

α -GLUCOSIDASE INHIBITORY ACTIVITIES AND ANTIOXIDANT PROPERTIES OF DEPSIDONE FROM *Garcinia* *parvifolia*

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ABSTRACT

Garcinia parvifolia, locally named “Aciu”, is commonly used by local people in East Kalimantan as a food additive and antibacterial, and for traditional therapies to treat high blood sugar levels, wounds, inflammation, and fever. In this study, in vitro, assays were carried out to evaluate extracts (*n*-hexane, dichloromethane, ethyl acetate, methanol residue) and an isolated compound from *G. parvifolia* fruits for their antidiabetic properties by inhibiting α -glucosidase and for their antioxidant activities by scavenging DPPH and ABTS^{•+} free radicals. Bioassay-guided isolation of *G. parvifolia* fruits resulted in a secondary metabolite, namely garcinisidone A. Furthermore, Garcinisidone A showed stronger antidiabetic properties than extracts and positive controls (acarbose and quercetin) with IC₅₀ values of 6.61 μ M. In addition, *G. parvifolia* fruits extract (dichloromethane) and garcinisidone A were found to have very strong antioxidant activities in comparison with the positive controls, namely ascorbic acid and quercetin. The results of this study confirmed the beneficial effects of *G. parvifolia* fruits as antidiabetic agents and free radical scavengers. This approach validates the ethnopharmacological use of *G. parvifolia* fruits for the treatment of diabetes and its complications caused by free radicals.

Keywords: Anti-diabetes, Antioxidant, Depsidone, East Kalimantan, *Garcinia parvifolia*.

RASĀYAN J. Chem., Vol. 16, No. 3, 2023

INTRODUCTION

Diabetes mellitus has become a chronic disease characterized by hyperglycemic circumstances due to the imbalanced demand for insulin.¹ Diabetes is known to be associated with severe complications, such as kidney failure, blindness, stroke, and heart disease.² Today, according to several studies, increasing free radical production due to hyperglycemia may partially initiate diabetes complications, such as neuropathy, nephropathy, and retinopathy.³ Recent studies reported that hyperglycemia can be controlled by inhibiting digestive enzymes such as α -glucosidase and α -amylase. It can be a significant and important strategy in treating diabetes mellitus.^{4,5} At present, α -glucosidase inhibitors such as acarbose and miglitol are commonly used as antidiabetic drugs. Nevertheless, these drugs may not be free of adverse effects, such as weight gain, diarrhea, gastrointestinal circumstances, and flatulence.^{6,7} In recent years, several studies showed that phytochemicals from medicinal plants have good prospects for inhibiting glycosidase and scavenging free radicals.⁸ *Garcinia* species is a highly well-known plant that is rich in secondary metabolites, such as xanthenes, flavonoids, benzophenones, and phenolic acids. Furthermore, the biological activities of *Garcinia* species have shown many potencies, such as antioxidant, antibacterial, anti-lipogenic, antifungal, cytotoxicity, and antidiabetic.⁹⁻¹² *Garcinia parvifolia* belongs to the Clusiaceae family. It grows naturally throughout the tropical region of East Kalimantan province and its fruits have been locally named “Aciu”. Based on ethnopharmacological information, these fruits have been used for food additives and

medical purposes.¹³ In the authors' continuous investigation to find natural inhibitors for diabetic disease, *G. parvifolia* fruits were recently investigated. Therefore, in this study, we investigated their antidiabetic and antioxidant activities *in vitro* by inhibiting α -glucosidase and scavenging free radicals respectively. To the best of the authors' knowledge, no scientific investigations have been conducted on the α -glucosidase inhibitory activity of *G. parvifolia* fruits.

EXPERIMENTAL

Materials

Ascorbic acid (Vitamin C), Na₂CO₃, *n*-hexane, dichloromethane, ethyl acetate, and MeOH were obtained from Merck, Darmstadt, Germany. Column chromatography was carried out using silica gel 60 (70-230 mesh) or Sephadex LH-20. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), quercetin, α -glucosidase (EC3.2.1.20) from *Saccharomyces cerevisiae* and *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG) supplied by Sigma-Aldrich. Acarbose was obtained from Bayer Vitol Leverkusen, Germany. Thin-layer chromatography was performed using silica gel 60 F₂₅₄ aluminum sheets, with spot detection using a UV lamp or *via* anisaldehyde staining followed by heating.

