).

Assessment of antioxidant activities

DPPH assay

The free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined following a previously published protocol with minor modifications (Traiyasuk et al. 2023). Briefly, the extract was mixed with freshly prepared 0.2 mM DPPH solution at a 1:1 ratio and incubated at

25°C in the dark for 30 min. Absorbance was then measured at 517 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard for the calibration curve. Results were expressed as micrograms of Trolox equivalents per gram of dry weight ($\mu\text{g TE/g DW}$).

Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM ferric chloride in a 10:1:1 ratio, as described previously (Benzie and Devaki 2017). A total of 20 μL of extract was added to 180 μL of FRAP reagent and incubated at 25°C in the dark for 30 min. The absorbance was recorded at 593 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Trolox served as the standard for the calibration curve. The antioxidant activity was expressed as micrograms of Trolox equivalents per gram of dry weight ($\mu\text{g TE/g DW}$).

ABTS assay

The ABTS radical scavenging assay was performed following the protocol of Hussien and Endalew with minor modifications (Hussien and Endalew 2023). The ABTS radical cation ($\text{ABTS}^{\bullet+}$) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate in a 1:1 ratio and incubating the mixture at 25°C in the dark for 12 h. The resulting solution (2.15 mL) was diluted with 50 mL of absolute ethanol and adjusted to an absorbance of 0.70 ± 0.05 at 734 nm to prepare the ABTS working solution. For the assay, 10 μL of extract was added to 190 μL of the ABTS working solution and incubated at 25°C in the dark for 15 min. Absorbance was recorded at 734 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Trolox was used as a reference standard for the calibration curve. Results were expressed as micrograms of Trolox equivalents per gram of dry weight ($\mu\text{g TE/g DW}$).

In vitro anti-aging assays

Antiglycation assay

The ability to inhibit advanced glycation end products (AGEs) formation was evaluated using the BSA–glucose model as previously described, with minor modifications (Starowicz and Zieliński 2019). Briefly, the extract was mixed with bovine serum albumin (BSA; 10 mg/mL) and glucose (90 mg/mL) in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.01% sodium azide at a ratio of 1:1:1. The mixtures were incubated

at 37°C in the dark for 7 days. Aminoguanidine (1 M) was used as the positive control. For the blank, 90% ethanol and 0.1 M sodium phosphate buffer were used in place of the extract and aminoguanidine, respectively. Fluorescence intensity (FI) was measured using a microplate reader (CLARIOstar, BMG Labtech, Germany) with excitation and emission wavelengths of 360 nm and 420 nm, respectively. The percentage inhibition of AGEs formation was calculated using the following equation:

$$\begin{aligned} &\% \text{ inhibition of AGEs formation} \\ &= \frac{\text{FI of blank} - \text{FI of extract}}{\text{FI of blank}} \times 100 \end{aligned}$$

Anti- α -glucosidase activity assay

The anti- α -glucosidase activity was assessed based on a previously established protocol (Chewchinda et al. 2021), using 0.1 M sodium phosphate buffer (pH 6.9) as the reaction medium. A stock solution of α -glucosidase enzyme (2 U/mL) was prepared in the buffer solution and subsequently diluted to a working concentration of 0.1 U/mL. The substrate, *p*-nitrophenyl α -D-glucopyranoside (*p*NPG), was prepared at a final concentration of 2 mM in the buffer solution. *C. grandis* ethanolic extracts were evaluated for their inhibitory activity. Acarbose served as the positive control, while the buffer solution alone was used as the negative control. Reaction mixtures were incubated at 37°C for 30 minutes, and the enzymatic reaction was terminated by adding 200 mM sodium carbonate (Na_2CO_3). The absorbance of the released *p*-nitrophenol was measured at 405 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany) and IC_{50} values were calculated using the equation:

$$\begin{aligned} &\% \alpha - \text{glucosidase inhibition} \\ &= \left[1 - \frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{control blank}})}{(\text{OD}_{\text{sample}} - \text{OD}_{\text{sample blank}})} \right] \times 100 \end{aligned}$$

where, $\text{OD}_{\text{control}}$ = Optical density of the control reaction

$\text{OD}_{\text{control blank}}$ = Optical density of the blank reaction

$\text{OD}_{\text{sample}}$ = Optical density of the sample

$\text{OD}_{\text{sample blank}}$ = Optical density of the sample blank

Anti-tyrosinase assay

The tyrosinase inhibitory activity of the extracts was evaluated following the method of Chai et al., with minor modifications (Chai et al. 2018). L-DOPA (3,4-dihydroxy-L-phenylalanine; 5 mM) was used as the substrate, and kojic acid (1 mM) served as a specific tyrosinase inhibitor and positive control. Reactions were performed in 50 mM sodium phosphate buffer (pH 6.8). For each assay, 10 μL of

extract was combined with 40 μ L of mushroom tyrosinase (0.2 mg/mL) and 20 μ L of L-DOPA. In the blank, DMSO was substituted for the extract. Absorbance was measured at 475 nm immediately after adding L-DOPA, using the kinetic method on a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Tyrosinase inhibition was determined by calculating the initial reaction slope over the first 10 min and comparing it to the control using the following equation:

%Tyrosinase inhibition

$$= \frac{\text{Slope of blank} - \text{Slope of extract}}{\text{Slope of blank}} \times 100$$

Anti-elastase assay

The elastase inhibitory activity of the extracts was assessed based on the method described by Wittenauer et al., with slight modifications (Wittenauer et al. 2015). The reaction was performed in 2 mM Tris buffer (pH 8.0), containing porcine pancreatic elastase (PPE; 5.1 units/mL) as the enzyme and N-Succinyl-Ala-Ala-Ala-p-nitroanilide (AAPVN; 0.25 mg/mL in 2 mM Tris buffer) as the substrate. Oleanolic acid (1 mM) served as a specific elastase inhibitor and was used as the positive control. For each assay, 10 μ L of extract was mixed with 10 μ L of PPE and pre-incubated at 25°C for 20 min. Following incubation, 40 μ L of AAPVN was added to initiate the reaction. In the blank, DMSO was substituted for the extract. The production of p-nitroaniline was monitored in kinetic mode by measuring the absorbance at 410 nm using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). The percentage of elastase inhibition was calculated by comparing the initial reaction slope over the first 10 min between the extract and the control using the following equation:

