ORIGINAL ARTICLE

Garcinia dulcis and *Garcinia forbesii* King fruit peel extract: Secondary metabolite composition, antioxidant, and elastase inhibitory activity evaluation

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ABSTRACT

Garcinia dulcis and *Garcinia forbesii* King are native plants from Indonesia and have tremendous potential as a source of raw medicines based on local wisdom. However, scientific data for strengthening pharmaceuticals are still limited. Therefore, it is necessary to conduct a study to strengthen and develop the potential of both plants using the approach of traditional medicine. This study aimed to explore the secondary metabolite composition and biological activity (antioxidant and antielastase) of both plants. Both samples were extracted using 70% ethanol and microwave-assisted extraction with a microwave power of 120 watts for 15 min. The extract obtained was then screened for phytochemicals using specific reagents. The total phenolic content (TPC) was determined using spectrophotometry with a 96-well microplate reader method. The total flavonoid content (TFC) was determined using the colorimetric method, whereas metabolite profiling analysis was conducted using the UPLC-QToF-MS/MS system. Meanwhile, biological activity was tested for antioxidant activity and antielastase as measured by a microplate reader 96-well spectrophotometry method at specific wavelengths. According to the results, *G. dulcis* and *G. forbesii* fruit peel extracts showed positive detection of particular secondary metabolites. TPC and TFC values were 13.98 ± 1.90 mg GAE/g and 10.33 ± 1.90 mg QE/g for *G. dulcis* and 11.98 ± 2.04 mgGAE/g and 1.96 ± 0.36 mgQE/g for *G. forbesii*. Metabolite profiling detected some compounds from *G. dulcis*, including ephedrannin B, hinokiflavone, mahuannin J, and candidate mass $C_9H_{12}O_8$, and *G. forbesii*, including 5-Hydroxy-7,8,2′-trimethoxyflavone, lucialdehyde B, candidate mass $C_{21}H_{39}NO_4$, candidate mass $C_{14}H_{10}O_6$, and candidate mass $C_{14}H_{12}O_6$. Meanwhile, the biological activities (antioxidant and antielastase) were 137.721 µg/mL and 108.893 µg/mL for *G. dulcis* and 481.948 µg/mL and 250.611 µg/mL for *G. forbesii*, respectively. Both plants showed different profiles of secondary metabolites and biological activities (antioxidant and antielastase) according to their respective characteristics.

Key words: Antielastase, antioxidant, *Garcinia dulcis*, *Garcinia forbesii* King, metabolite profiling

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INTRODUCTION

Garcinia belongs to the mangosteen tribe/family, Guttiferae or *Clusiaceae*, with polygamous green flowers of ± 435 species. Its distribution from South-east Asia extends to New Caledonia, Northern Australia, tropical Africa, Madagascar, Polynesia, Central and South-Central America, and South America. Several species of the genus *Garcinia* are used as a source of edible fruits, wood, resins, and other natural products and are used medicinally in various Indian Ayurvedic treatments. Some exciting examples studied for their potential efficacy as antioxidants that prevent premature aging of the *Garcinia* genus were *Garcinia dulcis* Kurz. and *Garcinia forbesii* King.

Both species are native to South-east Asia, especially from Indonesia and Malaysia, a tropical species that can adapt to humid, wet, and hot climates. Phytochemically, *Garcinia* species, mainly *G. forbesii* and *G. dulcis,* are a rich source of xanthones, anthocyanins, tannins, and phenolic compounds.[1] However, scientific data on these two plants are still very limited. Meanwhile, metabolite profiling studies and antioxidant and antielastase activity tests from the fruit skin of these two plants have never been reported.

G. dulcis and *G. forbesii* are plants known to have the potential to produce bioactive compounds that can benefit the health sector. Therefore, this study aimed to understand better the content of these secondary metabolites in both samples and their antioxidant activity and elastase inhibition ability. This research is essential because natural compounds with antioxidant and elastase inhibitory activity can have potential applications in developing health and cosmetic products.[2] As a first step, the capacity of raw material as a source of antioxidants related to total phenolic and flavonoid content was evaluated using 1,1-diphenyl-2-picrylhydrazine (DPPH) free radical scavenging and elastase enzymes' inhibitory activity assay. Therefore, conducting this study to learn more about it is essential.

