

Abstract

Background and Objective: Despite its traditional medicinal use, comprehensive data on the morphology, anatomy, and biological activities of Ma-eok (*Solanum lasiocarpum* Dunal) fruit remain limited. This study investigated the fruit's macroscopic and microscopic characteristics, alongside the phytochemical profile and biological activities of its lyophilized juice. **Methods:** Morphological and anatomical features were analyzed using stereomicroscopy, light microscopy, and scanning electron microscopy (SEM). Lyophilized fruit juice was evaluated for total phenolic (TPC) and flavonoid contents (TFC), as well as antioxidant (DPPH), anti-inflammatory (egg albumin denaturation), antimicrobial (agar diffusion), and DNA-protective (comet assay) activities. **Results:** The globose fruit (mean diameter 38 mm, weight 31 g) transitions from green to yellowish-orange upon ripening. SEM photographs revealed the presence of dense stellate trichomes of the porrect-geminate type on ripe fruits, whereas unripe fruits exhibited both non-glandular trichomes and additional glandular trichomes. Anatomical examination revealed that the exocarp consists of a single epidermal layer underlain by a hypodermal layer of calcium oxalate crystal-bearing cells, as indicated by field emission scanning electron microscope equipped with an energy dispersive X-ray spectrometer (FE-SEM-EDS) analysis, followed by 5–6 layers of parenchyma. The mesocarp consisted of parenchymatous tissue enclosing a thin and delicate endocarp. The locules contained a gelatinous matrix surrounding numerous kidney-shaped seeds with reticulate seed coats. Phytochemically, the lyophilized extract yielded a TPC of 68.13 ± 5.39 mg GAE/g and a TFC of 2.57 ± 0.01 mg QE/g. The fruit demonstrated strong antioxidant activity, with a value of 141.95 ± 0.02 mg TE/g, and significantly mitigated H₂O₂-induced DNA damage, as evidenced by comet assay results ($P < 0.05$). Antimicrobial testing demonstrated inhibitory activity against *Staphylococcus aureus* (12.0 ± 1.0 mm), *Escherichia coli* (9.7 ± 1.2 mm), and *Cutibacterium acnes* (9.7 ± 1.2 mm), whereas no inhibitory effect was observed against *Candida albicans*. Evaluation of anti-inflammatory activity was not feasible, as the high acidity of the extract induced premature protein precipitation during the assay. **Conclusion:** The established pharmacognostic data and biological activities provide robust evidence supporting the potential use of Ma-eok fruit juice in value-added foods, nutraceuticals, and cosmetics.

Keywords: *Solanum lasiocarpum* Dunal., Morphology of fruit, Anatomy of fruit, Bioactivity, Phenolic and Flavonoid contents

1 บทคัดย่อ

2 **ภูมิหลังและวัตถุประสงค์:** แม้จะมีการใช้ประโยชน์ทางยาแผนโบราณ แต่ข้อมูลเชิงลึกเกี่ยวกับสัณฐานวิทยา กายวิภาคศาสตร์
3 และฤทธิ์ทางชีวภาพของผลมะอึก (*Solanum lasiocarpum* Dunal) ยังคงมีจำกัด การศึกษาครั้งนี้จึงมุ่งศึกษาลักษณะทางมหภาคและ
4 จุลภาคของผลมะอึก ควบคู่ไปกับองค์ประกอบทางพฤกษเคมีและฤทธิ์ทางชีวภาพของน้ำผลมะอึกที่ผ่านการทำแห้งแบบแช่เยือก
5 แข็ง (lyophilized juice) **วิธีการศึกษา:** วิเคราะห์ลักษณะทางสัณฐานวิทยาและกายวิภาคศาสตร์โดยใช้กล้องจุลทรรศน์สเตอริโอ
6 กล้องจุลทรรศน์แบบใช้แสง และกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) นำน้ำผลมะอึกที่ผ่านการทำแห้งแบบแช่เยือก
7 แข็งมาประเมินหาปริมาณสารประกอบฟีนอลิกรวม (TPC) และฟลาโวนอยด์รวม (TFC) ตลอดจนทดสอบฤทธิ์ต้านอนุมูลอิสระ
8 (วิธี DPPH) ฤทธิ์ต้านการอักเสบ (การยับยั้งการเสียหายของโปรตีนไข่ขาว) ฤทธิ์ต้านจุลชีพ (วิธี agar diffusion) และฤทธิ์
9 ปกป้องดีเอ็นเอ (วิธี comet assay) **ผลการศึกษา:** ผลมะอึกมีลักษณะทรงกลม (เส้นผ่านศูนย์กลางเฉลี่ย 38 มม. น้ำหนัก 31
10 กรัม) โดยจะเปลี่ยนจากสีเขียวเป็นสีส้มเหลืองเมื่อสุก ภาพถ่ายจากกล้อง SEM แสดงให้เห็นการปรากฏของขน (trichome) ไม่
11 มีต่อมรูปดาว ชนิด porrect-geminate อย่างหนาแน่นบนผลสุก ในขณะที่ผลดิบพบทั้งขนชนิดไม่มีต่อมและขนต่อม การตรวจสอบ
12 ทางกายวิภาคศาสตร์พบว่า ผนังผลชั้นนอก (exocarp) ประกอบด้วยชั้นเอพิเดอร์มิสเพียงชั้นเดียว ซึ่งรองรับด้วยชั้นไตเนื้อเยื่อผิว
13 ที่ประกอบไปด้วยเซลล์ที่บรรจุคลอโรฟิลล์และเซลล์เยื่อหุ้มเซลล์ จากการบ่งชี้ด้วยการวิเคราะห์ด้วยเครื่อง FE-SEM-EDS ตามด้วยเซลล์
14 พาเรนไคมา 5–6 ชั้น ผนังผลชั้นกลาง (mesocarp) ประกอบด้วยเนื้อเยื่อพาเรนไคมาล้อมรอบผนังผลชั้นใน (endocarp) ที่มี
15 ลักษณะบางและสลายตัวง่าย ช่องว่างภายในผลบรรจุมวลสารลักษณะเป็นรู ซึ่งห่อหุ้มเมล็ดรูปไตจำนวนมากที่มีเปลือกหุ้มเมล็ด
16 เป็นลวดลายแบบร่างแห ในด้านพฤกษเคมี สารสกัดที่ผ่านการทำแห้งแบบเยือกแข็งให้ค่า TPC เท่ากับ 68.13 ± 5.39 mg GAE/
17 กรัม และค่า TFC เท่ากับ 2.57 ± 0.01 mg QE/กรัม ผลมะอึกแสดงฤทธิ์ต้านอนุมูลอิสระในระดับสูง โดยมีค่าเท่ากับ $141.95 \pm$
18 0.02 mg TE/กรัม และสามารถบรรเทาความเสียหายของดีเอ็นเอที่ถูกเหนี่ยวนำด้วย H_2O_2 ได้อย่างมีนัยสำคัญ ดังที่แสดงในผล
19 การทดสอบ comet assay ($P < 0.05$) การทดสอบฤทธิ์ต้านจุลชีพแสดงให้เห็นถึงฤทธิ์ยับยั้งเชื้อ *Staphylococcus aureus* (12.0
20 ± 1.0 มม.) *Escherichia coli* (9.7 ± 1.2 มม.) และ *Cutibacterium acnes* (9.7 ± 1.2 มม.) ในขณะที่ไม่พบผลการยับยั้งต่อเชื้อ
21 *Candida albicans* การประเมินฤทธิ์ต้านการอักเสบไม่สามารถดำเนินการได้ เนื่องจากความเป็นกรดสูงของสารสกัดเหนี่ยวนำให้
22 เกิดการตกตะกอนของโปรตีนก่อนเวลาอันควรในระหว่างการทดสอบ **ข้อสรุป:** ข้อมูลทางเภสัชเวทและฤทธิ์ทางชีวภาพที่ได้จาก
23 การศึกษานี้ เป็นหลักฐานสำคัญที่สนับสนุนศักยภาพในการนำน้ำผลไม้มะอึกไปประยุกต์ใช้ในผลิตภัณฑ์อาหารเพิ่มมูลค่า
24 ผลิตภัณฑ์โภชนเภสัช (nutraceuticals) และผลิตภัณฑ์เครื่องสำอางต่อไป

25 **คำสำคัญ :** มะอึก, สัณฐานวิทยาของผล, กายวิภาคศาสตร์ของผล, ฤทธิ์ทางชีวภาพ, ปริมาณสารประกอบฟีนอลิกและฟลาโ
26 นอยด์

INTRODUCTION

Solanum lasiocarpum Dunal [syn. *Solanum ferox* L., *Solanum stramoniifolium* Jacq.], locally known in Thailand as "Ma-eok," is a spine-bearing plant belonging to the Solanaceae family.^{1,2} Morphologically similar to typical eggplants, its fruits are uniquely covered in persistent epidermal hairs that must be removed prior to consumption. Due to its distinct acidic flavor, the fruit is widely used in Thai culinary applications, notably in traditional chili pastes and Northeastern-style papaya salads, often serving as a substitute for lime or tamarind.³⁻⁶ Nutritionally, a 100-g portion provides 66 kcal, containing 1.9 g of protein, 12.7 g of carbohydrates, 0.8 g of fat, and 3.6 g of crude fiber, alongside essential minerals (calcium, phosphorus, iron) and vitamins (A, B-complex, C).⁷

In traditional Thai medicine, various parts of Ma-eok serve distinct therapeutic functions: the roots alleviate pain and fever, leaves and roots soothe skin itching, seeds relieve toothaches, and the fruits are consumed as an expectorant to dissolve phlegm and relieve coughs.^{4,6} Phytochemically, the plant is characterized by a wealth of phenolic compounds.⁸

Despite its extensive utilization, scientific literature on the micro-anatomy of the Ma-eok fruit remains absent. While the fruit anatomy of several other *Solanum* species has been extensively documented,⁹⁻¹¹ structural studies on Ma-eok have been limited to macroscopic descriptions¹² or scanning electron microscopy (SEM) of pollen and seed coats solely for taxonomic identification.¹³⁻¹⁴

