Assessment of the ethanolic extract of *Coccinia grandis* on *in vitro* anti-tyrosinase and anti-inflammatory activities and its active chemical determination

1Chuchote, C. and 2*Somwong, P.

1Department of Pharmacognosy, College of Pharmacy, Rangsit University, Pathumthani 12000, Thailand

2Department of Pharmaceutical Chemistry, College of Pharmacy, Rangsit University, Pathumthani 12000, Thailand

**Abstract**

Cucurbitaceae plants have figured prominently in the world as edible medicinal plants, supplying essential elements and minerals to support human health and prevent some diseases caused by malnutrition. *Coccinia grandis* (L.) Voigt, also known as the ivy gourd, is one of the most popular cucurbitaceous plants that has spread throughout the tropics. For a long time, the plant has been cultivated as a food crop, and its aerial parts have been used as nutritious vegetables. *Coccinia grandis* extract has been scientifically reported to be efficient in various pharmacological investigations such as antioxidant, antimicrobial, and cell proliferative properties, and it was recently affirmed as a potential medicinal herb with antidiabetic properties. This suggests that developing plant extracts as functional ingredients in food and medicine is more advantageous. Thus, the goal of this study was to elaborate on previously unreported pharmacological activities as well as to identify the active phytochemical compound responsible for their action. In this study, *C. grandis* ethanolic extract was confronted with a variety of *in vitro* biological assays, including tyrosinase inhibition activity and anti-inflammatory activity. When compared to the positive controls, the plant extract displayed remarkable anti-tyrosinase activity with an IC\textsubscript{50} value of 0.29±0.06 mg/mL and anti-inflammatory activity with an IC\textsubscript{50} value of 9.63±1.10 mg/mL. The active triterpenoid compound lupeol was found in the sample at a level of 18.87±0.79 mg per 100 g of dry extract, as shown by the HPLC profile of the extract. The current study demonstrated the presence of an active chemical in *C. grandis* extract, which could support the prospect of integrating this extract into herbal food products that become beneficial to health.

1. **Introduction**

*Coccinia grandis* (L.) Voigt, also known as ivy gourd, is a type of edible plant that belongs to the genus *Coccinia* and is a perennial climber that has sturdy roots, slender stems, solitary tendrils, and hairless leaves. As a member of the family Cucurbitaceae, it is one of the most well-known plants that can have its entire plant prepared as a cooked vegetable. *Coccinia grandis* is an East African native plant that has spread by seed to various parts of tropical Asia and the Pacific region (Sapkota et al., 2021). Tam Leung, as it is called in Thailand, is a highly valued indigenous crop for diet planning because it contains significant amounts of essential minerals, such as calcium and iron, and important vitamins, including vitamins A and C (Patel et al., 2021). This plant has not only been identified as an important source of nutrients, but it also plays an important role in medicinal utilization. Almost any part of the plant has been used as a traditional medicine in Asian countries to treat skin diseases such as leprosy, acne, scabies, and wounds. It has also been used to treat poisoning, malaria, jaundice, and hepatitis, and it is now traditionally used to treat diabetes and obesity (Pekamwar et al., 2013). In Southeast Asia, the squashed fresh leaves of this plant are used as a traditional herbal treatment for bruises and itching from bug bites. This is done by applying the crushed leaves directly to the lesion. So, it was also added as one of the traditional home remedies used to treat common illnesses (Namchawi et al., 2021).

Previous research has shown that ivy gourd extract contains a significant concentration of phytochemicals (e.g., tannins, saponins, alkaloids, flavonoids,
triterpenoids and steroid compounds), and these phytoconstituents have been shown to be active principles in numerous pharmacological investigations (Laboni et al., 2017). As shown by previous studies, they may be responsible for the medicinal effects of the plant extract, such as its antioxidant, antiallergic, antiulcer, antimicrobial, antihelmintic, anti-inflammatory, analgesic, anti-diabetic, anti-hyperlipidemic and cytoxic properties (Sakharkar and Chauhan, 2017; Siddiqua et al., 2021). Recently, a herbal drug that was developed from the extract of C. grandis showed promise as a beneficial treatment for patients who had been diagnosed with type 2 diabetes mellitus. In randomized, controlled clinical trials, the drug improved glycemic and lipid profile boundaries significantly and was safe and well tolerated (Wasana et al., 2021). It also has evidence that the ethanolic extract of the plant could be used in herbal preparations safely for the clinical development of antibacterial activity for multidrug resistant strains (Alshahrani et al., 2022). As a matter of fact, it has been demonstrated that the plant extract has the potential to be used as a raw material in the manufacture of drugs and other health-related products.