MATERIALS AND METHODS

Materials

The materials utilized include DPPH, quercetin, elastase from porcine pancreas (porcine pancreatic elastase or PPE), N-Succinyl-Ala-Ala-Ala-p-nitroanilide (elastase substrate), gallic acid, and Trizma buffer, which were obtained from Sigma-Aldrich, USA; ethanol, ammonia, chloroform, HCl, and ether were purchased from PT. Smart Lab Indonesia, Indonesia; acetic acid anhydrous, sulfuric acid, magnesium powder, amyl alcohol, $FeCl_3$, formaldehyde, $C_2H_3NaO_3$, and NaOH were purchased from PT. Brataco, Indonesia; petroleum ether, methanol pro analyze, and distilled water were purchased from PT. Ikapharmindo Putramas, Indonesia.

Samples preparation and extraction process

Both samples were obtained from the Bogor Botanical Gardens. The samples (G.1/23/2021 for *G. dulcis* and G.2/23/2021 for *G. forbesii*) were collected from March to April 2021. They were determined and authenticated by Dr. Himmah Rustiami, SP., M.Sc., at the Biological Research Center of LIPI, Cibinong. Part of the fruit skin was dried and made of fibers. Then, both samples (250 g each) were extracted using microwave-assisted extraction with 70% ethanol for 15 min with a microwave power of 120 watts. Each filtrate was mixed and concentrated using a rotary evaporator at 40°C–45°C at 80 rpm until a thick extract was obtained.

Phytochemical screening

Phytochemical screening of each extract was carried out using the method from some literature,[3-5] which has been modified to include testing for alkaloid compounds, flavonoids, saponins, quinones, tannins, terpenoids, steroids, essential oils, and coumarins.

Determination of total phenolic content

The total phenolic content (TPC) was determined utilizing a microplate reader 96-well spectrophotometry method and Folin–Ciocalteu reagent.[6] Briefly, 20 µL of the test sample solution was added to 100 µL 25% Folin–Ciocalteu solution, and then homogenized for 5 min. Next, 50 µL Na_2CO_3 was added to the solution, incubated for 120 min at room temperature, and then measured at 750 nm wavelength. The TPC value in both extracts was determined using the equation formula from curve calibration of gallic acid (12.5–200 µg/L) as standard, where Y = 0.0658 + 0.013X, with R^2 = 0.9964, X was the absorbance of samples or standard, and Y was TPC value.

Determination of total flavonoid content

The total flavonoid content (TFC) was determined utilizing the $AlCL_3$ colorimetric method[7,8] with modification. In brief, 50 µl of standard solution or extracts (1 mg/ml) was added to 10 µl of 10% $AlCl_3$ solution, followed by 150 µl of 96% ethanol. The mixture on a 96-well plate was supplemented with 10 µl of 1 M $C_2H_3NaO_2$. As a reagent blank, 96% ethanol was utilized. After combining the chemicals, they were let to sit at room temperature for 40 min with no light. The absorbance was measured at 415 nm – quercetin solution with various concentrations (6.25–100 µg/L) as standard. The TFC value in both extracts was determined using the equation formula from curve calibration of quercetin, where Y = 0.005X–0.0017, with R^2 = 0.9971, X was the absorbance of samples or standard, and Y was the TFC value.

Metabolite profiling analysis

Metabolite profiling analysis was conducted using UPLC-QToF-MS/MS System.[9-11] Briefly, 1 µL sample solution was injected into the column (ACQUITY UPLC® BEH C_8) with acetonitrile (pump B) as the mobile phase. A full scan of 100–1200 m/z and a 0.3 mL/min flow rate for

electrospray ionization were employed. Data acquisition was processed with MassLynx 4.1 Software.

Antioxidant activity assay

The antioxidant activity was determined using a DPPH scavenging ability assay according to the literature[12,13] with slight modification. Each well received 180 µl of a 0.147 mM DPPH solution and a 20 µl stock solution of each extract in varying concentrations. The wells were then incubated for 30 min at room temperature in the dark. In addition, the absorbance was measured at 517 nm wavelength. Methanol was used as a blank. The scavenging ability (%) was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorption of standard} - \text{Absorption of sample}}{\text{Absorption of standard}} \times 100\%$$

Quercetin was used as a positive standard in this study in some amounts. Every test was run in triplicate. The sample concentration (half maximum inhibitory concentration [IC50] value) will block DPPH by 50% was determined.