Pharmacologically, extracts from different plant parts exhibit diverse bioactivities. Studies on non-edible parts (80% ethanolic extracts) revealed that the roots possess superior antioxidant and anti-inflammatory (nitric oxide reduction) properties, while leaf extracts demonstrate the highest antibacterial efficacy. Additionally, root and leaf extracts have shown targeted anticancer activities against various carcinoma cell lines.¹⁵ Regarding the fruit, an 80% methanolic extract exhibited notable total phenolic content (62.41 ± 1.18 mg GAE/g dw) and DPPH radical scavenging activity.¹⁶ Furthermore, aqueous flower extracts displayed allelopathic effects,¹⁷ and a targeted seed protein extract exhibited significant antibacterial action, primarily against Gram-positive strains.¹⁸

To address these knowledge gaps, this study sought to provide a comprehensive characterization of the macroscopic and microscopic morpho-anatomy of Ma-eok fruits through stereomicroscopic, light microscopic, and SEM analyses, thereby establishing reliable diagnostic markers for providing pharmacognostic reference data for plant material authentication. Furthermore, we evaluated the biological activities of freshly squeezed, finely homogenized

whole-fruit juice. This preparation maximizes the release of intracellular and partially water-soluble compounds, closely mirroring its traditional consumption. This lyophilized juice was assessed for total phenolic and flavonoid contents, antioxidant capacity (DPPH), anti-inflammatory properties (egg albumin denaturation), antimicrobial efficacy, and protection against oxidative DNA damage (comet assay). Ultimately, this research provides a crucial scientific foundation to support the future development of Ma-eok in the food, pharmaceutical, and cosmetic industries.

Objectives

1. To characterize the macroscopic morphology, micromorphology, anatomy, and organoleptic characteristics of Me-eok (*Solanum lasiocarpum*) fruit using visual observation, stereomicroscopy, light microscopy, and scanning electron microscopy (SEM), and to provide pharmacognostic reference data for plant material authentication.

2. To evaluate the antioxidant, anti-inflammatory, and antimicrobial and DNA-protective activities of lyophilized Me-eok fruit juice, and to determine its total phenolic and flavonoid contents.

MATERIALS AND METHODS

Materials

All chemicals and reagents used were of analytical grade. For the phytochemical and antioxidant assays, Folin-Ciocalteu's phenol reagent was purchased from Loba Chemie (Mumbai, India); anhydrous sodium carbonate from Merck (Darmstadt, Germany); gallic acid monohydrate from Fluka (Steinheim, Germany); aluminium chloride from Kemaus Chemicals (NSW, Australia); sodium hydroxide from J.T. Baker (NJ, USA); and DPPH, Trolox, sodium nitrite, and catechin from Sigma-Aldrich (MO, USA, and Darmstadt, Germany). For the anti-inflammatory assay, diclofenac sodium was generously provided by T.O. Chemicals (1979) Ltd. (Bangkok, Thailand). Phosphate-buffered saline (PBS) components were sourced from Ajax Finechem (Auckland, New Zealand) and QRëC (New Zealand). Agarose for the comet assay was supplied by Vivantis Biochemical. For the antimicrobial assays, standard microbial strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Cutibacterium acnes* ATCC 14916 and *Candida albicans* ATCC 10231) were obtained from the Department of Medical Sciences, Ministry of Public Health (Nonthaburi, Thailand). All culture media, including Tryptic soy agar/broth and Sabouraud dextrose agar/broth, were purchased from Difco (Detroit, MI, USA). For comet assay, agarose was purchased from Vivantis Biochemical (Cat. No. PC0701-500G).

Methods

1) Sample collection of Ma-eok fruit

Fresh Ma-eok fruits, aged approximately 20 and 45 days (1.5 months), were collected in November 2025 from Hom Kret Subdistrict, Sam Phran District, Nakhon Pathom Province, Thailand. The plant material was authenticated by Assoc. Prof. Dr. Uthai Sotanaphun. A voucher specimen (No. MA00028) was deposited at Department of Pharmacognosy Department, Faculty of Pharmacy, Silpakorn University.

2) Morphological study of Ma-eok fruit

2.1 Macroscopic and organoleptic methods

Fresh Ma-eok fruits were visually and organoleptically evaluated to determine their shape, color, surface and internal pulp textures, seed morphology, and placentation. For physical measurements, 15 fruits were randomly selected from three size categories (small, medium, and large). Fruit diameter and height were measured using a Vernier caliper, and fruit weight was recorded using an analytical balance. All quantitative data were expressed as mean $\pm$ standard deviation.

2.2 Microscopic method using light microscope

2.2.1 Anatomical observations using freehand sectioning.

Thin cross-sections of fresh, mature Ma-eok fruits were manually prepared using a scalpel, mounted on glass slides with a 1:1 (v/v) aqueous glycerol solution, and sealed with coverslips. Anatomical features were examined using an optical microscope (Model CH2, Olympus, Japan) at up to 400 $\times$ magnification. Micrographs were captured using a smartphone camera (Samsung Galaxy A26).

2.2.2 Anatomical observations using cryostat sections

Fresh, mature Ma-eok fruits were frozen overnight at -20 °C and trimmed into small sections (0.5–1 cm diameter, 0.2–0.5 cm thick). Samples were embedded in a frozen section compound (Leica Biosystems, USA) and sectioned at a thickness of 20–40 μ m using a cryostat microtome (LEICA CM 1850, Germany pre-chilled to -35 °C). The resulting sections were directly mounted onto glass slides, cover slipped, and examined microscopically for anatomical features.

2.3 Surface morphology using stereomicroscopy

Surface micromorphology of the excised Ma-eok fruit pericarp was examined under LED illumination using a stereomicroscope (Model SZX10, Olympus, Japan). Micrographs of the targeted surface structures were captured using a digital camera mounted on the microscope's trinocular head.

2.4 Microscopic method and elemental composition analysis using field emission scanning electron microscope equipped with an energy dispersive X-ray spectrometer (FE-SEM-EDS)

The microstructural morphology and elemental composition of the Ma-eok fruit were analyzed using a Field Emission Scanning Electron Microscope (Tescan Mira3, Czech Republic) equipped with an Energy Dispersive X-ray Spectrometer (EDAX Element EDS, USA). Excised samples (≤ 8 mm wide, < 1 mm thick) were dehydrated through a graded ethanol series (35-100%) and subjected to critical point drying. The dried specimens were mounted on aluminum stubs and sputter-coated under vacuum with a 6–10 nm layer of gold or tungsten to prevent surface charging. Following morphological imaging at optimal magnifications, EDS analysis was performed to determine the elemental composition via characteristic X-ray emission.

3) Preparation of lyophilized Ma-eok juice for phenolic, flavonoid, and biological evaluations

Fresh Ma-eok fruits (781.39 g) were de-haired, washed, and homogenized whole (including seeds). The homogenate was filtered through cheesecloth and centrifuged at 13,000 rpm for 30 min at 25°C. The clarified supernatant was frozen at -20°C , lyophilized for 4 days, and stored at -20°C prior to analysis.

4) Study of amount of principle compounds in lyophilized Ma-eok fruit juice

4.1 Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method.¹⁹ Briefly, 100 μL of aqueous lyophilized juice solutions (2.7–6.3 mg/ml) were mixed with 250 μL of 20% (v/v) Folin-Ciocalteu reagent and 100 μL of 3.75% (w/v) Na_2CO_3 . Following a 40-min incubation at room temperature, absorbance was measured at 765 nm using a VICTOR Nivo microplate reader. Assays were performed in triplicate. TPC was calculated from a gallic acid standard curve and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract).

4.2 Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method.²⁰ Briefly, 1.5 mL of the lyophilized juice solutions (20–60 mg/mL) or quercetin standards (20–100 µg/mL) was mixed with 1.5 mL of 2% (w/v) AlCl₃. Following a 30-min incubation in the dark at room temperature, absorbance was measured at 425 nm using a Cary 60 UV-Vis spectrophotometer. Assays were performed in triplicate. TFC was calculated using the quercetin calibration curve and expressed as mg quercetin equivalents per gram of extract (mg QE/g extract).

5) Evaluation of bioactivities of lyophilized Ma-eok fruit juice

5.1 Evaluation of antioxidant activity

Antioxidant capacity was evaluated using the DPPH radical scavenging assay.²¹ Briefly, 100 µL of the lyophilized juice solutions (2,700–6,300 µg/mL) or Trolox standards (50–200 mg/L) was mixed with 2.9 mL of 60 µM DPPH solution. Following a 60-min incubation in the dark, absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The DPPH radical scavenging activity (% inhibition) was calculated using the formula: $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} represents the absorbance of the sample-free DPPH solution. Results were expressed as % inhibition, EC₅₀ values, and mg Trolox equivalents per gram of extract (mg TE/g extract).

5.2 Evaluation of anti-inflammatory activity

In vitro anti-inflammatory activity was evaluated using the heat-induced egg albumin denaturation assay.²² Reaction mixtures consisted of 2 mL of the extract (up to 240 mg/mL) or diclofenac sodium (700–1,300 µg/mL; positive control), 0.2 mL of egg albumin, and 2.8 mL of PBS (pH 6.4). Distilled water served as the negative control. The mixtures were incubated at 37°C for 15 min, followed by heating at 70°C for 5 min to induce denaturation. After cooling, absorbance was measured at 660 nm in triplicate. The percentage of inhibition was calculated using the formula: $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} represents the absorbance of the sample-free egg albumin solution. IC₅₀ values were subsequently determined by plotting the percentage of inhibition against sample concentrations.

