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RESEARCH PAPER

Quantification of hydroxycitric acid in selected genotypes of *Garcinia atroviridis* Griff ex T. Anders. collected from four populations in Peninsular Malaysia

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Abstract

One of the potential food ingredients with anti-obesity benefits is hydroxycitric acid (HCA), which is a derivative of citric acid. It was reported that HCA can be obtained from fruits of garcinia species. In Malaysia, *Garcinia atroviridis* commonly known asam gelugur is a very popular fruit used as a flavouring agent in cooking and for traditional health care. It was also reported that the *G. atroviridis* fruits contain hydroxycitric acid (HCA) and flavonoids which possess remarkable hypolipidemic effects, promoting weight reduction by reducing lipogenesis and enhancing glycogen formation. Due to the health benefits, researchers from Forest Research Institute Malaysia (FRIM) intended to screen and identify *G. atroviridis* genotypes from several populations in Peninsular Malaysia with a high content of HCA. In this study, the fruits from selected genotypes were collected from four populations namely i) Tapah, Perak (AGA); ii) Yan, Kedah (AGY); iii) Bukit Gantang, Perak (AGBG) and iv) Jeli, Kelantan (AGJ). The ripe fruits were sliced and dried in an oven at 50°C for three days. Dried fruit with a moisture content of below 10% was finely ground before being analyzed for High-Performance Liquid Chromatography (HPLC). The amounts of HCA in each genotype showed a variation of non-detectable to detectable quantities and had an average HCA content in the range of 18.02-28.36% w/w (AGA), 21.91-27.03% w/w (AGY), 5.35-46.66% w/w (AGBG) and 31.65-57.21% w/w (AGJ). A total of 10 out of 69 genotypes have been selected as desirable genotypes due to acceptable amounts of HCA higher than 50% w/w. These superior genotypes will be used for clonal propagation and future clone development.

1. Introduction

Garcinia is a member of the Clusiaceae or Guttifereae family and has about 200 species worldwide, with the majority of them in Asia and Africa. Around 36 species of garcinia are native to India, including *Garcinia atroviridis* (GA) Griff ex T. Anders. *G. atroviridis* is indigenous to Thailand, Malaysia, Myanmar, and India (Arunachal Pradesh and Assam), where it can be found in humid forests up to an elevation of 600 meters a.s.l. (Mackeen *et al.*, 2000; Taher *et al.*, 2016). This species is a dioecious evergreen tree, up to about 25 m tall with a conical crown shape, drooping branches, and an enlarged trunk at the base. This species has female and male reproductive organs in separate individuals. The flowers have the organs of the opposite sex in a primitive form, and occasionally hermaphrodite flowers are present. The female plants fructify by parthenocarpy when the male flowers are absent. The fruits formed are subglobose berries with 9-13 ribs 7-10 cm in diameter and about 7 cm long, of initially green colour, then turn yellow when ripe. The pulp of the fruit has an intense yellow colour, with a sour taste and each fruit produces an average of 3 to 5 flattened seeds. It is locally known as "Asam keping" (dried slices) when the fruits are sliced and dried for consumption (Alsarhan *et al.*, 2014).

In Malaysia, fruits are frequently used as a flavouring agent while young leaves are used as traditional vegetables. Almost all of the tree's components, including its fruit, leaves, roots, and stem bark, have therapeutic properties as antioxidant, antiobesity, anti-inflammatory, and cytotoxic properties (Pangsuban *et al.*, 2009; Taher *et al.*, 2016). According to Rittirut and Siripatana (2006), the presence of

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hydroxycitric acid (HCA) in *G. atroviridis* has been shown to have strong antioxidant activity. Hydroxycitric acid (HCA) is the principal acid of the fruit rinds of *G. cambogia*, *G. indica*, and *G. atroviridis* (Chuah *et al*., 2013). HCA content in the fruits of *Garcinia* also would vary from region to region and across the genotypes (Apoorva *et al*., 2020). Seasonal factors also influence the secondary metabolite of this plants amd leading to the variation in the biosynthesis of its chemical compounds. Different parts of *Garcinia* species also gave a different amount of HCA and study did by Jayaprakasha *et al.* (2003) showed fruit rinds parts have contributed to higher HCA amounts.