%Elastase inhibition

$$= \frac{\text{Slope of blank} - \text{Slope of extract}}{\text{Slope of blank}} \times 100$$

Anti-collagenase assay

The collagenase inhibitory activity was assessed following a previously described protocol, with slight modifications (Wittenauer et al. 2015). N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) was used as the substrate, and collagenase from *Clostridium histolyticum* (ChC) served as the enzyme. 1,10-Phenanthroline (100 μ M), a specific collagenase inhibitor, was used as the positive control. In each reaction, 10 μ L of sample, 10 μ L of ChC, and 80 μ L of 0.05 M tricine buffer (containing 0.4 M NaCl and 0.01 M CaCl₂)

were mixed and incubated at 37°C for 20 min. For the blank, DMSO was substituted for the extract. The reaction was initiated by adding 20 μ L of FALGPA, and absorbance was measured immediately in kinetic mode at 335 nm for 20 min using a microplate reader (SPECTROstar^{Nano}, BMG Labtech, Germany). Collagenase inhibition (%) was calculated by comparing the initial slope during the first 10 min of the extract reaction with that of the control, using the following equation:

%Collagenase inhibition

$$= \frac{\text{Slope of blank} - \text{Slope of extract}}{\text{Slope of blank}} \times 100$$

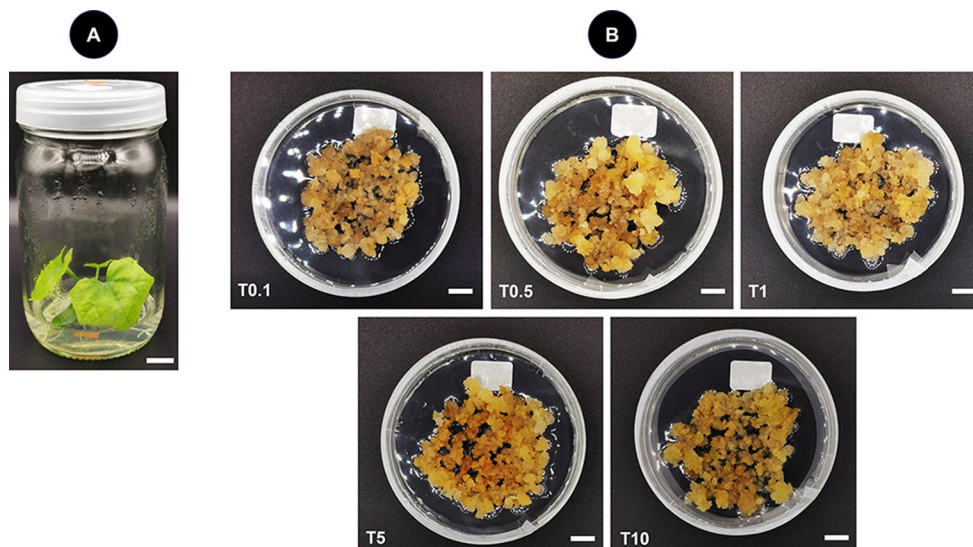
Qualitative analysis of phytochemicals by LC-ESI-QTOF-MS/MS

Phytochemical profiling of the *C. grandis* callus ethanolic extract was performed using liquid chromatography–electrospray ionization–quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS) on an Agilent 6575 A Revident QTOF system (Agilent Technologies, CA, USA). Chromatographic separation of the extract (1 μ L injection volume) was achieved on a reversed-phase Zorbax Eclipse Plus C18 column (3.0 $\times$ 100 mm, 1.8 μ m; Agilent, USA) equipped with a Zorbax Eclipse Plus C18 guard column (3.0 $\times$ 5 mm, 1.8 μ m). The mobile phases consisted of 0.1% (v/v) formic acid in water (solvent A) and 0.1% (v/v) formic acid in acetonitrile (solvent B). The gradient elution program was as follows: 0–20 min, A:B (95:5, v/v) to A:B (5:95, v/v); 20–25 min, A:B (5:95, v/v). A 5-min column re-equilibration was performed between runs. The flow rate was maintained at 0.5 mL/min, and the column temperature was set at 35°C.

Mass spectrometric detection was carried out in the m/z range of 100–1000 Da with a scan rate of 500 ms/spectrum, using a Dual AJS ESI ion source. The MS operating parameters were as follows: nitrogen flow rate, 8 L/min; gas temperature, 325 °C; nebulizer pressure, 30 psi; sheath gas temperature, 250°C; sheath gas flow, 11 L/min; nozzle voltage, 1000 V; capillary voltage (Vcap), 3500 V; fragmentor potential, 175 V; skimmer voltage, 45 V; and Oct 1 RF Vpp, 750 V. MS/MS analyses were performed in automatic mode using collision energies of 10, 20, and 40 V.

Data acquisition and processing were carried out using MassHunter software (Data Acquisition v12.1.98.0; Qualitative Analysis v12.0; Agilent Technologies, CA, USA). Peak identification was achieved by comparing retention times, mass spectra, and fragmentation patterns with published data and reference databases, including the MassHunter METLIN Metabolite Personal Compound Database/

Fig. 1 Callus culture of *C. grandis* on MS medium supplemented with NAA (0.2 mg/L) and varying concentrations of TDZ. (A) in vitro plant of *C. grandis* at 30 days, used for callus induction. (B) callus cultures of *C. grandis* grown on MS medium containing TDZ at 0.1 (T0.1), 0.5 (T0.5), 1 (T1), 5 (T5), and 10 (T10) mg/L. All scale bars represent 1 cm



Library (PCD/PCDL, Agilent Technologies) and the Human Metabolome Database (HMDB; <http://www.hmdb.ca>).