5.3 Evaluation of antimicrobial activity

The antimicrobial activity of fresh squeezed juice, blended/filtered juice, and dried juice extract (each at 300 mg/mL) was evaluated against *S. aureus*, *C. acnes*, *E. coli*, and *C. albicans* using the agar cylinder diffusion method.²³

Bacterial suspensions (adjusted to 0.5 McFarland) and fungal suspensions (adjusted to 2.0 McFarland) were uniformly swabbed onto Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) plates, respectively. Sterile stainless-steel cylinders (6 mm diameter) were placed on the agar surfaces and loaded with 100 μ L of the test samples. Doxycycline hyclate (0.01%) and a fluconazole disc (1 mg) served as positive antibacterial and antifungal controls, respectively. Following a 24-h incubation at 37°C (anaerobic for *C. acnes*; aerobic for all others), the diameters of the inhibition zones were measured in millimeters. All assays were conducted in triplicate.

5.4 Evaluation of antioxidative effect of Ma-eok extract against H₂O₂-induced DNA damage

The protective effect of the Ma-eok extract against H₂O₂-induced DNA damage in HCT116 (human colorectal carcinoma) cells was evaluated using the alkaline comet assay.²⁴ Cells ($\geq 1 \times 10^5$) were divided into five groups: an untreated control, an H₂O₂-treated positive control (10 μ M), and three extract pre-treatment groups (500, 800, and 1000 μ g/mL). Cells were pre-treated with the extract for 45 min, followed by a 10-min exposure to 10 μ M H₂O₂.

Treated cells were mixed with 0.8% low-melting agarose, sandwiched between two 0.8% agarose layers on pre-coated glass slides, and solidified at 4 °C. Slides were submerged in cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, pH 10, freshly supplemented with 1% Triton X-100 and 10% DMSO) for ≥ 2 h at 4 °C. Following DNA unwinding in alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH > 13) for ≥ 40 min, electrophoresis was performed at 300 mA for 30 min at 4 °C.

Slides were subsequently neutralized, rinsed, fixed in cold absolute methanol for 10 min, and stained with 1 μ M Hoechst for 15 min. Comets were visualized via fluorescence microscopy (Nikon ECLIPSE Ts2-FL). DNA damage, measured as tail length, was quantified using LUCIA Comet Assay software v4.81 (Laboratory Imaging). Statistical differences among groups were analyzed using one-way ANOVA followed by Tukey's post hoc test.

RESULTS AND DISCUSSION

2) Morphological study of Ma-eok fruit

2.1 Macroscopic observation

Macroscopically, both immature and mature Ma-eok fruits are globose (diameter: 38.26 ± 5.90 mm; height: 35.84 ± 5.55 mm; weight: 31.31 ± 14.86 g). Fruit color changed from green to yellowish-orange upon ripening. A dense layer of trichomes covers the entire fruit surface, including the pedicel and calyx (Figure 1).

Cross-sectional analysis (Figure 2) revealed axile placentation with numerous lenticular, broadly reniform seeds embedded within a gelatinous matrix filling the locules. Developmentally, the pericarp differentiates into three distinct structural layers: the exocarp, fleshy mesocarp, and endocarp.²⁵ These macroscopic traits and internal placentation patterns align with established botanical descriptions of the species.^{2,26} This macroscopic characterization provides a foundational framework for the subsequent micro-anatomical evaluations.

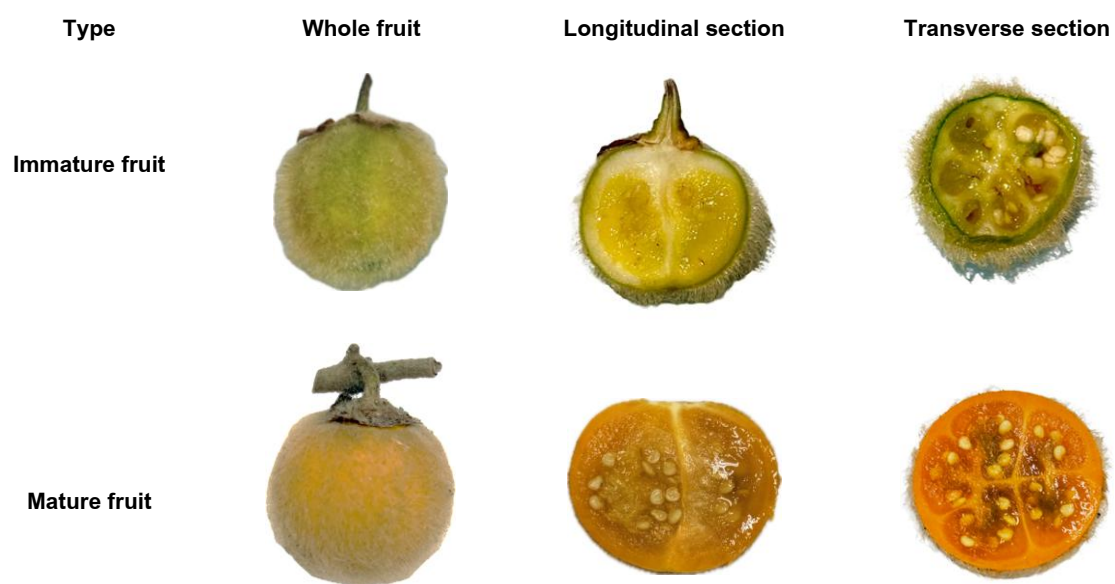


Figure 1 Macroscopic characteristics of Ma-eok fruit

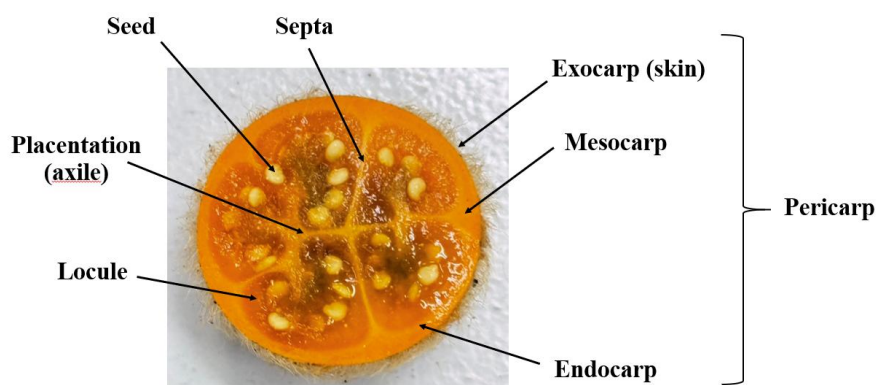


Figure 2 The components of Ma-eok fruit in cross section.

2.2 Light microscopic observations

1 Anatomical study of mature Ma-eok fruits was conducted using hand sectioning (Figures 3-4) and cryo-
2 sectioning (Figure 6). While cryo-sectioning successfully preserved the exocarp, cold-induced cellular rupture obscured
3 much of the mesocarp, endocarp, and locular contents; thus, paraffin embedding is recommended for future structural
4 investigations.

5 Both cryo-sectioning and scanning electron microscopy (SEM) revealed a dense indumentum of porrect-
6 geminate stellate non-glandular trichomes on the fruit surface, each bearing 5–12 subulate lateral rays and a prominent
7 multicellular uniseriate central ray (Figure 5), consistent with previous report for *Solanum*.²⁷ The exocarp consists of a
8 single layer of isodiametric epidermal cells underlain by a single-layered hypodermis composed of calcium oxalate
9 crystal-bearing cells (Figure 6G). However, whether these crystal-bearing cells are parenchymatous, collenchymatous,
10 fibrous, or otherwise lignified requires further histochemical validation, as variations exist among *Solanum* species.⁹⁻¹¹

11 Free-hand sectioning and SEM demonstrated the mesocarp was composed predominantly of elliptical
12 parenchymatous cells that were larger and more thick-walled than those surrounding the locules (Figure 3). This
13 aligned with anatomical reports for *Solanum* subgen. *Leptostemonum*.⁹⁻¹¹

14 Cryo-sectioning additionally revealed vascular bundles, numerous small refractive granules tentatively
15 interpreted as starch grains, and scattered yellow inclusions within the mesocarp (Figure 6).

16 The endocarp could not be clearly distinguished in the present material, likely owing to its delicate structure
17 and the limitations of the sectioning method employed. Interior to this region, the locules contained a gelatinous matrix
18 surrounding numerous seeds (Figures 3 and 4B). Transverse sections of the seeds were observed in cryosections
19 (Figure 6F). The seeds were yellowish-brown, broadly reniform, with thickened margins, a subterminal hilum, and a
20 reticulate seed-coat ornamentation (Figure 4B).

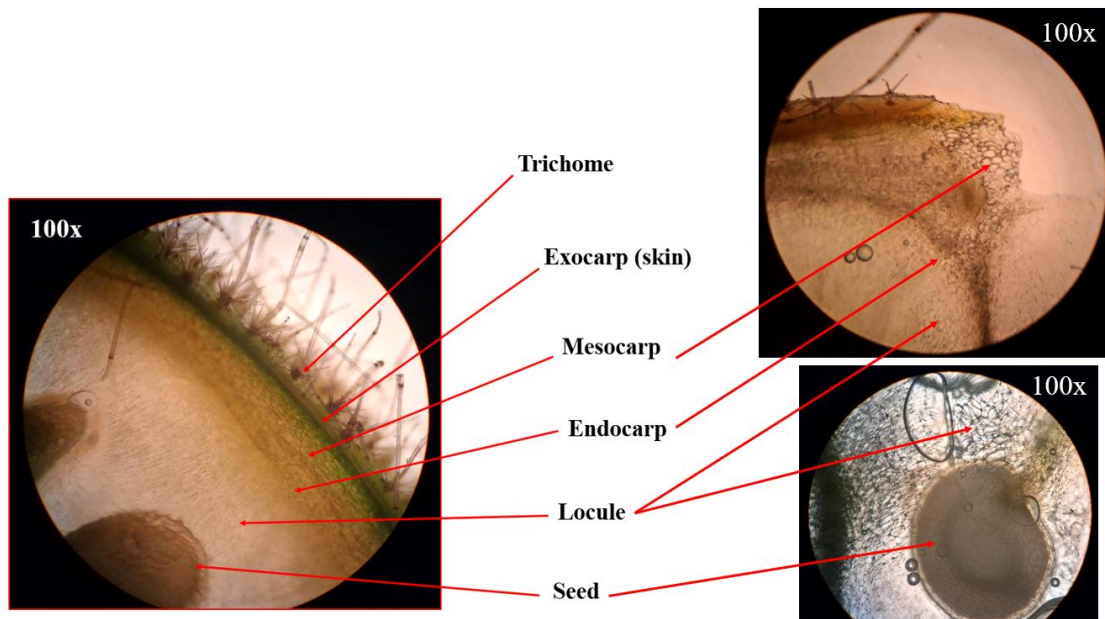


Figure 3 Microscopic characteristics of mature fruit tissue obtained by hand sectioning, showing the cellular features of the mesocarp, endocarp, locular gel, and seed.