Elastase inhibition activity assay

Each sample (20 µL) was added to wells holding 140 µL of a solution of 0.2 M Tris-HCl buffer (pH 8.0). After that, 20 µL of PPE solution was added and allowed to sit at room temperature for 15 min. After incubation, 20 µL of substrate SANA was added and re-incubated for 5 min under the same conditions. Subsequently, it was measured using a VersaMax® 96-well Microplate Reader at a wavelength of 405 nm.[13-15] Quercetin was used as the positive control at different concentrations. Every assay was run twice. Finally, equation (1) was used to determine the percentage of elastase inhibition, as follows:

$$\text{Percentage of elastase inhibition}\,(\%) = \frac{\text{Absorption of control} - (\text{Absorption of sample} - \text{Absorption of blank})}{\text{Absorption of control}} \times 100\%$$

To get the IC_{50} value, the extract concentration and percentage of inhibitory action were plotted.

RESULTS

Phytochemical screening

The results of the phytochemical screening showed that both samples contained secondary metabolites, as presented in Table 1, which are believed to be responsible for the biological activity, especially as antioxidants and antielastases.

Phenolic and flavonoid composition

Total phenol and flavonoid contents were determined using calibration curves of gallic acid for TPC (in GAE) and quercetin (in QE), respectively. The TPC and TFC values from both sample extracts showed different compounds, as presented in Table 2. In addition, the yield values between the two extracts were not significantly different and were directly proportional to the TPC and TFC values.

Table 1: Phytochemical screening of both samples

Skrining fitokimia	*Garcinia dulcis* fruit extract	*Garcinia forbesii* fruit extract
Alkaloids	−	+
Coumarin	−	+
Flavonoids	+	+
Essential oil	−	−
Kuinon	−	−
Tannins	+	+
Steroids and triterpenoids	+	+
Saponins	+	+

−: Present, +: Absent

Metabolite profiling analysis

As shown in Figure 1, the peaks in the two extracts have different metabolite profiles. Retention time (Rt) values between 1.17 and 7.10 min differentiate wells at a peak depth in Figure 1a, while Figure 1b shows an Rt value in the range of 4.79–9.3 min.

In Table 3, liquid chromatography results show retention time peaks, measured by mass spectra, and m/z values were obtained.

Antioxidant activity evaluation

The elastase enzyme inhibitory activity test and the DPPH radical scavenging assay IC_{50} values for the extract samples and quercetin as standard are displayed in Table 4. The quality of the activity offered increases with the concentration of the test sample. Based on the IC_{50} value derived from the linear regression equation, these data depict the activity of the two extracts. The findings indicate that while being weaker than the positive control, *G. dulcis* fruit extract has a more decisive action than *G. forbesii* fruit extract.

DISCUSSION

Based on the results of the phytochemical screening of the two plant extracts of *G. dulcis* and *G. forbesii*, the identification results of secondary metabolites did not differ much. Only alkaloids and coumarins were detected in *G. forbesii* fruit peel extract. Sutomo *et al*. have also reported a similar study,[1] which identified the results of secondary metabolites of *G. forbesii*, including alkaloids, flavonoids, phenols, tannins, and steroids. Apart from that, it also contains flavonoids, organic acids, and Vitamin C.[1,6,7]

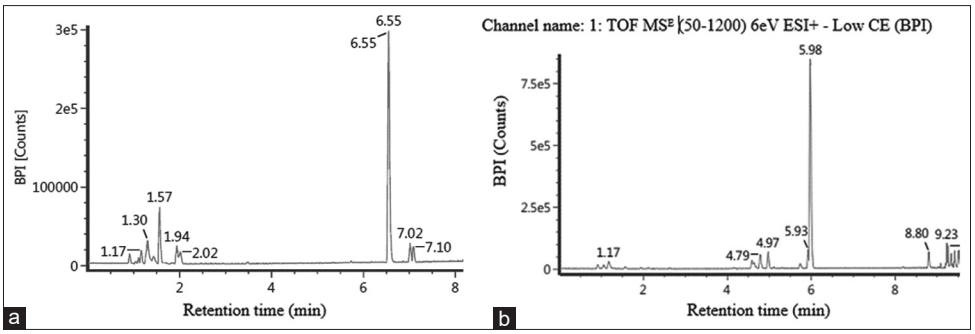

Figure 1: Peak data from both extracts' metabolite profiling analyses. Where (a) *Garcinia dulcis* fruit extract, and (b) *Garcinia forbesii* fruit extract