Besides that, this acid has been recognized as a viable supplement for weight loss and as an anti-obesity agent among all forms of organic acids. This bioactive compound can serve as a potent inhibitor of fatty acid and cholesterol synthesis (Chuah *et al*., 2013).

Looking at the current situation, the world is in a health transition. People are suffering from several diseases, and cardiovascular disease is one of the leading causes of death globally. Obesity has been shown to increase the risk of cardiovascular disease. According to the National Health and Morbidity Survey (Institute for Public Health, 2020), Malaysia's adult obesity rate is at 19.7% and the rate is projected to increase to 41% by 2035. In recent years, herbs have been used as an alternative treatment all over the world to prevent obesity. However, many herbal-based products that are being sold in the market are developed from unknown sources of low-quality raw materials. Thus, this study aims to support the herbal industry in obtaining good-quality raw materials for sustainable production of quality end products.

Thus, the main objective of this study was to quantify hydroxycitric acid (HCA) from selected genotypes of *G. atroviridis* collected from four populations in Peninsular Malaysia. Quantification of HCA will be one of the main criteria in the selection of superior clones for the development of future breeding strategies.

2. Materials and methods

2.1 Materials

Fresh fruits from the selected genotype of *G. atroviridis* were collected from four populations namely (i) Tapah, Perak; (ii) Yan, Kedah; (iii) Bukit Gantang, Perak; and (iv) Jeli, Kelantan. Individual selection of genotypes from each population was done based on their superior phenotypic characteristics., The selected trees were at the stage of mass flowering and fruiting season during sample collection (Table 1). The collected genotypes were named based on their population and number of trees i.e.; AGA (Tapah); AGY (Yan); AGBG (Bukit Gantang) and AGJ (Jeli). All the collected fruits were brought back to the laboratory for quantification of HCA through HPLC analysis.

2.2 Sample preparation and high-performance liquid chromatography analysis

Fresh and fully riped fruits *G. atroviridis* were washed with running tap water to remove dirt and impurities from the rind of the fruits. The fruits were thinly sliced and oven-dried at 50°C for 3 days. Dried samples were ground and sieved using a 500 µm laboratory test sieve to obtain the samples in a powder form. Dried plant powder (0.5 g each) of the samples was weighed accurately, then dissolved in 5 mL methanol and sonicated using an ultrasonicator for 15 mins. The solutions were filtered through a 0.45 µm PTFE cartridge. The filtrates were diluted with methanol to the final working concentration. HCA reference compound was prepared by dissolving it in methanol to produce solutions with concentrations ranging from 6.3 to 2000 µg/mL for the calibration plot. At a flow rate of 1 mL/min, a 20 µL sample volume was injected into the HPLC system (HPLC WATERS 2535, USA) for analysis. Retention time data and UV spectra for clear and distinct peaks were analyzed and recorded.

3. Results and discussion

Qualitative analysis for the determination of the presence of HCA in all test samples was conducted. The results of the analysis showed the presence of HCA in all tested samples. Therefore, quantitative analysis for the determination of HCA content in the test samples was carried out based on High-performance liquid chromatogram (HPLC) of standard hydroxy citirc (HCA) as shown in Figure 1. Results represented for three replicate samples with 3 injections for each replicate. The amounts of HCA showed a variation from non-detectable to detectable quantities and the average range of HCA content is presented in Table 2.