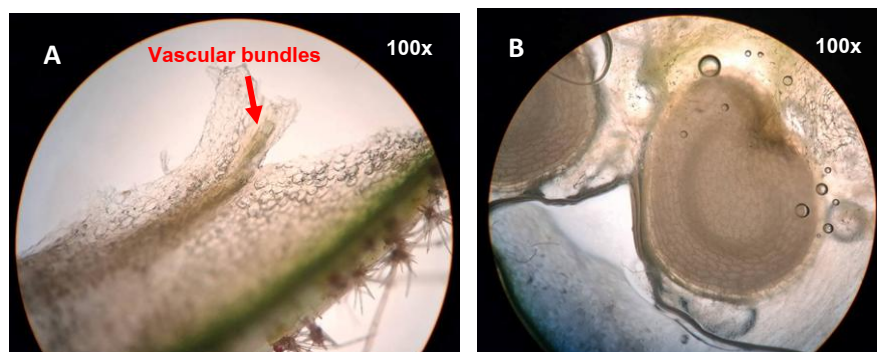


Figure 4 Microscopic characteristics of vascular bundles observed in the mesocarp layer of mature fruit (A) and seeds of mature fruit (B)

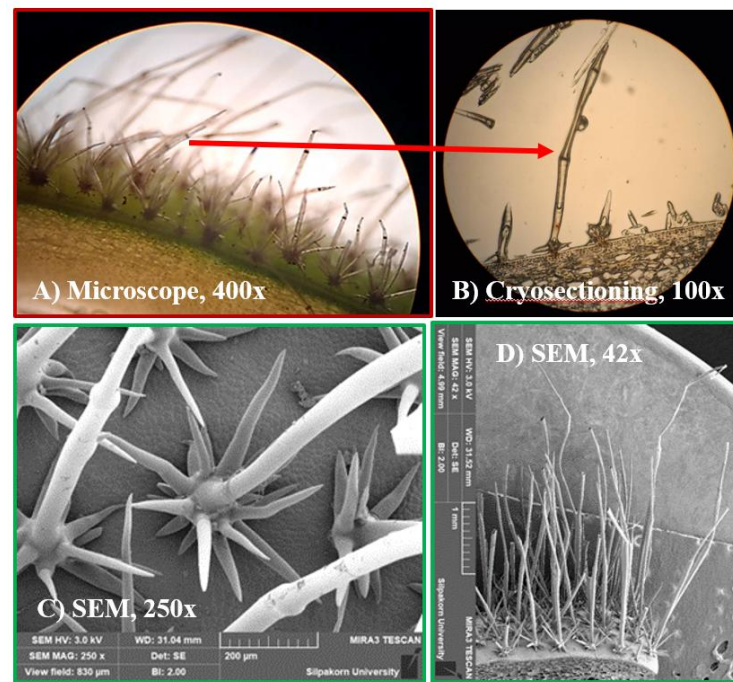
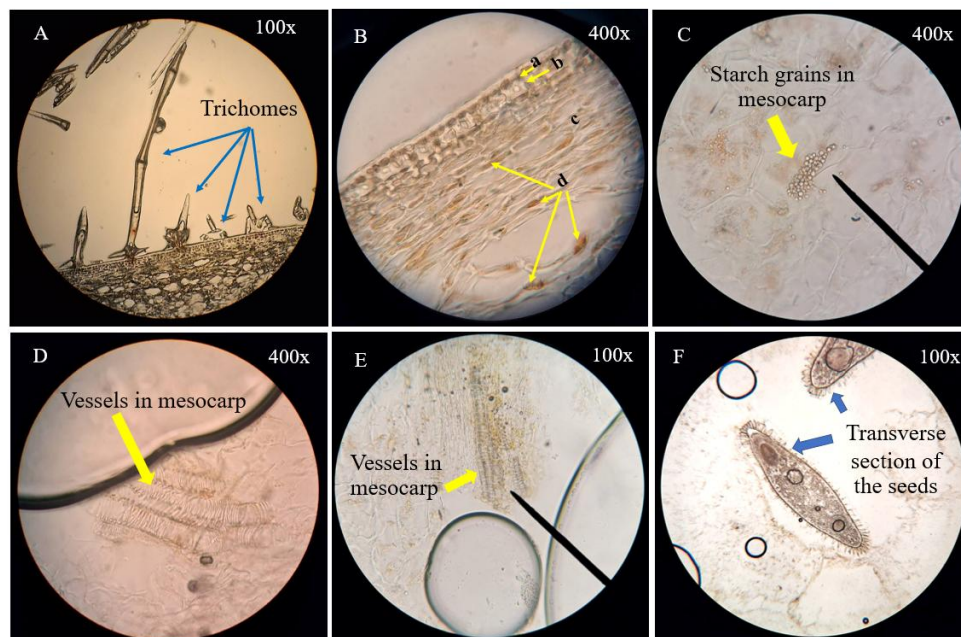


Figure 5 Microscopic characteristics of trichomes on the surface of mature fruit of Ma-eok observed under: (A) light microscopy using hand-sectioning and (B) cryo-sectioning, showing the longest multicellular trichome arranged in a single row with an incompletely developed base. An SEM image is also presented (C and D).



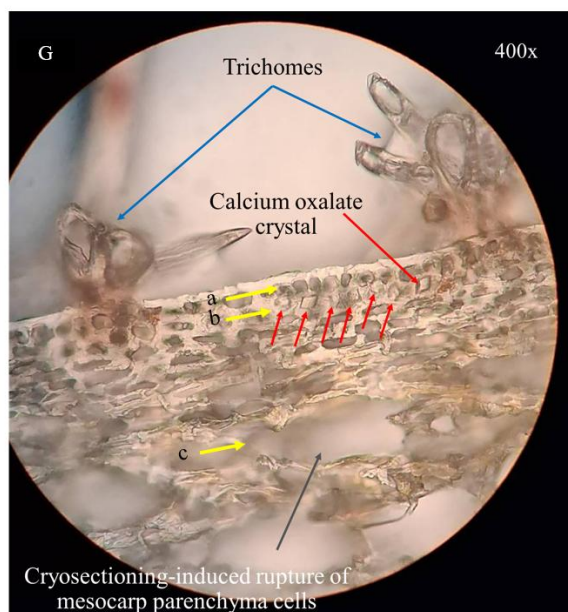


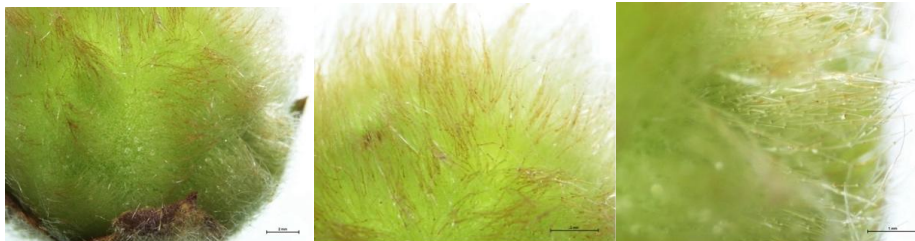
Figure 6 Micrographs of cryosectioned tissues: (A) Characteristics of trichomes, (B) Exocarp and mesocarp layers, where a, represents isodiametric cells (epidermal cells); b, represents cells containing calcium oxalate crystals (hypodermis); c, represents the mesocarp; and d, represents yellow contents, (C) Clusters of starch granules, (D, E) Vessels. (F) A transverse section of the seeds within the locule, (G) A magnified view of the epicarp and mesocarp layers.

2.3 Stereomicroscopic observations

Stereomicroscopic observations focused primarily on the indumentum of fruits, leaves, pedicels, calyces, inflorescences and flowers, providing supplementary pharmacognostic information. The surfaces of these organs were densely covered with trichomes, although their density and relative abundance varied among organs and developmental stages. Three trichome morphotypes were recognized: (1) stellate trichomes, occurring in both sessile and stalked forms; (2) simple, uniseriate, multicellular non-glandular trichomes; and (3) glandular trichomes. The stellate trichomes, found in almost all plant organs and densely covering throughout, consisted of 5 to 15 arms without or with stalked up to ca. 1 mm long. Stellate trichomes were widely distributed on the surfaces of most organs, where they formed a dense indumentum. These trichomes possessed 5–15 rays and occurred in both sessile and stalked forms, the latter bearing stalks up to ca. 1 mm long. Simple non-glandular trichomes were uniseriate, consisting of approximately 3–6 elongated cells with an acute terminal cell. They were considerably longer than the stellate trichomes, reaching up to ca. 5 mm in length, although their exact point of attachment was frequently obscured by the dense indumentum. This trichome

morphotype was particularly conspicuous on the surfaces of both immature and mature fruits (Figures 7A–C). Glandular trichomes were composed of 1-3 uniseriate stalk and 1 ellipsoid head cell, containing yellow-brownish substance. The glandular trichomes consisted of a 1–3-celled uniseriate stalk terminating in a single ellipsoid head cell. The glandular head appeared translucent, with yellowish-brown contents clearly visible under stereomicroscopy. Glandular trichomes were present on the surfaces of nearly all aerial organs but were rarely observed on the surface of mature fruits. The marked reduction in glandular trichomes on mature fruits may reflect a developmental shift in fruit function. In many plant species, glandular trichomes are known to produce or accumulate defensive secondary metabolites that deter herbivores and pathogens. Their abundance on immature fruits may therefore contribute to fruit protection during early development, whereas their reduced occurrence on mature fruits may be associated with the transition from defense to seed dispersal. However, this hypothesis requires further investigation through chemical and functional analyses. Young inflorescences were densely covered with stellate and glandular trichomes, particularly on the outer surface of the calyx, where both trichome types were conspicuous (Figures 7F–G). The abaxial surface of the corolla was similarly covered with stellate trichomes and simple multicellular non-glandular trichomes, although at a lower density than the calyx. In contrast, the adaxial corolla surface was nearly glabrous, and the anthers were also glabrous (Figure 7H).