Statistical analysis

Each experiment was conducted with five biological replicates, and each replicate included three technical replicates. All experimental data are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, MA, USA). One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was conducted as recommended by the software. A significance level of $p < 0.05$ was considered statistically significant for all tests. Correlation analysis was performed using IBM SPSS Statistics for Windows, version 30.0.0.0 (IBM Corp., Armonk, NY, USA).

Results and discussion

Effect of thidiazuron (TDZ) on *C. grandis* callus induction and biomass production

C. grandis is a well-known plant widely consumed as a daily food source. Surprisingly, only limited studies have focused on its plant cell and tissue culture, particularly on callus induction. In this study, TDZ was evaluated for the first time as a cytokinin-like regulator to induce callus formation in *C. grandis*. Five TDZ concentrations (0.1, 0.5, 1, 5, and 10 mg/L) were tested in Murashige and Skoog (MS) medium supplemented with a constant concentration of NAA (0.2 mg/L). The NAA concentration was fixed based on previous studies reporting its best efficacy in promoting callus induction (Jayalakshmi et al. 2019). Thirty-day-old

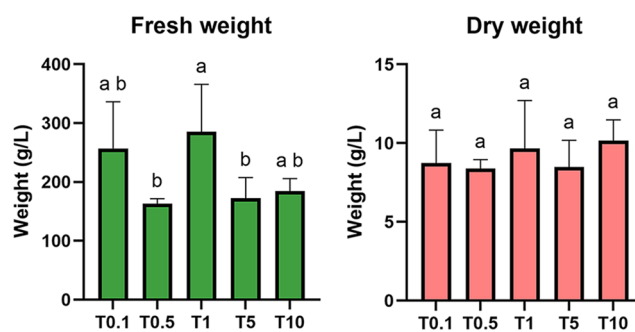


Fig. 2 Callus culture of *C. grandis* on MS medium supplemented with NAA (0.2 mg/L) and varying concentrations of TDZ. (A) in vitro plant of *C. grandis* at 30 days, used for callus induction. (B) callus cultures of *C. grandis* grown on MS medium containing TDZ at 0.1 (T0.1), 0.5 (T0.5), 1 (T1), 5 (T5), and 10 (T10) mg/L. All scale bars represent 1 cm

in vitro plants of *C. grandis* were used to initiate callus cultures (Fig. 1A).

Callus formation was first observed after 7 days of culture, with a callogenic frequency of 100% at all TDZ concentrations tested (Supplementary Fig. S1). As a control, cultures containing only NAA (0.2 mg/L) in the absence of TDZ were also established; however, no callus formation was observed, and root development occurred instead (Supplementary Fig. S2).

After the second subculture passage, calli from all TDZ concentrations displayed similar morphological characteristics, appearing as yellow, friable tissue (Fig. 1B). Maximum fresh weight was recorded in calli grown on MS medium containing NAA 0.2 mg/L supplemented with TDZ 1 mg/L (285.40 g/L), while the highest dry weight was observed at TDZ 10 mg/L (10.17 g/L) (Fig. 2). In contrast, the lowest biomass, both in terms of fresh and dry weights, was obtained at TDZ 0.5 mg/L, with a fresh weight of 162.87 g/L and a dry weight of 8.39 g/L (Fig. 2). Although fresh weight

values exhibited high variability commonly reported in similar experiments (Alsafran et al. 2022; Choi et al. 2025), dry weight was considered a more reliable parameter for evaluating biomass for pharmaceutical and cosmeceutical applications.

Despite TDZ 10 mg/L yielding the highest dry weight, no statistically significant differences were observed between TDZ concentrations. This finding suggests that lower TDZ levels may be more advantageous for callus culture, not only to minimize potential disruptions of endogenous hormonal balance during long-term maintenance through successive subcultures but also for cost-effectiveness (Anjum et al. 2017).

The concentration and balance of phytohormones are critical for callogenesis and the production of plant specialized metabolites (Tariq et al. 2024). Our results confirm that both auxin and cytokinin are essential in appropriate proportions for efficient callus induction, consistent with previous studies (Ishii et al. 1998; Ikeuchi et al. 2013; Bakhtiar et al. 2025). Cytokinin such as TDZ can modulate auxin responses, influencing gene expression involved in callogenesis (Abdelazeez et al. 2025). Notably, supplementing NAA with other phytohormones has been widely reported to enhance callus induction efficiency in numerous plant species (Nazir et al. 2020).

TDZ has demonstrated superior performance in promoting callus induction and biomass production compared to other cytokinins such as kinetin and BAP in several plant species, including *Ocimum basilicum* cv. Purple Basil, and *Solanum virginianum* L (Nazir et al. 2019; Usman et al. 2022). Successful callus induction with TDZ has also been reported in *Afgekia mahidoliae* B.L. Burt & Chermis, *Alcea rosea* L., *Nothapodytes nimmoniana* (J.Graham) Mabb., *Curcuma longa* L., *Hibiscus syriacus* L., and *Paeonia lactiflora* Pall (Seo et al. 2017; Kaewthip et al. 2021; Traiyasuk et al. 2023; Kadam et al. 2023; Song et al. 2023; Tariq et al. 2024). TDZ at 0.5 and 1 mg/L exhibited a robust

capacity for callus induction of *Sophora flavescens* Aiton, aligning with the results observed in our study (Park et al. 2020). In comparison to previous studies using kinetin, BAP, 2,4-D, or IAA (Abul Kashem and Mahbubur Rahman 2018; Jayalakshmi et al. 2019), our results achieved a 100% callogenic frequency, underscoring the higher potency of TDZ as a cytokinin relative to BAP and kinetin (Huetteman and Preece 1993).