The purpose of this study was to elaborate on the efficacy of the plant extract in order to establish the value of the extract of this plant and explore the novel indication of its medicinal use. In this study, the ethanolic extract from the aerial part of C. grandis was put through a tyrosinase inhibition activity assay as well as an albumin denaturation inhibition assay to determine its ability to act as a skin-depigmenting agent and an anti-inflammatory agent, respectively. Both of these tests were carried out because there were only a few reports available that provided the pertinent information. Furthermore, HPLC was used to perform phytochemical analysis on this plant extract in order to discover the active ingredient responsible for the biological activities and to serve as an example for developing guidelines for the chemical quality control of the extract. In our previous reports on the identification of active phytochemical compounds in plant extracts and herbal crude drugs, we focused on the triterpene lupeol, which exhibited noticeable antioxidant, anti-inflammatory, and wound-healing properties (Somwong and Theanphong, 2021; Somwong and Kamkaen, 2022). The compound was therefore chosen as a chemical marker for the chemical analysis of C. grandis extract, and the amount of this active compound in the plant extract was reported herein. This research would be useful in providing important information regarding the efficacy of C. grandis for the future incorporation of the plant extract into nutraceuticals and herbal medicine production.

2. Materials and methods

2.1 Chemicals and reagents

The lupeol (99% purity) was supplied by NSABE Co., Ltd. (Nanjing, China). All of the analytical reagents and disposable accessories for the HPLC analysis were furnished by Honeywell Burdick and Jackson (North Carolina, US) and S.N.P. Scientific Co., Ltd. (Bangkok, Thailand), respectively. The necessary chemical reagents for performing the tyrosinase inhibition experiments such as mushroom tyrosinase and tyrosine and for protein denaturation inhibition assays such as egg albumin, as well as the positive controls used in these in vitro biological assays, i.e. kojic acid and diclofenac diethylammonium, respectively, were provided by Manose Health and Beauty Research Center Co., Ltd. (Chiang Mai, Thailand).

2.2 Plant materials

Raw C. grandis aerial parts were collected at one year of maturity in August 2021 in the area of Rangsit University's arboretum in Pathumthani province, Thailand. Asst. Prof. Chomnapas Chuchote, one of the authors from the Department of Pharmacognosy, College of Pharmacy, Rangsit University, Thailand, identified the plant sample. The plant sample was kept at the herbarium of the Department of Pharmacognosy at Rangsit University. A voucher specimen has been given the code RSU-PG-CG01.

2.3 Preparation of extracts

To obtain the pulverized samples, the C. grandis raw material was dried and ground. The material was accurately weighed in a Soxhlet apparatus thimble (10 g). Extraction took three hours with 300 mL of absolute ethanol. This experiment was employed three times on the plant powder sample. In a rotary evaporator, the ethanolic extract collected from the reservoir of a Soxhlet apparatus was evaporated to dryness. The extract was then accurately weighed to determine the percentage of extraction yield.

2.4 Preparation of sample solutions

The concentrated extract from the plant sample was accurately weighed and reconstituted with limited amounts of methanol to provide a concentration of 50 mg/mL for use as sample solutions for HPLC analysis. Furthermore, the crude extract was divided in order to prepare sample solutions by dilution with the appropriate solvent for each biological assay. Both biological and chemical sample solutions were prepared in triplicate.

2.5 Tyrosinase inhibition assay

Serial solutions of extracted materials at
concentrations of 0.00025, 0.0025, 0.025, 0.25, and 2.5 mg/mL and the standard anti-tyrosinase agent, kojic acid, were diluted with 10% (v/v) DMSO and evaluated using the modified dopachrome technique using L-tyrosine as a substrate, as described previously (Chang, 2009). In brief, 96-well microplates were filled with 40 µL of sample or standard solutions, 40 µL of 0.1 mg/mL L-tyrosine, 50 µL of 0.1 mg/mL mushroom tyrosinase, and 80 µL of 0.1 M phosphate buffer. A 10% (v/v) DMSO solution served as the negative control. The mixture was incubated for 60 mins at 37°C. Using a microplate reader, the quantity of dopachrome generated in the reaction mixture was measured at 450 nm before and after incubation. The experiment was performed three times. The percentages of tyrosinase inhibition were calculated by using an equation described in a documented protocol (Winitchai et al., 2011), and the sample concentrations providing 50% inhibition (IC_{50}) were defined using a graph plotted between the percentages of tyrosinase inhibition activity and the tested concentrations.