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A) Surface of the immature fruits

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B) Surface of mature fruits

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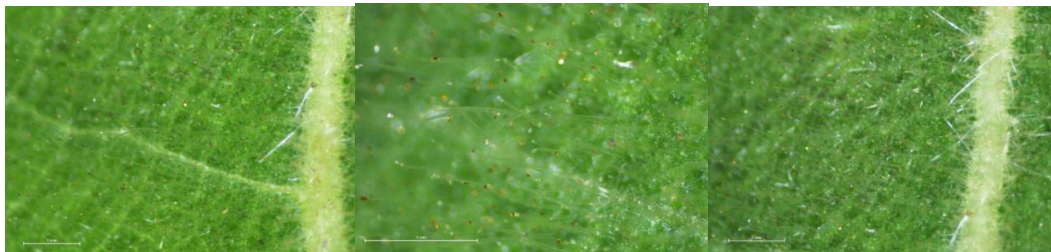
C) Surface of persistent calyx and pedicel of mature fruit



D) Surface of abaxial (lower) of the leaves



E) Surface of adaxial (upper) of the leaves



F) Surface of the inflorescence showing floral buds and unopened flowers.



G) Surface of sepal



H) Petal, anther, and stigma

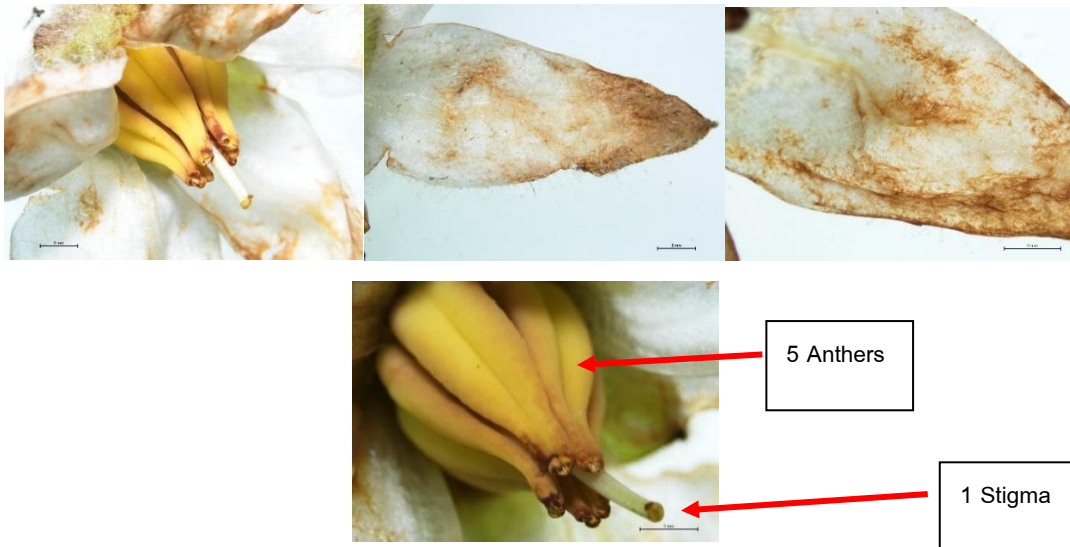


Figure 7 Stereo micrograph of the surface of Ma-eok fruits, leaves, and flowers

2.4 Scanning electron microscopic observations

SEM provided detailed observations of the fruit surface micromorphology, particularly the morphology of trichomes and seed coat ornamentation. The epidermal surfaces of both immature and mature fruits were densely covered with sessile porrect-geminate stellate non-glandular trichomes.²⁷ Individual trichomes consisted of multiple rays radiating from a central point (Figures 8A-C, 8H). In contrast, glandular trichomes, characterized by a uniseriate stalk terminating in a single ellipsoid head cell, were readily observed on immature fruits (Figures 8D-G) but were not detected on the surfaces of mature fruits (Figure 9). This observation is consistent with stereomicroscopic examination and demonstrate a distinct difference in trichome composition between immature and mature fruits. However, higher-magnification SEM examination clarified that the conspicuous elongate hairs, previously interpreted as simple uniseriate multicellular non-glandular trichomes based on stereomicroscopic observations, do not represent a separate trichome type. Instead, they correspond to the prominent elongate central ray of sessile porrect-geminate stellate trichomes, with the remaining shorter rays arising from the same basal point.

Consistent with light microscopy, SEM transverse sections (Figure 10) revealed an exocarp comprising a single layer of isodiametric epidermal cells, subtended by a subepidermal layer of calcium oxalate-containing cells. Beneath this layer were several layers of ovoid parenchymatous cells forming the outer mesocarp. The arrangement of these tissues corresponded well with that observed in free-hand and cryo-sectioned materials, while SEM provided clearer visualization of the surface architecture and crystal-bearing cells.

SEM analysis (Figure 11) demonstrated that the seeds are broadly reniform—a shape shared with *S. diphylum*, *S. forskalii*, *S. melongena*, and *S. schimperianum*. The seed coat exhibited a reticulate pattern of nearly isodiametric cells with raised boundaries and papillate sculpturing, closely resembling *S. schimperianum*.²⁸ The spermoderm cells varied irregularly in shape and size, displaying sinuate-undulate walls. A distinctive large, deep lumen resembling a floral lobe (5–9 lobes oriented parallel to the seed outline) was observed, aligning with previous SEM characterizations of *S. stramoniifolium* seeds.¹⁴ As highlighted by Mahdy et al., 2019,²⁸ these specific micromorphological features of the seed coat provide valuable diagnostic criteria for species authentication.

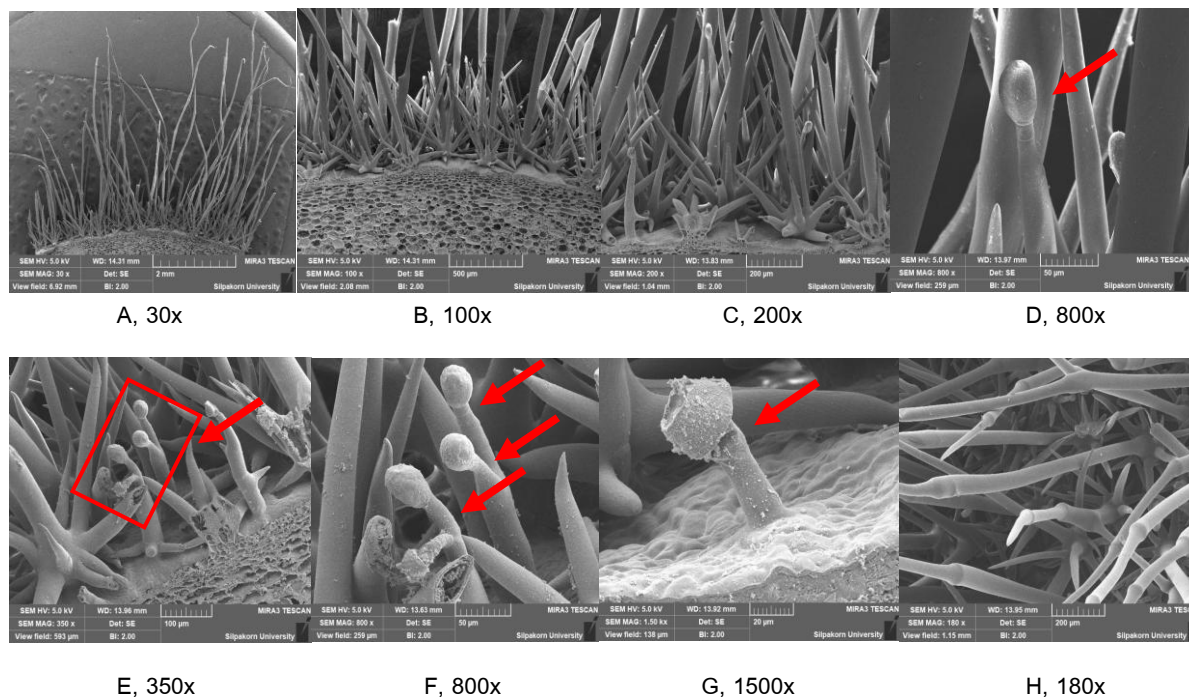


Figure 8 Scanning electron micrograph of the surface of an immature Ma-eok fruit, with the red arrow indicating a glandular trichome.