However, determining the optimal TDZ concentration for callus culture requires considering factors beyond biomass production. In this study, further analyses including quantification of total phenolic and flavonoid content and evaluation of antioxidant and anti-aging activities were performed to identify the most suitable culture conditions for generating biologically active extracts.

Total phenolic and flavonoid contents (TPC and TFC)

Phenolic and flavonoid contents were quantified, as these two major phytochemical groups are well recognized for contributing to a range of biological activities, particularly antioxidant and anti-aging effects (Namchaiw et al. 2021; Fan et al. 2022). The levels of TPC and TFC in callus cultures were observed to vary depending on the concentration of phytohormones. Notably, TDZ has previously been reported to enhance TPC and TFC in *Solanum virginianum* callus cultures, outperforming other phytohormones such as BAP and NAA (Usman et al. 2022). This enhancement is attributed to TDZs ability to stimulate the phenylpropanoid pathway by activating phenylalanine ammonia-lyase (PAL), a key enzyme involved in the biosynthesis of phenolics and flavonoids (Usman et al. 2022; Tariq et al. 2024).

In the present study, the highest TPC (311.35 $\mu\text{g GAE/g DW}$) and TFC (545.06 $\mu\text{g QE/g DW}$) were observed in callus cultures grown on MS medium containing NAA 0.2 mg/L supplemented with TDZ 0.5 mg/L (Fig. 3). Conversely, the lowest TPC was recorded at TDZ 0.1 mg/L (215.92 $\mu\text{g GAE/g DW}$), while the lowest TFC was observed at TDZ 10 mg/L (251.03 $\mu\text{g QE/g DW}$) (Fig. 3). Although TDZ 0.5 mg/L appeared to yield the highest phenolic and flavonoid content, no statistically significant differences in TPC were found among the five TDZ concentrations (Fig. 3). For TFC, no significant differences were observed among TDZ concentrations of 0.5, 1, and 5 mg/L. Based on these findings, TDZ 0.5 mg/L appears to be the optimal concentration for promoting phenolic and flavonoid accumulation in *C. grandis* callus cultures (Fig. 3).

Interestingly, while increasing TDZ concentrations initially enhanced TPC and TFC, further increases beyond an optimal point resulted in decreased or fluctuating levels. Similar patterns have been reported in callus cultures of *A. rosea*, *S. virginianum*, *Linum usitatissimum*, and *Sophora*

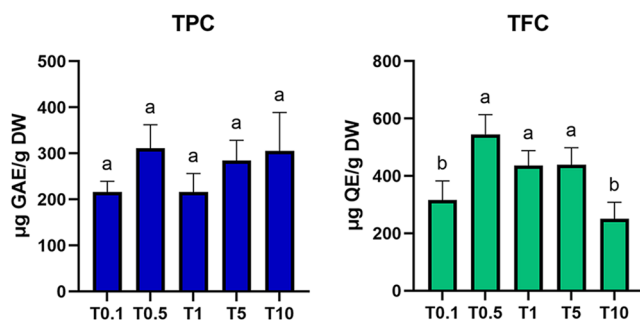


Fig. 3 Total phenolic (TPC) and flavonoid content (TFC) of *C. grandis* callus cultured on MS medium supplemented with NAA (0.2 mg/L) and varying concentrations of TDZ. Data are expressed as mean $\pm$ standard deviation ($n=5$). Different superscript letters within the same column indicate statistically significant differences ($p<0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test

flavescens (Park et al. 2020; Khan et al. 2020; Usman et al. 2022; Tariq et al. 2024). This phenomenon is not unique to TDZ; comparable trends were observed with other phytohormones, such as 2,4-D and kinetin, in *Ajuga xylorrhiza* callus cultures, where higher phytohormone levels led to reduced TPC and TFC accumulation (Orcan and Orcan 2024).

Moreover, the TPC in *C. grandis* callus extracts was considerably lower than that reported in wild *C. grandis* leaves ($1200.0 \pm 31.0 \mu\text{g GAE/g DW}$) (Thummajitsakul et al. 2021). Similarly, TFC levels in the callus cultures were reduced compared to wild plants. This trend aligns with findings from other species, such as *Saussurea costus*, where callus cultures also exhibited lower flavonoid content than wild-grown plants (Khan et al. 2021). Such reductions may result from the controlled environmental conditions of laboratory cultures (e.g., constant temperature, humidity, and carbon sources), which lack the biotic and abiotic stresses that stimulate secondary metabolite production in wild plants (Yeshe et al. 2022; Tariq et al. 2024). Under stress or extreme environmental conditions, plants often enhance phenolic and flavonoid synthesis as part of their defense mechanisms (Yeshe et al. 2022).

In vitro cell-free antioxidant activities

Reactive oxygen species (ROS) are among the primary contributors to cellular damage, leading to lipid peroxidation, protein and DNA damage, and the onset of various oxidative stress-related diseases, including accelerated cellular aging (Xiao et al. 2020). Given this, evaluating antioxidant potential is critical, particularly using both electron transfer (ET)-based and hydrogen atom transfer (HAT)-based assays to account for the multifaceted mechanisms of antioxidant action (Xiao et al. 2020). In the present study, the in vitro antioxidant capacity of *C. grandis* callus extracts was assessed using three complementary assays: DPPH,

FRAP, and ABTS. DPPH reflects both ET and HAT mechanisms, while FRAP and ABTS primarily assess ET- and HAT-based antioxidant activities, respectively (Nazir et al. 2020; Usman et al. 2022). The combined use of these assays provides a comprehensive understanding of the extract's potential to scavenge diverse ROS types.