Table 2: The results of the total phenolic and flavonoid content of both samples

Test sample	TPC (mg GAE/g±SD)	TFC (mg QE/g±SD)	Yield (%w/w)
Garcinia dulcis fruit extract	13.98±1.90	10.33±1.90	19.68
Garcinia forbesii fruit extract	11.98±2.04	1.96±0.36	18.72

TPC: Total phenolic content, TFC: Total flavonoid content, SD: Standard deviation, GAE: Gallic acid equivalent, QE: Quercetin equivalent

Table 3: Results of both extracts' ultra-high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (m/z value) spectra

Test sample	Component name	m/z [H$^+$]	Retention time	Formula
Garcinia dulcis fruit extract	Ephedrannin B	541.1137	7.02	$C_{30}H_{20}O_{10}$
	Hinokiflavone	539.0979	7.10	$C_{30}H_{18}O_{10}$
	Mahuannin J	557.1089	6.56	$C_{30}H_{20}O_{11}$
	Candidate mass $C_9H_{12}O_8$	249.0604	1.57	$C_9H_{12}O_8$
Garcinia forbesii fruit extract	5-hydroxy-7,8,2′-trimethoxyflavone	329.1014	8.81	$C_{18}H_{16}O_6$
	Lucialdehyde B	453.3361	9.23	$C_{30}H_{44}O_3$
	Candidate mass $C_{21}H_{39}NO_4$	369.2879	5.98	$C_{21}H_{39}NO_4$
	Candidate mass $C_{14}H_{10}O_6$	275.0546	4.79	$C_{14}H_{10}O_6$
	Candidate mass $C_{14}H_{12}O_6$	277.0702	4.98	$C_{14}H_{12}O_6$

Table 4: The result of antioxidant activity assay using diphenyl-2-picrylhydrazine radical scavenging assay and elastase inhibition activity assay from *Garcinia dulcis* and *Garcinia forbesii* fruit extracts

Test sample	IC_{50} (μg/mL)	
	Antioxidant activity	Elastase inhibitory activity
Garcinia dulcis fruit extract	137.721	108.893
Garcinia forbesii fruit extract	481.948	250.611
Quercetin	76.208	15.812

IC_{50}: Half-maximal inhibitory concentration

The assay method using the Folin–Ciocalteu reagent was the most straightforward method available for measuring phenolic content and has been used to measure polyphenols in natural products. However, this method was susceptible and precise because the maximum absorption of the chromophore depends on the base solution and the concentration of phenolic compounds.

The m/z value was adjusted to the database on the tool, and the possible compounds were detected. At the peak of 7.02, an m/z value of 541.1137 was obtained, possibly the detected compound was ephedrannin B, and at the peak of 6.56 with an m/z value of 557.1089, the detected compound might have been mahuannin J. Meanwhile, the results of the metabolite profiling analysis showed that 5-hydro-7,8,2′-trimethoxy-flavanone with a molecular weight of 329.1014 m/z with a retention time of 8.81 min, lucialdehyde B with a molecular weight of 453.3361 with a retention time of 9.23, as well as other suspected compounds, such as $C_{21}H_{39}NO_4$, $C_{14}H_{10}O_6$, and $C_{14}H_{12}O_6$. The presumptive compound can only be suspected because the database available for this test was limited; hence, the exact compound in the sample cannot be known.

In the DPPH test, the IC_{50} obtained was 137.721 μg/mL for *G. dulcis* fruit extract, better than *G. forbesii* fruit extract with an IC_{50} of 481.948 μg/mL. This was in line with the results of testing the total phenolic and flavonoid levels in *G. dulcis* fruit extract, which were higher than the results of *G. forbesii* fruit extract. These results indicate the great potential of *G. dulcis* fruit extract as a source of antioxidants.

The parameter used to determine the inhibitory activity of PPE in this study was IC_{50}. The inhibitory activity of the PPE in *G. dulcis* rind was strengthened by the results of phytochemical screening, where the *G. dulcis* and *G. forbesii* fruit peels contained flavonoids and tannins, which played an important role in inhibiting the PPE.

CONCLUSIONS

The present study found the potency of *G. dulcis* and *G. forbesii* fruit peel extract. The two samples showed different profiles of secondary metabolites and biological activities (antioxidant and antielastase) according to their respective characteristics. This research is an early stage of developing and utilizing the *Garcinia* family as a source of potential pharmaceutical raw materials based on local wisdom. More research must be done to expand our understanding of these two plants from a scientific standpoint.

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Conflicts of interest

There are no conflicts of interest.

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