2.6 In vitro anti-inflammatory assay

This study used an albumin denaturation assay to determine the dried C. grandis extract’s anti-inflammatory efficacy in vitro (Theanphong and Somwong, 2022). The various doses of the tested extract, about 2 mL, were diluted with 20% Tween 20 and mixed with 0.2 mL of fresh hen's egg albumin and 2.8 mL of phosphate buffered saline (pH 6.4) to make concentrations of 0.25, 0.5, 1, 2 and 4 mg/mL. Similar to the negative and positive controls, 2 mL of the examined extract was replaced with double-distilled water and diclofenac diethylammonium, respectively. In an incubator for five minutes, the resulting mixture was warmed to 70°C. An ultraviolet spectrophotometer (UV-1800, Shimadzu, Japan) was used to measure the bioactivity of the combination after it had been chilled to room temperature. The absorbance of the tested samples was compared to that of the control sample, and the percentage inhibition of protein denaturation was computed based on a reference study’s equation (Chandra et al., 2012). This plant extract and the conventional diclofenac diethylammonium were both compared using the IC_{50} value as a means of assessing their anti-inflammatory effects. By plotting the percentage of inhibition against the concentration of the treatment, it was possible to figure out the sample concentration at which 50% of albumin denaturation inhibition was demonstrated.

2.7 HPLC apparatus and chromatographic conditions

An HPLC system (1260 Infinity Series, Agilent Technologies, US) equipped with a photodiode array detector (DAD) was used for the chromatographic operation. The equipment was controlled using OpenLab ChemStation software (Agilent Technologies, US). A nylon membrane with a 0.45 µm mesh size was utilized to filter the sample and working standard solutions. An Accucore XL C18 packed column was used in the HPLC system to separate the 20-µL samples in the isocratic mode, with a mobile phase consisting of methanol and acetonitrile at a 90:10 ratio. At 1.0 mL/minute, the flow rate of the mobile phase was maintained in the HPLC instrument’s column chamber. Data on the absorbance of the lupeol compound at 210 nm was acquired by DAD in the chromatogram of tested materials. The HPLC system included a 12-minute time constraint for analyzing each injection.

2.8 Verification of the analytical method for lupeol determination

Lupeol (20 mg) was dissolved in methanol and then poured into a volumetric flask containing 50 mL to make a stock standard solution with a concentration of 400 µg/mL. The stock standard solution was diluted with methanol to make the working standard solutions at concentrations ranging between 10 and 400 µg/mL. The method of analysis used in this investigation was based on a previously published protocol, and the analytical approach was verified in accordance with the guidelines stated in our previously published report (Somwong and Theanphong, 2021). The lupeol standard was spiked into the plant extract to test the accuracy and precision of the analytical method used. The percentages of recovery and the relative standard deviation (RSD) were used to verify the method’s accuracy and precision. Furthermore, the analytical approach was checked for its specificity to guarantee it could be used to evaluate this plant extract.

2.9 Determination of lupeol content in extracts

The lupeol concentration contained in the extract was estimated using a linear regression equation derived from the calibration curve of working standard solutions. The extracted sample was tested in triplicate, and the lupeol concentration was expressed as milligrams per 100 g of dried crude extract. The content was shown along with its standard deviations (SD).

3. Results and discussion

3.1 Tyrosinase inhibition activity

Extracts from the aerial parts of C. grandis were examined for tyrosinase inhibitory activity using the modified dopachrome technique (Winitchai et al., 2011). Figure 1 depicts the percentage of tyrosinase inhibition of the plant extracts and a positive control. The IC_{50} values of the plant ethanolic extracts obtained from the
Soxhlet procedure were estimated using the linear regression equations from the tyrosinase inhibition graph, which was done from the three-time experiments, yielding an average value of 0.29±0.06 mg/mL. The IC\textsubscript{50} value for kojic acid, the anti-tyrosinase agent used as a positive control in this study, was 0.005±0.00 mg/mL. As a result, the \textit{C. grandis} extract had the ability to prevent tyrosinase from performing its normal function. It was discovered that the plant extract could be one of the naturally active anti-tyrosinase agents. However, its effectiveness was approximately 0.017 times less than that of standard kojic acid, the commercial chemical agent that is utilized for skin-lightening in cosmeceutical anti-aging products.