All antioxidant activities were expressed as $\mu\text{g Trolox equivalent (TE)/g dry weight (DW)}$ (Fig. 4). Among the tested extracts, TDZ 10 mg/L yielded the highest DPPH scavenging activity ($283.53 \mu\text{g TE/g DW}$), although the difference was not statistically significant compared to TDZ 0.5 and 5 mg/L. The lowest DPPH activity was recorded at TDZ 1 mg/L ($206.35 \mu\text{g TE/g DW}$). Interestingly, the highest FRAP ($605.16 \mu\text{g TE/g DW}$) and ABTS ($255.80 \mu\text{g TE/g DW}$) values were observed in extracts from callus cultures grown on MS medium containing NAA 0.2 mg/L supplemented with TDZ 0.5 mg/L. In contrast, the lowest FRAP and ABTS activities were observed at TDZ 10 mg/L ($464.93 \mu\text{g TE/g DW}$) and TDZ 0.1 mg/L ($189.38 \mu\text{g TE/g DW}$), respectively. Taken together, these findings suggest that callus cultures induced with TDZ 0.5 mg/L exhibited the most robust antioxidant potential across all assays. This observation correlates strongly with the elevated total phenolic and flavonoid contents (TPC and TFC) in these extracts, supporting the notion that phenolic and flavonoid compounds contribute significantly to antioxidant capacity. Similar positive correlations between TPC/TFC and antioxidant activity have been reported in various plant species such as *O. basilicum* L. cv 'Thai Basil', *S. virginianum* (Nazir et al. 2020; Usman et al. 2022). These results also align with previous studies on *C. grandis* callus, which demonstrated potent antioxidant activity with low IC_{50} values in DPPH and FRAP assays (Jayalakshmi et al. 2019; Singh et al. 2023).

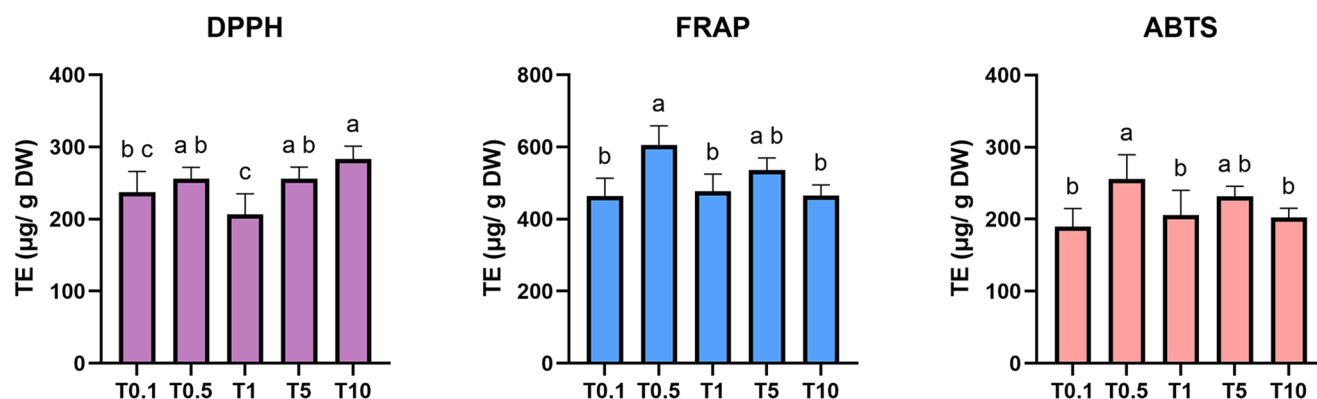


Fig. 4 In vitro cell-free antioxidant activities of *C. grandis* callus cultured on MS medium supplemented with NAA (0.2 mg/L) and varying concentrations of TDZ. Data are expressed as mean $\pm$ standard

deviation ($n=5$). Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test

Table 1 Inhibition of advanced glycation end-products (AGEs) by *C. grandis* ethanolic extract

Sample	%inhibition*
Aminoguanidine	89.83 ± 1.10 ^a
T0.1	55.85 ± 1.27 ^b
T0.5	58.53 ± 1.05 ^b
T1	50.67 ± 2.06 ^c
T5	50.46 ± 2.72 ^c
T10	42.07 ± 2.09 ^d

*Values are presented as mean ± standard deviation ($n=5$). Different superscript letters within the same column indicate statistically significant differences ($p<0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test

Discovery of anti-aging activities

Inhibition of advanced glycation end products (AGEs) formation (anti-AGEs)

Advanced glycation end products (AGEs) are harmful molecules generated through irreversible, nonenzymatic reactions between reactive carbonyl groups of reducing sugars and nucleic acids, lipids, or proteins (Rungratanawanich et al. 2021). This process leads to the production of reactive oxygen species (ROS), triggering oxidative stress and chronic inflammation (Rungratanawanich et al. 2021). Ultimately, AGEs contribute to protein dysfunction, cellular apoptosis, and tissue damage, playing a key role in the pathogenesis of various age-related disorders, including cardiovascular and neurodegenerative diseases, diabetes, immune system dysregulation, cancer, and musculoskeletal conditions (Li et al. 2021; Chen et al. 2022; Zhu et al. 2024). In dermatology, AGEs are directly associated with skin aging manifestations such as wrinkle formation, loss of elasticity, and dull or yellowed skin tone due to protein cross-linking and structural deterioration (Zheng et al. 2022).

To our knowledge, this study is the first to report the anti-AGEs activity of *C. grandis* callus culture extracts. Among the tested conditions, the extract from callus induced with TDZ 0.5 mg/L exhibited the highest inhibition of AGEs formation (58.53%), while the lowest inhibition was observed in extracts from TDZ 10 mg/L cultures (42.07%) (Table 1). Although these values are lower than the positive control, aminoguanidine (89.83% inhibition), they still demonstrate promising anti-glycation potential.