Extraction and Isolation Procedure

G. parvifolia fruits (2.1 kg) were collected from June to August 2020 from tropical forests in East Kalimantan. Plant specimens were deposited at the Forest Product Chemistry Laboratory at Mulawarman University, Indonesia. *G. parvifolia* fruits were dried in shade-dried at ambient temperature, then grounded and extracted with methanol (3 x 1L) for 3 days. After the extraction process, the filtrates were evaporated to obtain concentrated MeOH extracts (500 gr, 23.80%). Afterward, a portion of the MeOH crude extract was fractionated with liquid-liquid extraction successively using organic solvents with increasing polarity i.e., *n*-hexane, dichloromethane, and ethyl acetate. Thus, from the fractionation, *n*-hexane (30.73 gr, 6.14%), dichloromethane (119.25 gr, 23.85%), ethyl acetate (154.99 gr, 30.99%), and MeOH residue (195.03 gr, 39.0%) extracts were obtained. Thereafter, the extracts of *G. parvifolia* fruits were subjected to antidiabetic and antioxidant bioassays and then further isolated to obtain bioactive compounds. These extracts were tested for their biological activities, namely antidiabetic and antioxidant activities using the TLC (thin-layer chromatography) technique. Among these extracts, the dichloromethane fraction was further investigated. The dichloromethane extract was fractionated with vacuum column chromatography (Silica gel 60G Merck) and eluted with *n*-hexane: ethyl acetate with ratios of 100:0, 90:10, 70:30, 60:40, 50:50, 40:60, 20:80, 0:100 to provide 8 major fractions (Fr RR1 – Fr RR8). The Fr RR4 was further chromatographed on Silica gel (230-400 mesh) with isocratic solvent dichloromethane: ethyl acetate (8:2, v/v) to obtain 6 subfractions (Fr RR4-1 – Fr RR4-6) according to the comparison of TLC profiles. Thus, subfraction Fr RR4-4 was chromatographed on Sephadex LH-20 (100% MeOH) to obtain garcinisidone A (420 mg). The isolated compound was identified using 1D and 2D NMR (¹H, ¹³C, HMQC, and HMBC), UV-Vis, IR, and HR-ESIMS experiments and *via* comparison with those of previous studies.¹⁴

Yeast α -glucosidase Inhibition Assay

The α -glucosidase inhibition assays on the *n*-hexane, dichloromethane, ethyl acetate, methanol residue extracts, and the isolated compound was carried out according to a previously published method.¹⁵ Briefly, mixtures of phosphate buffer (pH 6.9), 20 μ L of crude extract, and the isolated compound (6.25 – 100 μ g/mL) were pre-incubated with enzyme solution at 37°C for 10 minutes in a 96-well plate. Afterward, 50 μ L of *p*-NPG was added to each well at 37°C for 20 minutes. The mixture reaction was terminated by adding 100 μ L of Na₂CO₃ (1 M). The absorbance of the reaction mixture was determined spectrophotometrically at 405 nm in a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader. Acarbose and quercetin were used as the standard references. The inhibitory activity percentage was calculated using the following formula: % Inhibition = [(A₀-A₁)/A₀] x 100, with A₀ as the absorbance without the sample and A₁ as the absorbance with the sample. The IC₅₀ value was obtained from a plot of % inhibition versus the final concentration of the reaction.

DPPH Free Radical Scavenging Assay

The ability of the different extracts and the isolated compound to scavenge DPPH free radicals was determined using the previous method.¹⁶ Briefly, different concentrations of extracts and the isolated

compound were mixed with 100 μL of DPPH solution (0.10 mM) in a 96-well, which was then incubated in a dark place at ambient temperature. After 30 minutes of incubation, the absorbance was measured at 517 nm. Ascorbic acid and quercetin were used as the standards. Finally, the percentage of inhibition (PI) was calculated with the following formula (1): % Inhibition = (Absorbance of Control – Absorbance of Sample)/Absorbance of Control $\times$ 100. The data were illustrated as the concentration of samples required to inhibit the formation of DPPH radicals by 50% (IC_{50}).