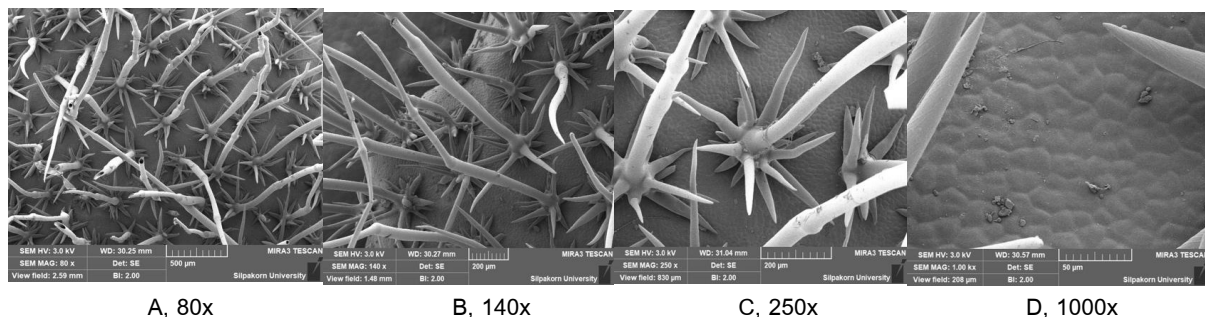


Figure 9 Scanning electron micrograph of surface of mature Ma-eok fruit

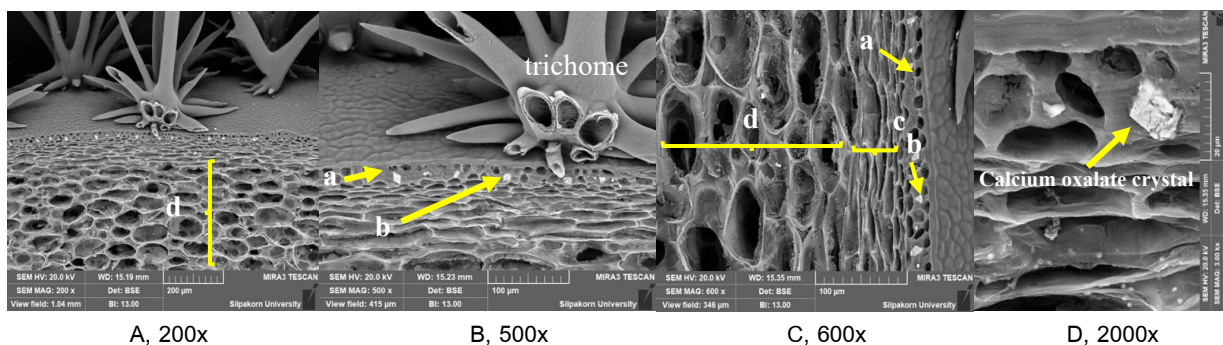


Figure 10 Scanning electron micrograph of the mature fruit surface of Ma-eok. a, Single layer of isodiametric cells; b, single layer of cells containing calcium oxalate; c, unidentified cell layers; d, mesocarp composed of multiple rows of ovoid cells, likely parenchymatous.

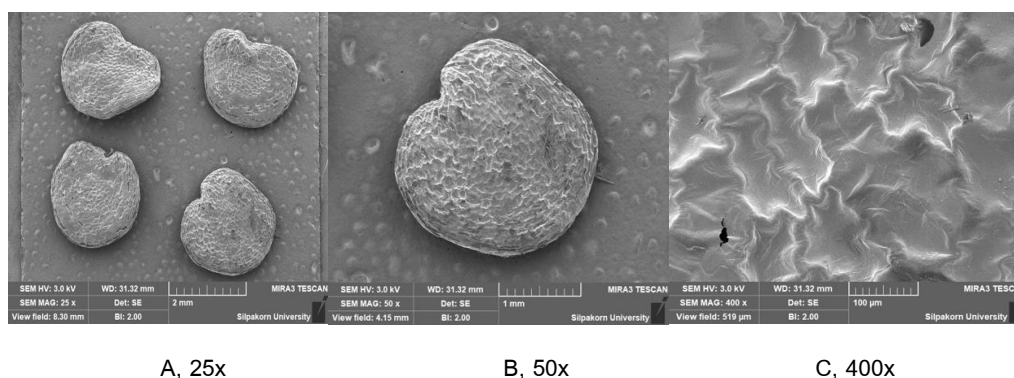


Figure 11 Scanning electron micrograph of surface of mature seed of Ma-eok

SEM-EDX (Energy Dispersive X-ray Spectroscopy) was utilized to characterize the elemental composition of opaque white amorphous crystals (Selected area 1) embedded within the epicarp, tissues of epicarp (Selected area 2) of immature fruit (Figure 12A), and crystals (EDS spot 1-4), tissues of epicarp (Selected area 1-3) and tissues of trichome (Selected area 4-5) of mature fruit (Figures 12B-C). These crystals, which were notably more abundant in mature fruits, were primarily composed of calcium (Ca), oxygen (O), and carbon (C). In immature fruits, EDX analysis of the amorphous crystal region yielded a weight percentage (wt%) dominated by O (50.91%), Ca (27.68%), and C (12.50%) (Tables 1). The EDX analysis of mature fruit crystals exhibited even higher concentrations of Ca (44.13–59.58%) and O (21.10–45.11%), alongside C (1.48–3.94%) (Tables 2).

Conversely, regular cellular tissues lacking crystals of immature fruit were predominantly composed of essential organic elements (wt%): C (39.89%), O (36.92%), N (7.07%), Ca (4.19%), and S (1.08%) (Table 1). Similar elemental profiles were observed in mature epicarp tissues. Notably, analysis of the trichomes revealed only C (47.86–

48.70%), O (34.95–40.04%), and minor aluminum (Al, 1.8%), with a complete absence of Ca (Table 3). (Note: Tungsten [W] and Gold [Au] detected across samples were sputtering artifacts from SEM-EDX preparation).

Elemental mapping of mature epicarp and mesocarp tissues (Figure 13) visually corroborated these quantitative findings. The mapping area was dominated by C (58%) and O (31%), followed by N (4%), Ca (4%), and K (3%). Dense accumulations of O (cyan) and Ca (orange) signals precisely aligned with the structural outlines of the crystals.

The combined quantitative and spatial EDX data strongly indicate that the epicarp crystals are composed of Ca, O, and C. While this profile fits both calcium carbonate and calcium oxalate, extensive anatomical literature confirms that calcium oxalate is significantly more prevalent in the *Solanaceae* family.⁹⁻¹¹ Meanwhile, the bulk fruit tissues are primarily composed of non-metallic macromolecules (C, O, N, S). The unexpected detection of Al in the trichomes requires further validation with larger sample sizes to rule out environmental contamination and confirm its biological relevance.

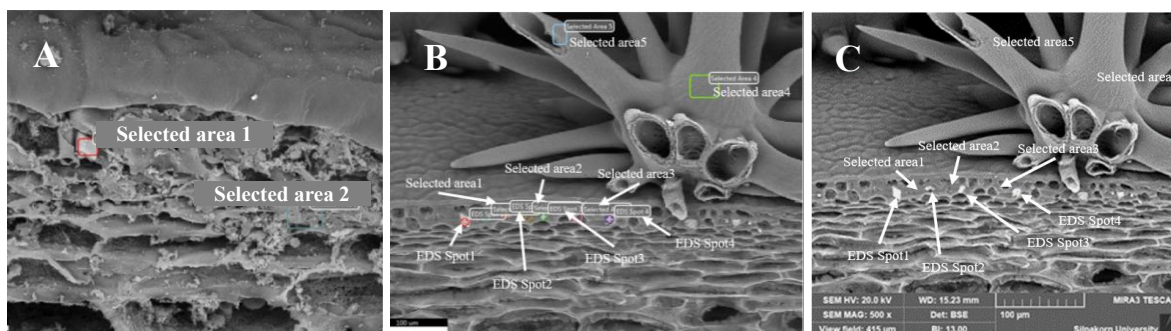


Figure 12 Scanning electron micrograph of A) immature fruit of Ma-eok showing Selected areas 1 (white amorphous crystal) and Selected area 2 (tissues of epicarp) used for EDX analysis, B) mature fruit of Ma-eok showing spotted white crystal; EDS Spot 1, EDS spot 2, EDS spot 3, EDS spot 4, and selected arear of tissues of epicarp; Selected Area 1, Selected Area 2, Selected Area 3, and selected arear of trichomes tissue; Selected area 4, and Selected area 5 for EDX analysis (the positions were marked by EDX program); and C) positions from the picture 10B were marked by the researcher for showing equivalent positions).

Table 1 Elemental composition of Selected area 1 and Selected area 2 in immature fruit of Ma-eok determined by EDX analysis

Selected area 1-white crystal			Selected area 2-tissues of epicarp		
Element	Weight %	Atomic %	Element	Weight %	Atomic %
C K	12.50	20.92	C K	39.89	52.46
O K	50.91	63.99	N K	7.07	7.97

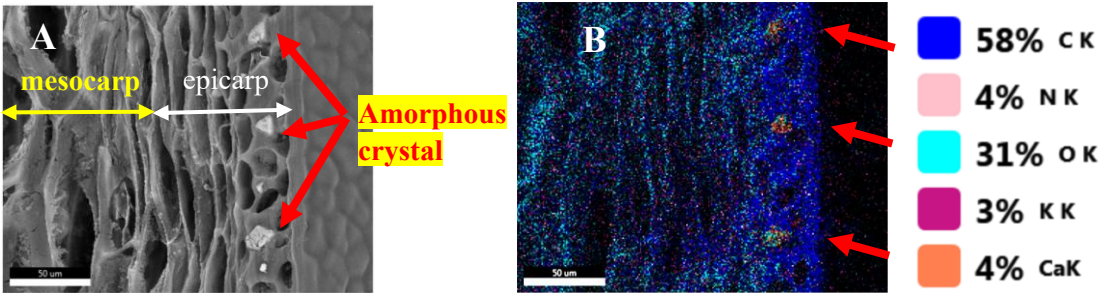
W M	8.36	0.91	O K	36.92	36.45
K K	0.55	0.28	W M	10.85	0.93
Ca K	27.68	13.89	S K	1.08	0.53
			Ca K	4.19	1.65

Table 2 Elemental composition of EDS Spot 1, EDS Spot 2, EDS Spot 3, and EDS Spot 4 (white crystal) in mature fruit of Ma-eok determined by EDX analysis