Notably, the antiglycation activity of the callus extracts was lower than that reported for wild *C. grandis* leaf ethanolic extracts, which achieved 95.9% inhibition (Kaewnarin et al. 2014). Since phenolic and flavonoid compounds are well known for their pivotal roles in inhibiting AGEs formation, as demonstrated in numerous previous studies (Yeh et al. 2017; Yang et al. 2018; Chociey et al. 2024; El Allaoui et al. 2025), this disparity may be attributed to the comparatively

Table 2 IC₅₀ values of α -glucosidase inhibitory activity of *C. grandis* callus extracts treated with various TDZ concentrations (0.1–10 mg/mL)

Sample	IC ₅₀ (μg/mL) *
Acarbose	624.90 ± 5.02 ^d
T0.1	850.64 ± 27.47 ^{bc}
T0.5	1287.07 ± 9.36 ^a
T1	790.10 ± 8.20 ^c
T5	1243.93 ± 36.77 ^a
T10	865.09 ± 30.46 ^b

*Values are presented as mean ± standard deviation ($n=5$). Different superscript letters within the same column indicate statistically significant differences ($p<0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test

lower total phenolic and flavonoid contents (TPC and TFC) in the callus cultures. Additionally, the absence of certain secondary metabolites often synthesized in wild plants under natural environmental stressors may also contribute to the reduced antiglycation activity observed in the callus extracts.

Despite this, the moderate inhibition of AGEs formation observed in the *C. grandis* callus extracts suggests potential utility as an anti-aging agent in cosmeceutical formulations. Moreover, to enhance the value of these extracts for skin care applications, we further investigated their inhibitory effects on key skin-aging-related enzymes, including tyrosinase, elastase, and collagenase, as described in the following sections.

Anti- α -glucosidase activity of *C. grandis* callus extracts

The α -glucosidase inhibitory activity of *C. grandis* callus extracts treated with various concentrations of TDZ was evaluated and compared to the standard inhibitor, acarbose. The inhibitory activities were expressed as IC₅₀ values, with lower IC₅₀ indicating stronger inhibition (Table 2). Acarbose exhibited potent activity, with an IC₅₀ of 624.90 ± 5.02 μg/mL. Among the TDZ-treated callus samples, the extract derived from callus grown with TDZ 1 mg/L showed the highest inhibition (IC₅₀ = 790.10 ± 8.20 μg/mL), followed by TDZ 0.1 mg/L (850.64 ± 27.47 μg/mL) and TDZ 10 mg/L (865.09 ± 30.46 μg/mL). In contrast, the samples TDZ 0.5 and 5 mg/L demonstrated the weakest activity, with IC₅₀ values of 1287.07 ± 9.36 μg/mL and 1243.93 ± 36.77 μg/mL, respectively. Among the extracts, TDZ 0.5 and 5 mg/L were statistically different from TDZ 0.1, 1 and 10 mg/L, with higher IC₅₀ values indicating significantly weaker inhibition. TDZ 1 mg/L was significantly more active than TDZ 0.5 and 5 mg/L but not significantly different from TDZ 0.1 and 10 mg/L. These findings indicate that the callus extracts could be grouped into three activity levels: high (TDZ 1 mg/L), moderate (TDZ 0.1 and 10 mg/L), and

low (TDZ 0.5 and 5 mg/L). The results demonstrate that TDZ treatment has a substantial influence on the antidiabetic potential of *C. grandis* callus, likely by modulating the production of bioactive secondary metabolites. TDZ is known to act as a plant hormone in plant tissue cultures, often enhancing the accumulation of phenolic compounds and flavonoids, both of which are reported to contribute to α -glucosidase inhibition (Ahmad and Faisal 2018). The stronger activity observed at lower to moderate TDZ concentrations (particularly at 1 mg/L) suggests that this level may promote the optimal biosynthesis of such compounds. Conversely, the reduced activity at 0.5 and 5 mg/L may indicate either suboptimal stimulation or inhibitory effects on metabolite synthesis due to hormonal imbalance or stress. Interestingly, the extract from callus treated with the highest TDZ concentration (10 mg/L) showed improved activity compared to the mid-range concentrations, though it did not surpass that of TDZ 1 mg/L. This may suggest a non-linear dose-response relationship where only specific TDZ levels favor the accumulation of active phytochemicals. Such patterns are consistent with other studies where plant growth regulators induced varying effects on secondary metabolite profiles, depending on their concentrations and the species involved (Patel and Ishnava 2015; Yusakul et al. 2020). Overall, although the *C. grandis* callus extracts did not exhibit inhibitory potency comparable to acarbose, they demonstrated distinct α -glucosidase inhibitory activity that varied with TDZ concentration, an aspect that, to our knowledge, has not been previously investigated. These findings suggest that *C. grandis* callus, particularly when cultured with 1 mg/L TDZ, holds potential as a source of antidiabetic agents. Future studies focusing on phytochemical profiling and identification of active constituents will help elucidate the molecular basis of the observed inhibitory effects. Moreover, optimizing culture conditions to enhance

the production of these metabolites could further improve the bioactivity of the extracts for potential therapeutic applications, especially antidiabetic purpose.

Potential of skin anti-aging: tyrosinase, elastase and collagenase inhibition

To identify natural agents with skin anti-aging and whitening properties, the inhibition of three key enzymes, including tyrosinase, elastase, and collagenase, is widely employed as a fundamental screening approach for plant extracts (Eun Lee et al. 2019; Younis et al. 2022; Piyasena et al. 2024). These assays generate crucial data for the development of cosmeceutical products. Although *C. grandis* is not traditionally recognized for skin anti-aging or whitening applications in ethnomedicine, a recent study (2024) has reported (Chuchote and Somwong 2024), for the first time, tyrosinase inhibition by *C. grandis* leaf extract. Notably, the present study is the first to comprehensively evaluate the inhibitory potential of *C. grandis* callus extracts on all three enzymes across different TDZ concentrations (Fig. 5).

All *C. grandis* callus extracts demonstrated inhibitory activity against tyrosinase, elastase, and collagenase, albeit with varying potencies. Among these, the extracts showed particularly strong inhibition of elastase and collagenase. The highest elastase ($70.24 \pm 1.36\%$) and collagenase ($72.48 \pm 3.74\%$) inhibition were observed in callus cultured on medium supplemented with TDZ 0.5 mg/L. Conversely, TDZ 5 mg/L yielded the lowest elastase inhibition ($60.46 \pm 0.07\%$), while TDZ 10 mg/L exhibited the lowest collagenase inhibition ($51.86 \pm 4.06\%$). This pattern may correlate with reduced total phenolic and flavonoid contents (TPC and TFC) at higher TDZ concentrations, as these phytochemicals are widely reported to play critical roles in elastase and collagenase inhibition.