ABTS Free Radical Scavenging Assay

The free radical scavenging assay on the ABTS free radical of the extracts and the isolated compound was conducted using the previously described method with a slight modification.¹⁷ Briefly, in a 96-well microplate, 100 μL of ABTS⁺ solution and 20 μL of the samples (extracts and the isolated compound) were plated individually. The absorbance was measured at a wavelength of 750 nm after a 60-minute incubation at room temperature. The free radical scavenging activity was calculated using formula (1).

RESULTS AND DISCUSSION

In this study, the authors evaluated the α -glucosidase inhibitory activity and antioxidant potency of four different fractions of *G. parvifolia* fruits, namely *n*-hexane, dichloromethane, ethyl acetate, methanol, and their isolated compound. The IC_{50} values for α -glucosidase inhibitory activity and antioxidant potency on the *n*-hexane, dichloromethane, ethyl acetate, methanol extracts, and the isolated compound are summarized in Table-2. The dichloromethane extract was further investigated based on the thin layer chromatography profile and the results showed that it had promising inhibition activities. The dichloromethane extract of *G. parvifolia* was chromatographed over a silica gel column and further purified using Sephadex LH-20 to give a depsidone, namely garcinisidone A, whose structure was characterized based on spectroscopic data and via comparison with those of previous studies.¹⁴ White powder (420 mg); UV-Vis (EtOH) λ_{maks} (log ϵ): 280(3.43), 315(3.93); R_f = 0.58 (Dichloromethane: ethyl acetate 4.5:0.5); FTIR (DRIFT, KBr, $\bar{\nu}$ (cm^{-1})): 3348 (O-H), 3043 (C-H Sp^2), 2968 (C-H Sp^3), 1651 (C=O), 1589 (C=C), 1069 (C-O). HR-ESI-MS spectrum of Garcinisidone A is shown in Fig.-3. Garcinisidone A was obtained as a white powder. The molecular formula was deduced from the HRESIMS analysis of pseudomolecular ion ($\text{M}+\text{H}$)⁺ at m/z 427.2142 (calcd 427.1757), which accounted for the molecular formula of $\text{C}_{24}\text{H}_{26}\text{O}_7$. The UV spectrum of garcinisidone A showed maxima absorption bands at 280 and 315 nm. Meanwhile, its IR spectrum showed the vibration band of hydroxyl groups at ν_{max} 3348 cm^{-1} (*br*) and the vibration band of a lactone carbonyl group at ν_{max} 1651 cm^{-1} . The ^1H NMR spectrum showed the presence of signal due to a methoxy group [δ_{H} 3.70 (3H, s)], two sets of aromatic protons [δ_{H} 6.39 (1H, s) and δ_{H} 6.69 (1H, s)], four methyl groups [δ_{H} 1.56 (3H, s); 1.60 (3H, s); 1.68 (3H, s); 1.76 (3H, s)], two methylene groups [δ_{H} 3.42 (2H, m) and δ_{H} 3.24 (2H, d, $J=7.8$)], and two vinyl groups [δ_{H} 5.13 (2H, m) $\times$ 2]. Moreover, the ^{13}C NMR spectrum also contained a lactone carbonyl group [δ_{C} 168.63], one methoxy group [δ_{C} 60.42], four methyl carbons [δ_{C} 17.14, 17.38, 25.11, and 25.17], and two methylene carbons [δ_{C} 21.67 and 23.82]. The spectroscopic data suggested that the isolated compound is substituted with two prenyl side chains, one methoxy group, and three hydroxyl groups. The NMR data (Table-1) analysis including HMQC (*Heteronuclear Multiple Quantum Coherence*) and HMBC (*Heteronuclear Multiple Bond Correlation*) (Fig.-2) led to confirmation that the structure of the isolated compound is that of a depsidone group, namely Garcinisidone A (Fig.-1).

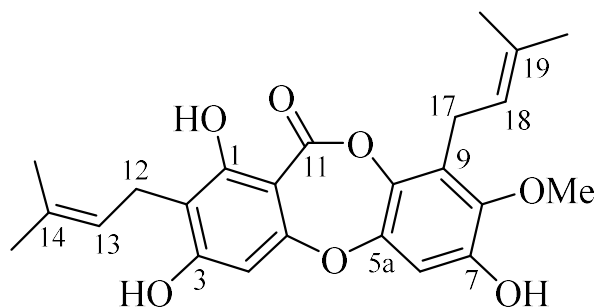
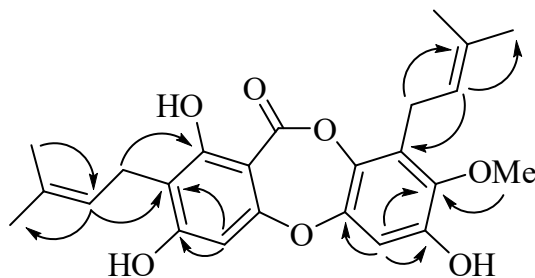