\begin{figure}[h]
  \centering
  \includegraphics[width=0.8\textwidth]{figure1.png}
  \caption{Plots of the tyrosinase inhibitory activity against different doses of \textit{C. grandis} extract (A) and kojic acid (B). The experiments in triplicate were each represented by a plot line in the graph.}
\end{figure}

The findings of this study could support the utility of \textit{C. grandis} extract in tyrosinase inhibition, which was first demonstrated in this report. The results were demonstrated in the same way as previous studies on this plant that showed its efficacy on antioxidant effects, and it was identified as a useful plant with promising antioxidant sources (Umanaheswari and Chatterjee, 2008). Previous investigations have shown that the alcoholic extracts of the leaves and fruits of this plant have strong antioxidant activities compared to various antioxidant standard agents (Kondhare and Lade, 2017; Laboni \textit{et al}, 2017). Their effects were linked to the high amounts of phenolic and flavonoid compounds in the plant extract, and pharmacological research has shown that they are considerable natural antioxidants. Due to the high anti-oxidative activity demonstrated in the \textit{in vitro} radical scavenging experiments, the extract of \textit{C. grandis} was encouraged to be subjected to a number of biological experiments, one of which was an assessment of its anti-diabetic properties. It was discovered that the feasible anti-diabetic function of the plant extract was attributed to its antioxidant effect (Lee \textit{et al}, 2015; Meenatchi \textit{et al}, 2017). Currently, the extracts derived from this plant's parts have enough scientific evidence to be developed as a functional diet and a safe treatment for diabetes (Chanda \textit{et al}, 2020). Similarly to antioxidant and anti-diabetic properties, the plant extract has the potential to be further developed as an ingredient in nutraceuticals for anti-aging properties, as it possessed the remarkable effect in this study as a tyrosinase inhibitor, which is one of the important properties other than antioxidant and anti-glycation activities to be carried out in the pharmacological study for the assessment of plant-based materials to be used in functional food for rejuvenation (Nurkolis \textit{et al}, 2021; Saleem \textit{et al}, 2021).

### 3.2 \textit{In vitro} anti-inflammatory activity

The anti-inflammatory property of \textit{C. grandis} extract in the present study was evaluated by an \textit{in vitro} experiment of the denaturation inhibition of egg albumin. Using linear regression models based on the graphs of percentage inhibition versus treatment shown in Figure 2, the 50\% inhibitory effect of different concentrations of the tested extract and the control of an albumin denaturation assay were calculated. \textit{Coccinia grandis} extracts exhibited inhibitory activity in a three-time protein denaturation assay, with an average IC\textsubscript{50} value of 9.63±1.10 mg/mL. As a consequence, the plant extract was discovered to have anti-inflammatory properties, although its effect was less apparent than that of the positive control. Compared to the nonsteroidal anti-inflammatory medication (NSAID) diclofenac diethylammonium, which was often utilized as a positive control in this assay and had an IC\textsubscript{50} value of 0.54±0.01 mg/mL, in this analysis, the assessed extract from the plant had anti-inflammatory activity with IC\textsubscript{50} values 0.06 times smaller than diclofenac.

This study provides additional evidence for the anti-inflammatory properties of \textit{C. grandis} extract, which were demonstrated in a preceding experiment involving egg albumin denaturation (Majumder \textit{et al}, 2017). The \textit{in vitro} anti-inflammatory activity of the plant extract was demonstrated in the same manner as in the prior report and the current investigation. Both experiments demonstrated that the plant extract effectively inhibited protein denaturation. However, it was less potent than the comparator drug, diclofenac. The anti-inflammatory qualities of the plant extract that might be used in herbal medicine and functional foods were confirmed after the scientific validity of the research supporting its usage.

https://doi.org/10.26656/fr.2017.8(4).427 © 2024 The Authors. Published by Rynnye Lyan Resources
was strengthened. Our findings can be used as a rational justification for its anti-inflammatory function. Nevertheless, this study also suggested that the plant extract should be combined with other potential anti-inflammatory medicinal plants for enhanced pharmacological actions than when used alone. As noted in previous research, *C. grandis* extract was combined with *Clerodendrum inerme* and *Acanthus ebracteatus* to develop an herbal combination that demonstrated a synergistic effect on an antimicrobial property and the potential to be utilized as a novel option for skin therapy (Pratoomsoot et al., 2020). In *in vivo* anti-diabetes studies, the plant extract was evaluated in combination with various plant extracts, and the herbal combinations showed greater anti-hyperglycemic and anti-hyperlipidemic activity than the *C. grandis* extract individually (Putra et al., 2021). Additionally, the blending of the plant and *Blumea balsamifera* extracts had great antioxidant activity as measured by the FRAP, DPPH, and ABTS methods (Putra et al., 2022). These results demonstrate a synergistic interaction between *C. grandis* and other medicinal plants and recommend the use of the plant extract as an ingredient in combined preparations.