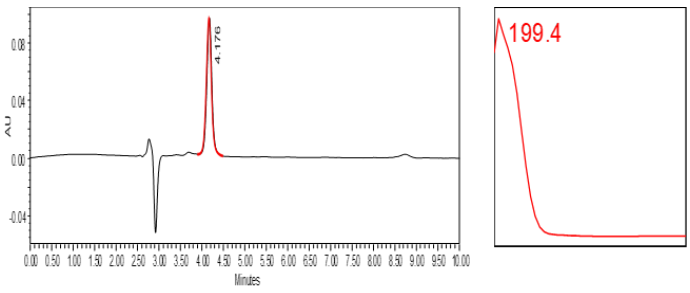

Figure 1. High-performance liquid chromatogram (HPLC) of standard hydroxy citirc (HCA).

The highest concentration of HCA was found in genotype AGJ15 from Jeli, Kelantan (57.21±0.85% w/

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Table 1. Morphological data of selected genotypes *Garcinia atroviridis* and their GPS locations.

Genotypes	Flowering and fruiting status		Height (m)	Diameter (cm)	GPS location	
	Flowering	Fruiting				
AGA3	No	Yes	30.0	32.0	041244.6° N	1011901.5° E
AGA4	No	Yes	10.0	20.0	040830.5° N	1010946.3°E
AGA7	No	Yes	20.0	12.0	042313.9° N	1011248.8° E
AGY5	Yes	Yes	10.4	48.0	05.81012° N	100.40366° E
AGY12	Yes	Yes	24.7	68.0	05.79960° N	100.38321° E
AGBG11	No	Yes	6.6	20.5	04.76274° N	100.77834° E
AGBG12	Yes	Yes	7.1	23.0	04.74604° N	1078080° E
AGBG13	No	Yes	5.2	24.1	04.76071° N	100.78091° E
AGBG14	Yes	Yes	7.7	35.8	04.76633° N	100.76656° E
AGBG17	No	Yes	5.6	22.6	04.75802° N	100.76631° E
AGBG22	Yes	Yes	7.2	36.0	04.76377° N	100.74789° E
AGBG23	Yes	Yes	6.2	37.2	04.75744° N	100.74569° E
AGBG24	Yes	Yes	6.7	48.0	04.75642° N	100.74482° E
AGBG25	Yes	Yes	8.3	22.5	04.75672° N	100.74494° E
AGBG28	No	Yes	7.5	16.1	04.75827° N	100.74856° E
AGBG29	No	Yes	6.2	38.0	04.75853° N	100.74963° E
AGBG30	Yes	Yes	5.7	25.0	04.75836° N	100.74892° E
AGBG31	Yes	Yes	8.3	56.8	04.80286° N	100.75007° E
AGBG32	Yes	Yes	8.9	45.5	04.80116° N	100.74796° E
AGBG33	Yes	Yes	6.3	106.5	04.80316° N	100.75121° E
AGJ10	Yes	Yes	16.8	34.4	05.68669° N	101.71729° E
AGJ11	Yes	Yes	16.0	28.1	05.68080° N	101.71030° E
AGJ12	Yes	Yes	16.0	52.0	05.68020° N	101.71048° E
AGJ13	No	Yes	16.0	41.4	05.74820° N	10174279° E
AGJ14	Yes	Yes	11.4	26.8	05.75046° N	101.74561° E
AGJ15	No	Yes	11.4	28.0	05.75031° N	101.74564° E
AGJ16	No	Yes	14.9	29.5	05.75043° N	101.74761° E
AGJ17	Yes	Yes	12.4	33.8	05.71705° N	101.74638° E
AGJ20	No	Yes	24.6	40.9	05.70465° N	101.77563° E
AGJ24	No	Yes	13.6	36.7	05.66259° N	101.76433° E
AGJ27	No	Yes	13.2	41.0	05.72775° N	10186122° E
AGJ28	No	Yes	15.2	28.0	05.77213° N	101.89172° E
AGJ30	No	Yes	13.2	38.4	05.72909° N	10190606° E
AGJ31	No	Yes	18.4	38.0	05.73450° N	101.93679° E