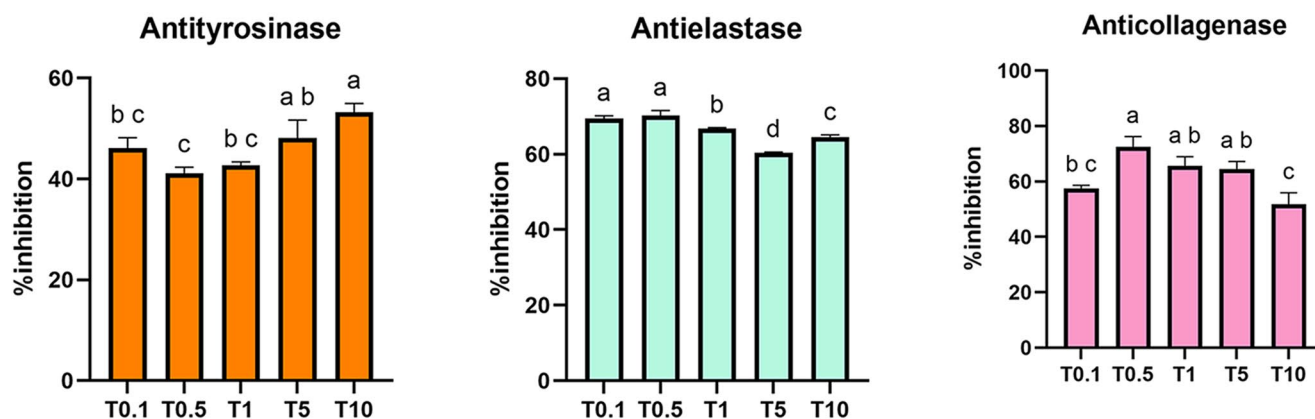


Fig. 5 Anti-aging potential of *C. grandis* callus cultured on MS medium supplemented with NAA (0.2 mg/L) and varying concentrations of TDZ, assessed via tyrosinase, elastase, and collagenase inhibition assays. Kojic acid (1 mM), oleanolic acid (1 mM), and 1,10-phenanthroline (100 μ M) were used as specific inhibitors for tyrosinase,

elastase, and collagenase, showing inhibition rates of $75.97 \pm 0.59\%$, $90.41 \pm 0.29\%$, and $80.89 \pm 3.93\%$, respectively. Values are presented as mean $\pm$ standard deviation ($n = 5$). Different superscript letters within each column indicate statistically significant differences ($p < 0.05$), determined by 1-way ANOVA followed by Tukey's post hoc test

In contrast, the highest tyrosinase inhibition was observed in callus cultured with TDZ 10 mg/L ($53.24 \pm 1.73\%$), while TDZ 0.5 mg/L exhibited the lowest activity ($41.06 \pm 1.27\%$). This result suggests that compounds other than phenolics and flavonoids may contribute to antityrosinase activity in *C. grandis* callus extracts. Importantly, our findings demonstrate that *C. grandis* callus, like the leaf extracts reported previously, possesses notable tyrosinase inhibitory potential (Chuchote and Somwong 2024).

Overall, these findings underscore the promising anti-aging potential of *C. grandis* callus extracts for development into phytocosmeceutical products. Among the tested conditions, callus cultures grown on MS medium supplemented with 0.5 mg/L TDZ and 0.2 mg/L NAA emerged as the most optimal system for producing extracts with superior antioxidant and anti-aging activities. Beyond their bioactivity, callus cultures offer significant advantages over wild-harvested plants as a sustainable raw material source (Nazir et al. 2020). They minimize environmental impact, enable precise control over metabolite production under standardized culture conditions, maintain sterility, and remain unaffected by external factors such as temperature, soil composition, humidity, and light fluctuations (Bojko et al. 2024). Furthermore, callus systems can be scaled up to cell suspension cultures and enhanced with elicitors such as monochromatic light, methyl jasmonate, yeast extract, or microorganisms to stimulate the accumulation of bioactive phytochemicals particularly phenolics and flavonoids thereby further boosting their anti-aging efficacy (Kumari et al. 2023).

Correlation analysis

To explore the relationship between phytochemicals (i.e., TPC and TFC) and biological activities, Pearson correlation coefficients (PCCs) were computed (Table 3). The analysis revealed that TPC was highly correlated with DPPH antioxidant activity and showed moderate correlations with FRAP, ABTS, and anti- α -glucosidase activities. Weak correlations were observed between TPC and anti-tyrosinase

and anti-collagenase activities. However, none of these correlations reached statistical significance.

In contrast, TFC exhibited a strong correlation with anti-collagenase activity, which was statistically significant ($p < 0.01$). TFC also displayed high correlations with antioxidant activities, particularly FRAP and ABTS, suggesting that these assays are more closely associated with TFC than TPC. Moderate correlations were observed between TFC and both anti-AGEs and anti- α -glucosidase activities, while low correlation was found with anti-elastase activity; however, these were not statistically significant.

This finding aligns with previous reports highlighting the pivotal role of flavonoids in inhibiting collagenase activity (Bo and Hyun 2005; Nutho and Tungmunthum 2023). To support this observation, LC-ESI-QTOF-MS/MS analysis was conducted to identify flavonoids present in *C. grandis* callus extracts, providing valuable insights for future pharmaceutical and cosmeceutical product development.