EDS Spot 1			EDS Spot 2			EDS Spot 3			EDS Spot 4		
Element	Weight	Atomic	Element	Weight	Atomic	Element	Weight	Atomic	Element	Weight	Atomic
O K	45.11	70.93	C K	3.94	11.18	C K	3.09	6.39	C K	1.48	4.03
Ca K	44.13	27.70	O K	21.10	44.95	O K	41.75	64.76	O K	21.96	44.73
Au L	10.76	1.37	Ca K	45.63	38.80	Ca K	44.41	27.50	Ca K	59.58	48.43
			Au L	29.33	5.08	Au L	10.74	1.35	Au L	16.98	2.81

Table 3 Elemental composition of tissues of epicarp; Selected area 1, Selected area 2, Selected area 3, and tissues of trichome; Selected area 4 and Selected area 5 in mature fruit of Ma-eok determined by EDX analysis

Selected area 1			Selected area 2			Selected area 3			Selected area 4			Selected area 5		
Element	Weight	Atomic	Element	Weight	Atomic	Element	Weight	Atomic	Element	Weight	Atomic	Element	Weight	Atomic
C K	47.63	77.37	C K	33.02	66.34	C K	25.23	58.84	C K	47.86	63.69	C K	48.70	60.76
O K	14.92	18.19	O K	15.81	23.84	O K	17.48	30.61	O K	34.95	34.92	O K	40.04	37.51
Ca K	1.89	0.92	Ca K	7.40	4.45	Ca K	4.33	3.03	Au L	17.19	1.39	Al K	1.81	1.01
Au L	35.56	3.52	Au L	43.78	5.36	Au L	52.96	7.53				Au M	9.45	0.72



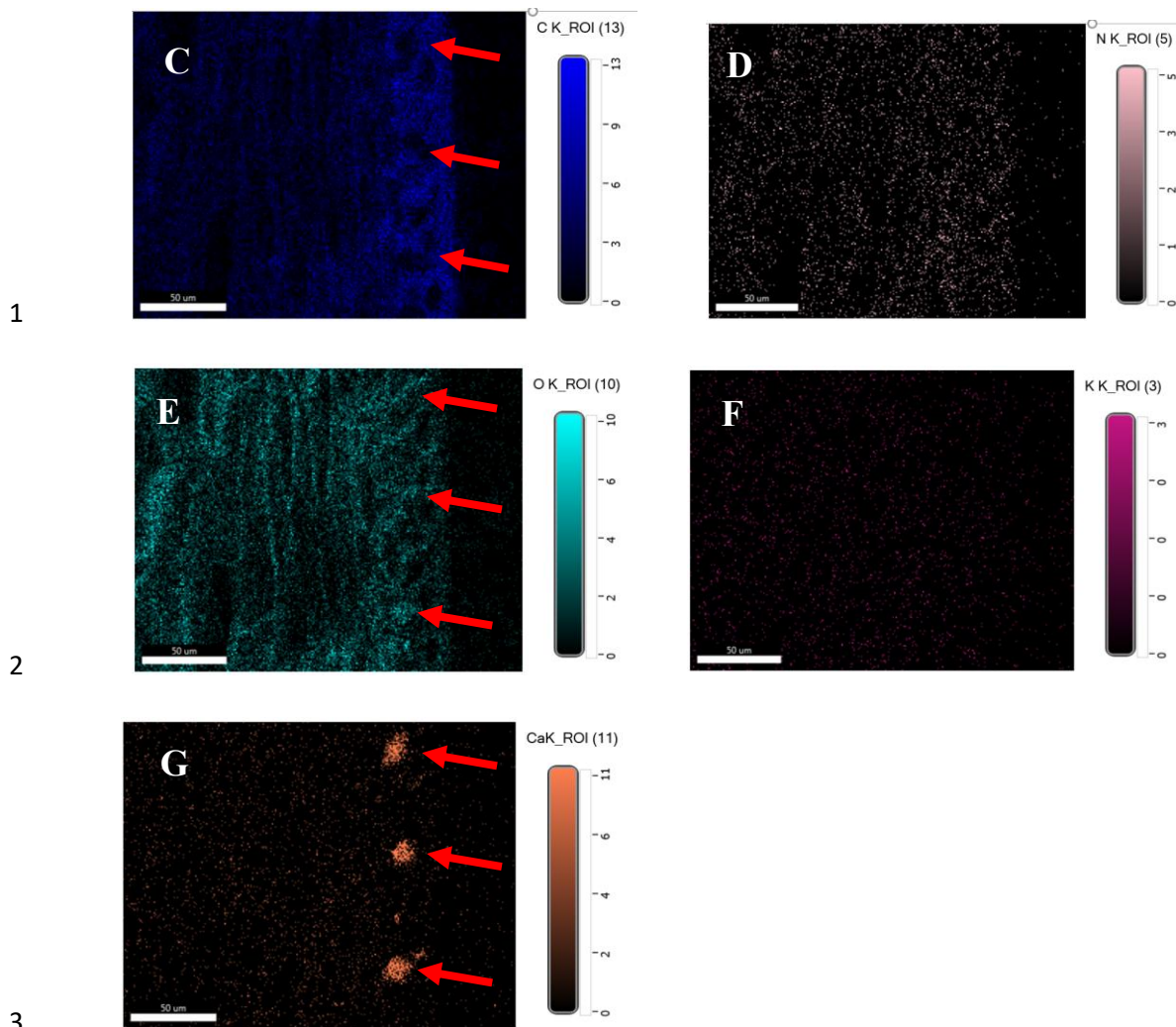


Figure 13 Elemental mapping of A) tissues of epicarp and mesocarp of mature fruit of Ma-eok, B) Overlay mapping of overall elements, C)-E) Overlay mapping of carbon, nitrogen, oxygen, potassium and calcium, respectively with original area determined by EDX analysis

4) Study of amount of principle compounds in lyophilized Ma-eok fruit juice

4.1 Determination of total phenolic content

Total Phenolic Content (TPC) of the lyophilized Ma-eok fruit juice, determined via the Folin-Ciocalteu assay using a gallic acid standard curve ($y = 0.0033x + 0.1188$, $R^2 = 0.9919$), was 68.13 ± 5.39 mg GAE/g extract (Table 4). This finding is highly consistent with a previous study, which reported a comparable TPC of 62.41 ± 1.18 mg GAE/g dry weight for an 80% methanolic extract of the fruit.²⁹

Table 4 The total phenolic content (mg GAE/1 g extract), total flavonoid content (mg QE/1 g extract), DPPH scavenging activity, and anti-inflammatory activity by inhibition of egg albumin denaturation of lyophilized Ma-eok fruit juice (n=3)

Tests	Mean $\pm$ SD
Total Phenolic Content (mg GAE/1 g extract)	68.13 $\pm$ 5.39
Total Flavonoid Content (mg QE/1 g extract)	2.57 $\pm$ 0.01
DPPH radical scavenging activity	
Lyophilized Ma-eok fruit juice, EC ₅₀ value (μ g/mL)	718.12 $\pm$ 2.17
Trolox, EC ₅₀ value (μ g/ml)	103.68 $\pm$ 0.04
mg TE/1 g extract	141.95 $\pm$ 0.02
Anti-inflammatory activity	
Lyophilized Ma-eok fruit juice at 240 mg/mL (%Anti-inflammation)	-38.59 $\pm$ 2.51
Diclofenac sodium, IC ₅₀ value (μ g/mL)	874.60 $\pm$ 5.82

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4.2 Determination of total flavonoid content

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The total flavonoid content (TFC) of the lyophilized fruit juice was evaluated using the aluminum chloride colorimetric assay. Based on the quercetin standard calibration curve ($y = 0.0099x + 0.3075$, $R^2 = 0.9900$), the TFC of the extract was found to be 2.57 ± 0.01 mg QE/g extract ($n = 3$), as shown in Table 4. This result agreed with previous report that TFC (1.689 ± 0.17 mg QE/g extract) of *S. lasiocarpum* water extract by ultrasonic technique.³⁰

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Quantitative analysis demonstrated that the extract's total phenolic content (68.13 ± 5.39 mg GAE/g) significantly exceeded its total flavonoid content (2.57 ± 0.01 mg QE/g). This disparity indicates that non-flavonoid phenolic compounds are the predominant antioxidants in the lyophilized Ma-eok juice, while flavonoids constitute only a minor fraction. Notably, this study represents the first reported data on the flavonoid content of Ma-eok fruit.

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5) Evaluation of bioactivities of lyophilized Ma-eok fruit juice

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5.1 Evaluation of antioxidant activity by DPPH assay

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Both the lyophilized Ma-eok extract and Trolox exhibited dose-dependent DPPH radical scavenging activity (Table 4). The extract's EC₅₀ was 718.12 ± 2.17 μ g/mL, demonstrating approximately 7-fold lower antioxidant activity than Trolox (103.68 ± 0.04 μ g/mL). This moderate efficacy is consistent with the extract's low flavonoid content. Based on the Trolox standard curve ($y = 0.5101x - 2.9229$, $R^2 = 0.9943$), the extract's antioxidant capacity was calculated as

141.95 ± 0.02 mg TE/g extract. The previous reported that the ultrasonic water extract of Sarawak Ma-eok fruit and Trolox presented IC₅₀ value = 10.2 ± 0.11 mg/ml and 0.69 ± 0.14 mg/ml of DPPH radical scavenging activity, respectively [30]. A direct comparison between ultrasonic water extract of Sarawak Ma-eok and lyophilized fruit juice in this study was unfeasible due to the differ concentration, however the water extract of Sarawak Ma-eok was 14-fold lower antioxidant activity than Trolox.

5.2 Evaluation of anti-inflammatory activity

In the egg albumin denaturation assay (Table 4), the lyophilized extract at its maximum testable concentration (240 mg/mL) yielded an inhibition of −38.59 ± 2.51%. In contrast, the positive control diclofenac sodium demonstrated a typical dose-dependent anti-inflammatory response (IC₅₀ = 874.60 ± 5.82 µg/mL).