### 3.3 Lupeol content in extracts

The peak of the selected triterpenoid molecule lupeol can be seen in the HPLC chromatogram of the ethanolic extract of *C. grandis*. This peak appeared at a retention time of 9.6 minutes, which corresponded to the signal of the lupeol standard and was also displayed in Figure 3. The standard-spiked approach in three-time analyses with acceptable recovery and relative standard deviation percentages of average values of 92.38 and 1.98% for the determination of lupeol demonstrated that the analytical method that was utilized in this study was correct and precise. This was shown by the fact that the percentages of average recovery and relative standard deviation were within the acceptable ranges. In the extracts that were tested in triplicate, the average amount of lupeol compound per dried weight of the extract (100 g) was calculated to be expressed as a value of 18.87 mg with a standard deviation of 0.79 mg. The signal of the lupeol compound, as shown in Figure 3, was separated specifically from the other interferences found in the plant extract, and the compound lupeol was quantified clearly in the plant samples by the analytical method used in this study. This suggests that the compound is a considerable chemical marker of the *C. grandis* extract, and the method could be applied as a decent analytical tool to assess the quality of this plant extract.

![Figure 2](image1.png)

**Figure 2.** Plots showing the albumin denaturation assay's percentage inhibition against various concentrations of *C. grandis* extract (A) and diclofenac diethylammonium (B). Each plot line in the graph represents one of the three replicated studies.

![Figure 3](image2.png)

**Figure 3.** Chromatograms of the chemical marker lupeol with a retention time of 9.6 mins in the standard (A) and *C. grandis* extract (B).

The pentacyclic triterpene lupeol has been discovered to be a significant chemical constituent in members of various plant families, and it can be found in many parts of plants, including the root, stem, leaf, and fruit. The lupeol compound is now being touted as one of the most important sources of nutraceuticals, which provide numerous advantages for human health. It continues to pique the interest of researchers as a promising agent due to successive pharmacological experiments that revealed its defensive efficacy against various types of cancer, diabetes, obesity, inflammation, and skin problems. Various molecular studies and preclinical tests were carried out to elucidate its actions, and the results confirmed that the compound can exert these biological functions by controlling a variety of
cellular pathways (Sohag et al., 2022). The compound was also reported to be present in C. grandis (Pekamwar et al., 2013), therefore it was chosen as a marker for chemical analysis in this study, in which its amount found in the plant's aerial portion was first demonstrated.

Exploration of the lupeol molecule and determination of its quantity in C. grandis extract in the current study are important for future research, which could focus on utilizing the plant extract as an element in dietary supplements and compounded medications. This study was linked to previous reports on other medicinal plants that their extracts had antioxidant, anti-diabetic, enzymatic inhibition, and anti-inflammatory properties, and that these plants contained this compound as a critical chemical component (Gurupriya et al., 2018; Rathinavel et al., 2021; Somwong and Theanphong, 2021). As a consequence, the biological activities of the plant investigated in this study might well be related to the apparent lupeol compound in the extract. However, prior to employing this plant extract, substantial pharmacological and toxicity studies should be carried out to ensure its efficacy and safety.

4. Conclusion

Coccinia grandis was proven to be a healing property plant due to the significant antioxidant capabilities and anti-diabetes activity of its extract, which was observed in all plant sections. This experiment contributed to the efficacy of the plant extract's anti-tyrosinase and anti-inflammatory characteristics to its value. In addition, the HPLC profile of the extract of the current investigation revealed the presence of the prospective pharmacological triterpenoid agent, lupeol, a molecule whose biological activities, such as enzyme inhibition and anti-inflammation, have been addressed. This research indicates that the plant extract has the ability to be exploited in pharmaceutical products. Based on the bioactivities described in this paper and the fact that all plant parts are eaten as food, it might also be possible to use plant extracts as ingredients in nutraceuticals.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

All of this research was supported by the Rangsit University Research Institute, and the Rangsit University College of Pharmacy provided the facilities needed for it.

References


Pharmaceutical Technology and Research, 12(2), 147-151. https://doi.org/10.4103/japtr.JAPTR_13_21