Phytochemical analysis by LC-ESI-QTOF-MS/MS

As this is the first study to investigate *C. grandis* callus extract induced by TDZ and NAA, the phytochemical composition of the extract was qualitatively analyzed. The callus extract obtained from cultures grown on MS medium supplemented with 0.5 mg/L TDZ and 0.2 mg/L NAA was selected for LC-ESI-QTOF-MS/MS analysis, as it demonstrated superior biomass production, total phenolic and flavonoid contents, antioxidant capacity, and anti-aging activities. A total of eleven metabolites were identified, encompassing both primary and secondary metabolites (Table 4). Among the secondary metabolites, the flavonol glycoside rutin (quercetin-3-*O*-rutinoside) was detected. Rutin has been previously reported in both the leaves and fruit extracts of *C. grandis*, with recent studies showing a higher concentration in leaves compared to fruit (Lee and Joo 2022, Hossain et al. 2024; Lee et al. 2025). However, the diversity and quantity of phytochemicals present in the callus extract appeared lower than those of the wild plant, likely due to the controlled in vitro culture conditions, which lack environmental stressors that can enhance secondary metabolite production. Based on previous reports, rutin is a key bioactive compound known for its antioxidant and anti-aging properties, including antiglycation, anti- α -glucosidase, anti-tyrosinase, anti-elastase, and anti-collagenase activities, and is hypothesized to contribute substantially to the bioactivities observed in *C. grandis* callus extracts (Choi et al. 2016, 2021; Enogieru et al. 2018; Amnuaykan et al. 2024; El-Sharkawy et al. 2024).

Table 3 Pearson correlation coefficients (PCCs) between total phenolic content (TPC) and total flavonoid content (TFC) and the biological activities of *C. grandis* callus extracts. Statistically significant correlations are shown in bold. $p < 0.01$ was considered significant

	TPC	TFC
DPPH	0.844	−0.337
FRAP	0.597	0.86
ABTS	0.677	0.837
AGEs	−0.211	0.685
α -glucosidase	0.667	0.696
Tyrosinase	0.289	−0.831
Elastase	−0.239	0.193
Collagenase	0.102	0.997*

Table 4 Metabolic profiling by LC-ESI-QTOF-MS/MS of *C. grandis* ethanolic callus extract

No.	Compound	RT (min)	Formula	MW	m/z	Error (ppm)	Adduct	Fragment ions (MS/MS)
1	L-Malic acid	1.008	C ₄ H ₆ O ₅	134.0215	133.014	1.64	[M-H] ⁻	115.0034
2	Succinic acid	1.608	C ₄ H ₆ O ₄	118.0266	117.0191	1.6	[M-H] ⁻	73.0291
3	Leucine	1.664	C ₆ H ₁₃ NO ₂	131.0946	132.1019	0.12	[M-H] ⁺	86.0963
4	Coumaric acid	3.657	C ₉ H ₈ O ₃	164.0473	163.0399	1.03	[M-H] ⁻	119.0500, 93.0342
5	Rutin	5.102	C ₂₇ H ₃₀ O ₁₆	610.1533	609.1452	1.57	[M-H] ⁻	447.0920, 301.0273
6	Phytosphingosine	10.309	C ₁₈ H ₃₉ NO ₃	317.2929	318.3004	-0.41	[M-H] ⁺	256.2635
7	Cuscohygrine	11.019	C ₁₃ H ₂₄ N ₂ O	224.1888	225.1962	-0.26	[M-H] ⁺	143.1179, 100.1120
8	Sphinganine	11.775	C ₁₈ H ₃₉ NO ₂	301.2980	302.3056	-1.01	[M-H] ⁺	106.0859, 59.0696
9	Ceramide(t18:0/16:0)	19.056	C ₃₄ H ₆₉ NO ₄	555.5226	556.5312	-2.21	[M-H] ⁺	494.4944, 371.2254, 283.2636
10	GlcCer(d18:1(8Z)/16:0(2OH[R]))	19.842	C ₄₀ H ₇₇ NO ₉	715.5598	716.5685	-1.94	[M-H] ⁺	554.5150, 536.5046, 282.2774
11	Ceramide(d18:0/18:0)	21.074	C ₃₆ H ₇₃ NO ₃	567.5590	568.5674	-1.88	[M-H] ⁺	311.2950, 284.2950, 88.0752

Conclusion and future perspectives

Coccinia grandis (L.) Voigt, a well-known edible plant, is rich in phytochemicals, particularly phenolics and flavonoids. This study reports, for the first time, the successful induction of *C. grandis* callus cultures using thidiazuron (TDZ), a potent cytokinin-like phytohormone, in combination with the auxin, i.e. 1-naphthaleneacetic acid (NAA). The optimal phytohormone concentrations for callus induction and culture were identified as 0.5 mg/L TDZ and 0.2 mg/L NAA in MS medium. The resulting ethanolic callus extract exhibited promising biological activities, including antioxidant potential (evaluated via DPPH, FRAP, and ABTS assays) and anti-aging effects such as inhibition of advanced glycation end products (AGEs), α -glucosidase, tyrosinase, elastase, and collagenase. Notably, the extract showed strong anti-elastase and anti-collagenase activities, highlighting its potential for applications in phytocosmeceutical development. Phytochemical profiling via LC-ESI-QTOF-MS/MS revealed rutin as the predominant flavonoid in the callus extract, supporting its contribution to the observed bioactivities. For future work, scaling up callus cultures to cell suspension systems in bioreactors should be explored to enable sustainable and large-scale production of bioactive metabolites. The use of elicitors to enhance secondary metabolite accumulation, particularly phenolics and flavonoids, warrants investigation to improve extract quality and potency. Moreover, comprehensive toxicity assessments are essential to ensure the safety of *C. grandis* callus extracts for pharmaceutical or cosmeceutical applications. Overall, this study provides valuable insights into *C. grandis* callus

culture and its bioactivities, offering a foundation for future research and development of innovative phytocosmeceutical products.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate This study did not involve any experiments on human participants or animals performed by any of the authors. All authors have provided their consent for participation and publication.

Conflict of interest The authors declare that they have no conflicts of interest.

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