The extract's negative inhibition is likely a false negative stemming from a methodological limitation. Because the fruit juice is highly acidic, and acids are known to induce protein denaturation,³¹ the extract prematurely precipitated the egg white proteins. This turbidity falsely elevated absorbance readings, resulting in a negative calculated inhibition. Given that Ma-eok root extracts possess anti-inflammatory properties via nitric oxide inhibition,¹⁵ the fruit juice may still exert anti-inflammatory effects through alternative pathways not captured by this specific protein denaturation model.

5.3 Evaluation of antimicrobial activity

Antimicrobial activity was evaluated via the agar diffusion method (Table 5). Fresh juice, centrifuged fresh juice, and lyophilized juice (tested at 300 mg/mL) all inhibited *S. aureus* (inhibition zones: 10.0 ± 1.0, 9.3 ± 0.6, and 12.0 ± 1.0 mm, respectively). However, only the more concentrated lyophilized juice exhibited activity against *E. coli* and *C. acnes* (9.7 ± 1.2 mm for both). None of the samples inhibited *C. albicans*. The assay was successfully validated using doxycycline hyclate (antibacterial) and fluconazole (antifungal) as positive controls.

In summary, all Ma-eok juice preparations were most effective against *S. aureus*. The broader antibacterial efficacy of the lyophilized juice against *E. coli* and *C. acnes* indicates that higher concentrations of active compounds are required to inhibit these strains. Regardless of the preparation method, the juice lacked antifungal activity. While previous studies have reported the antibacterial properties of *S. stramonifolium* seed extracts³² and non-edible plant parts,¹⁵ to our knowledge, this is the first study documenting the antimicrobial activity of Ma-eok fruit juice.

Table 5 Antimicrobial activities of different Ma-eok juice preparations and the positive control. (n=3)

Formulation	Inhibition zone diameter $\pm$ S.D. (mm)			
	<i>S. aureus</i> 6538	<i>E. coli</i> 8739	<i>C. acnes</i> 14916	<i>C. albicans</i> 10231
Fresh Ma-eok juice	10.0 $\pm$ 1.0	-	-	-
Centrifuged fresh juice (non-freeze-dried)	9.3 $\pm$ 0.6	-	-	-
Lyophilized Ma-eok juice (300 mg/ml)	12.0 $\pm$ 1.0	9.7 $\pm$ 1.2	9.7 $\pm$ 1.2	-
0.01% Doxycycline hyclate	25.0 $\pm$ 1.0	17.0 $\pm$ 1.0	51.3 $\pm$ 2.3	-
Fluconazole disc (1 mg)	-	-	-	27.7 $\pm$ 0.12

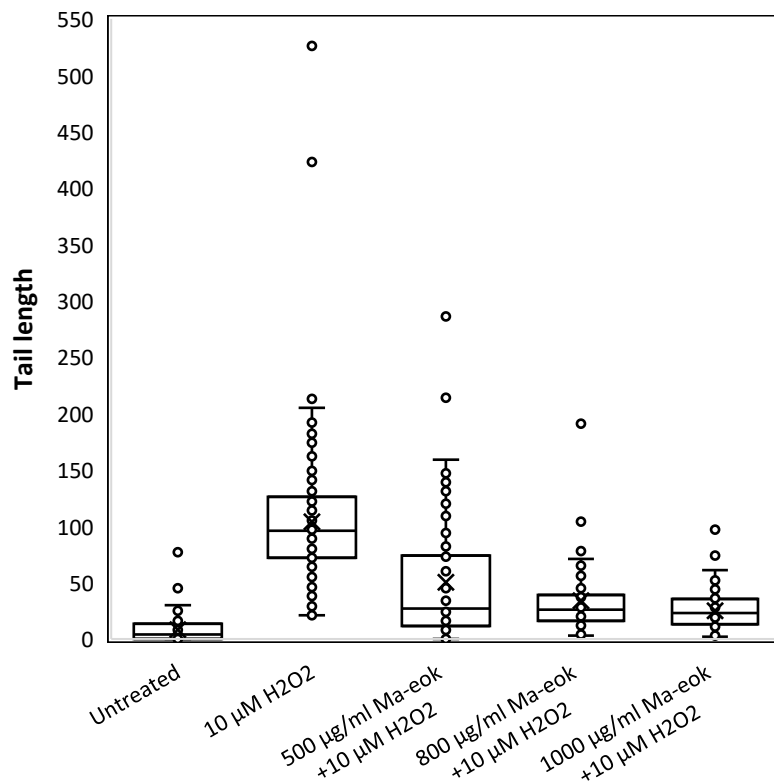
1 (-) no inhibition zones

2 **5.4 Evaluation of antioxidative effect of Ma-eok extract against H₂O₂-induced DNA damage**

3 The protective effect of Ma-eok extract against oxidative DNA damage in HCT116 cells was evaluated using
 4 the alkaline comet assay (Figure 14). Compared to the minimal DNA migration observed in untreated cells (negative
 5 control), exposure to 10 μ M H₂O₂ alone (positive control) caused extensive DNA strand breakage, evidenced by
 6 significantly increased comet tail lengths. However, pretreating the cells with Ma-eok extract (500, 800, and 1000
 7 μ g/mL) for 45 min prior to a 10-min H₂O₂ co-treatment significantly reduced comet tail lengths at all concentrations
 8 relative to the H₂O₂-only control ($P < 0.05$).

9 These findings demonstrate that the Ma-eok extract effectively mitigates H₂O₂-induced oxidative DNA
 10 fragmentation. These results are consistent with previous studies on other *Solanum* species that have exhibited
 11 antigenotoxic potential as evaluated by the comet assay. Specifically, similar protective effects were observed in the
 12 70% aqueous ethanolic leaf extract of *Solanum cernuum*,³³ the 80% aqueous ethanolic fruit extract of *S. lycocarpum*,³⁴
 13 and the purified anthocyanin extract from the fruit skin of *S. melongena*.³⁵ Conversely, it is important to note that
 14 certain members of the genus *Solanum* have been reported to act as genotoxic agents, particularly in the form of polar
 15 extracts (e.g., ethanol, aqueous ethanol, and water) derived from various fruit components, including seeds, peels, and
 16 pulp.³⁶ The protective antioxidative capacity observed in this study is likely driven by the extract's phenolic compounds,

- 1 which corroborates our total phenolic content (TPC) results. Furthermore, this finding aligns with the antioxidant activity
2 reported for Ma-eok from Phitsanulok (Thailand)¹⁶ and Palangkaraya, Central Kalimantan (Indonesia).³⁷



3 **Figure 14** Ma-eok extract significantly reduces H₂O₂-induced DNA strand damage using the comet assay (n=77-108)

4 CONCLUSIONS

5 This study successfully elucidated the morphological, anatomical, and sensory characteristics, alongside the
6 biological activities and phytochemical profiles, of the Ma-eok (*Solanum lasiocarpum* Dunal) fruit. The distinct anatomical
7 features, including specific stellate trichomes and calcium oxalate crystals, along with its sensory attributes, will serve
8 as a fundamental basis for the future botanical authentication of this plant. Furthermore, the lyophilized fruit juice extract
9 demonstrated promising antioxidant properties, DNA-protective effects, and antibacterial activities, supported by its
10 phenolic and flavonoid contents. Despite the limitation in the anti-inflammatory assay due to the extract's natural acidity,
11 these overall findings provide crucial scientific evidence to support the future application and formulation of Ma-eok fruit
12 juice into value-added products, including foods, dietary supplements, and cosmetics.

13 Disclosure

1 The authors report no conflicts of interest in this work.

2 **Author contributions**

3 P.S. conducted the investigation and wrote the manuscript section regarding oxidative DNA by Comet assay. W.W.
4 investigated the microstructural morphology and elemental composition of the Ma-eok fruit by FE-SEM-EDS and
5 performed the data analysis and interpretation. Y.S. conducted the stereomicroscopy of the Ma-eok fruit, leaves,
6 inflorescence, and flowers. J.C., T.S., and W.T. investigated the antimicrobial activity and contributed to the review and
7 editing of the manuscript. T.P. contributed to conceptualization, resources, methodology, investigation, editing
8 manuscript, funding acquisition, project administration, and supervision. W.N. supervised the undergraduate research,
9 reviewed and edited the senior project, wrote the original draft of the manuscript, and conducted the microscopic analysis.
10 P.W. was conducted the microscopic assay and edited the manuscript. All authors have read and agreed to the published
11 version of the manuscript.

12 **Funding**

13 This work was supported by the Undergraduate Senior project Grant for the Bachelor of Pharmacy Program, Faculty of
14 Pharmacy, Silpakorn University (Academic Year 2025).

15 **Statement of human and animal rights**

16 This research did not involve human participants, human data, human tissue, or animal models.

17 **Declaration of competing interest**

18 The authors declare no competing interests. This independent study was conducted without external influence, and all
19 data collection, analyses, and interpretations were performed objectively.

20 **Declaration of generative AI and AI-assisted technologies in the writing process**

21 During the preparation of this work, the authors used Gemini (version 3.1 pro-preview) to improve the English language
22 and readability of the manuscript. After using this tool, the authors thoroughly reviewed and edited the content as needed
23 and take full responsibility for the final content of the publication.

24 **Data availability statement**

1 Data will be made available on request.

2 Acknowledgments

3 This work was financially supported by the Undergraduate Senior Project Grant, Bachelor of Pharmacy Program, Faculty
4 of Pharmacy, Silpakorn University (Academic Year 2025). We thank Ms. Kotchawan Saiwong, Mr. Chatchai Puangrak
5 and Ms. Thansinee Ruttanatubtimtong for their support and help. The authors thank the Faculty of Pharmacy, the Faculty
6 of Science, and the scientific instrument facilities at Silpakorn University for equipment support. We also extend our
7 gratitude to Asst. Prof. Dr. Phuvamin Suriyaamporn, Ph.D. for providing the tissue embedding reagents and sectioning
8 advice, and to Ms. Anchalee Kumsum (Scientist, Faculty of Pharmacy, Silpakorn University) for her technical instruction
9 and assistance with microtome sectioning.

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