Fig.-1: Garcinisidone A from the Fruits of *Garcinia parvifolia*

Table-1: 1D (^1H and ^{13}C) and 2D (HMQC and HMBC) NMR of Garcinisidone A

Position	δ _H (ppm) (multiplicity, integrity, <i>J</i> in Hz)	δ _C (ppm)	δ _C (ppm) ^a	HMBC
1	-	162.70	161.06	-
2	-	112.72	112.22	-
3	-	162.99	164.17	-
4	6.39 (<i>s</i> ,1H)	99.63	99.6	C-11a, C-2, C-4a, C-3
4a	-	159.8	159.09	-
5a	-	146.44	145.57	-
6	6.66 (<i>s</i> ,1H)	105.9	105.68	C-9a, C-5a, C-7, C-8
7	-	148.56	148.14	-
8	-	143.58	142.94	-
9	-	128.34	127.46	-
9a	-	135.38	134.38	-
11	-	168.63	167.17	-
11a	-	97.5	92.98	-
12	3.24 (<i>d</i> , 2H, 7,8)	21.67	21.43	C-2, C-13, C-14, C-3
13	5.13 (<i>m</i> ,2H)	122.08	122.31	C-15, C-12, C-16, C-14
14	-	131.08	130.48	-
15	1.68 (<i>s</i> ,3H)	25.11	25.42	C-16, C-13, C-14
16	1.56 (<i>s</i> ,3H)	17.14	17.66	C-15, C-13, C-14
17	3.42 (<i>m</i> ,2H)	23.82	23.48	C-18, C-19, C-9a, C-8, C-9
18	5.13 (<i>m</i> ,2H)	122.22	121.83	C-20, C-12, C-21, C-19
19	-	132.16	131.64	-
20	1.76 (<i>s</i> ,3H)	25.17	25.46	C-21, C-18, C-19
21	1.60 (<i>s</i> ,3H)	17.38	17.46	C-21, C-18, C-19
1-OH	11.12 (<i>s</i> ,1H)	-	-	C-11a, C-2, C-1
8-OMe	3.70 (<i>s</i> ,3H)	60.42	60.05	C-8

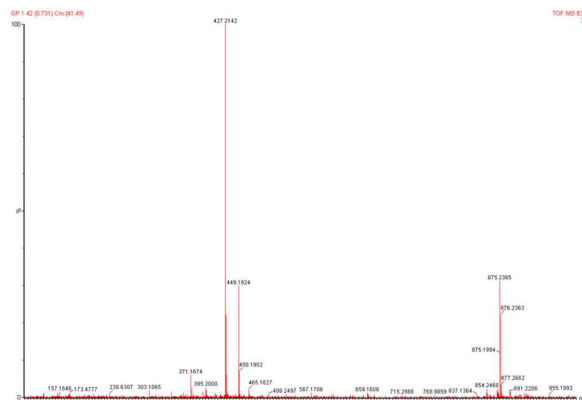
^aPrevious study¹⁴

Fig.-3: HR-ESI-MS Spectrum of Garcinisidone A

Table-2: α -Glucosidase Inhibitory Activity and Antioxidant Potency of *G. parvifolia*

No	Samples ^a	IC ₅₀		
		α -Glucosidase	DPPH	ABTS ^{•+}
Extracts ^b				
1	<i>n</i> -hexane	7.69 $\pm$ 0.24	NI	NI
2	Dichloromethane	3.15 $\pm$ 0.30	NI	63.01 $\pm$ 2.53
3	Ethyl acetate	11.12 $\pm$ 0.48	NI	NI
4	MeOH	7.84 $\pm$ 0.51	NI	NI
Isolated compound ^c				
5	Garcinisidone A	6.61 $\pm$ 0.40	NI	18.92 $\pm$ 0.03
Positive controls ^c				
6	Acarbose	22.55 $\pm$ 0.97	ND	ND
7	Quercetin	24.39 $\pm$ 0.69	100.65 $\pm$ 1.85	99.68 $\pm$ 0.88
8	Ascorbic acid	ND	54.20 $\pm$ 2.07	93.71 $\pm$ 0.32

^aAll assays were repeated three times (mean $\pm$ SD)^bIC₅₀ value of extracts (μ g/mL)^cIC₅₀ value of the isolated compound and positive controls (μ M)^dNI: No inhibition, inhibitory effects less than 35% at 100 μ g/mL^eND: Not determined

In this study, the *n*-hexane, dichloromethane, ethyl acetate, methanol extracts, and the isolated compound, namely garcinisidone A, were obtained from the fruits of *G. parvifolia*, which have been evaluated for their antidiabetic and antioxidant activities by inhibiting α -glucosidase and scavenging free radicals. These activities are summarized in Table-2. Among the four extracts tested, the dichloromethane extract exhibited the greatest inhibitory effect against α -glucosidase with inhibitory activity (IC₅₀) value of 3.15 μ g/mL, followed by *n*-hexane, methanol, and ethyl acetate extracts with IC₅₀ values of 7.69 μ g/mL, 7.84 μ g/mL, and 11.12 μ g/mL respectively. As seen in Table-2, Garcinisidone A, the isolated compound from dichloromethane extract, exhibited a very strong inhibitory effect on α -glucosidase with an IC₅₀ value of 6.61 μ M. Interestingly, garcinisidone A displayed α -glucosidase inhibitory activity that was 3-4 times more potent than acarbose (IC₅₀ 22.55 μ M) and quercetin (IC₅₀ 24.39 μ M) as positive standards. However, to the best of the authors' knowledge, there has been no study on α -glucosidase inhibition using extracts and isolated compound garcinisidone A from *G. parvifolia* fruits. Furthermore, to continue the authors' investigation on the potency of *G. parvifolia* fruits for preventing diabetic complications caused by free radicals, the authors examined the antioxidant activities of the extracts and isolated compounds as well. As shown in Table-2, unfortunately, the extracts and isolated compound did not demonstrate potent activities against DPPH free radicals with a percentage inhibition effect of less than 30% at 100 μ g/mL. Meanwhile, among the four examined extracts, dichloromethane extract exhibited good scavenging activities against ABTS⁺⁺ free radical with an IC₅₀ value of 63.01 μ g/mL. The order of free radical scavenging activities of the four fractions against ABTS⁺⁺ free radical from the weakest to the strongest was as follows: *n*-hexane; ethyl acetate; methanol > dichloromethane fraction extracts. This finding is in line with the finding of a previous study, which reported that flesh and peel of *G. parvifolia* fruits showed potency to scavenge free radicals, namely DPPH and ABTS.¹¹ Furthermore, the antioxidant potency of garcinisidone A against ABTS⁺⁺ free radical was examined, resulting in an IC₅₀ value of 18.92 μ M. Garcinisidone A displayed the strongest inhibition activities, which were 5 times more potent than those of ascorbic acid (IC₅₀ 93.71 μ M) and quercetin (IC₅₀ 99.68 μ M) as positive controls. Based on the results, it can be concluded that the *G. parvifolia* fruits, including their fractions and isolated compound, have great antidiabetic and antioxidant properties *via* inhibiting α -glucosidase and free radicals. The fruits may also have positive effects on human health as a food additive.

CONCLUSION

In summary, this study reported that dichloromethane fraction and isolated compounds (Garcinisidone A) from *G. parvifolia* fruits showed the highest inhibitory activities against α -glucosidase and free radicals (DPPH and ABTS⁺⁺). Garcinisidone A exhibited α -glucosidase inhibitory activities that were five times as potent as those of acarbose and quercetin. Meanwhile, on free radical scavenging activity, dichloromethane

fraction and isolated compound Garcinisidone A showed the highest scavenging activities on DPPH and ABTS⁺ free radicals. Based on the results of this study, it can be concluded that *G. parvifolia* fruits as a local food additive are beneficial for human health due to their antidiabetic and antioxidant potentials. However, further studies are necessary to identify other secondary metabolites from *G. parvifolia* fruits that may have other biological activities.

ACKNOWLEDGMENTS

The authors are grateful to the Universitas Airlangga for the 2020 Grant for Mandate Research Group (347/UN3.14/PT/2020).

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC- 8385/2023]