Table 2. Range of hydroxycitric acid (%) in *Garcinia atroviridis* from four populations

Population	Range of hydroxycitric acid (%) w/w
Tapah, Perak (AGA)	18.02- 28.36
Yan, Kedah (AGY)	21.91- 27.03
Bukit Gantang, Perak (AGBG)	5.35- 46.66
Jeli, Kelantan (AGJ)	31.65- 57.21

w), whereas, genotype AGBG9 from Bukit Gantang, Perak recorded the lowest HCA content (5.35±0.07% w/w) compared to the other genotypes. Genotype AGA3 (28.36±0.42% w/w) and AGY12 (27.03±1.86% w/w) recorded the highest HCA content within the population of Tapah, Perak, and Yan, Kedah respectively. For the population Bukit Gantang, Perak, genotype AGBG11 gave the highest HCA content (46.66±1.79% w/w) compared to the other 33 genotypes within the population. This population has the highest number of samples that have been screened for chemical analysis. Generally, all the selected genotypes have HCA content of more than 22% w/w (Table 3). All the variation of HCA amounts between genotypes and populations indicated that the trait may have influenced by different environment factors in that particular populations as mentioned by Apoorva *et al.* (2020). The result also indicated that evaluated genotypes of *G. atroviridis* have higher HCA content than the one that has been quantified by Jayaprakasha and Sakariah (1998). In that study, it has been discovered that range of HCA in *G. cambogia* (Malabar tamarind) was about 16–18%. A study by Kumar *et al.* (2012) also found that methanolic extract from fruit rinds of *G. indica* is higher 90.43±2.45

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Table 3. The average concentration of hydroxy citric acid (% w/w) in *Garcinia atroviridis* collected from four populations

Genotype	Average of hydroxycitric acid±RSD (% w/w)	Genotype	Average of hydroxycitric acid±RSD (% w/w)	Genotype	Average of hydroxycitric acid±RSD (% w/w)	Genotype	Average of hydroxycitric acid±RSD (% w/w)
AGA3	28.36±0.42	AGY12	27.03±1.86	AGBG11	46.66±1.79	AGJ15	57.21±0.85
AGA7	25.88±0.29	AGY5	24.76±1.35	AGBG29	46.28±0.09	AGJ14	56.75±0.38
AGA4	24.31±0.56	AGY17	23.85±0.22	AGBG13	46.21±6.93	AGJ31	56.32±0.09
AGA9	23.20±0.11	AGY8	22.03±0.05	AGBG30	45.41±1.65	AGJ16	55.05±0.72
AGA18	21.91±0.21			AGBG31	42.77±0.27	AGJ12	54.71±0.45
AGA15	21.49±0.19			AGBG24	41.79±0.14	AGJ11	53.46±0.14
AGA14	21.23±0.17			AGBG23	40.54±0.62	AGJ28	53.19±0.15
AGA17	20.18±0.11			AGBG22	39.95±1.22	AGJ24	53.12±0.44
AGA16	19.74±0.21			AGBG28	39.55±0.96	AGJ20	52.71±0.19
AGA1	18.02±0.42			AGBG32	38.95±0.63	AGJ30	52.61±0.42
				AGBG12	37.89±13.04	AGJ27	52.43±0.42
				AGBG25	37.15±0.60	AGJ17	52.29±0.38
				AGBG33	35.14±0.30	AGJ10	51.23±0.34
				AGBG14	31.33±0.74	AGJ2	48.05±0.48
				AGBG17	30.42±0.69	AGJ4	47.85±0.07
				AGBG34	28.60±2.51	AGJ18	47.80±0.48
				AGBG10	25.86±0.45	AGJ21	47.15±0.26
				AGBG20	24.76±0.55	AGJ34	46. 69 ±0.16
				AGBG21	22.48±0.83	AGJ5	45.22±0.20
				AGBG19	19.62±0.79	AGJ9	43.78±0.02
				AGBG15	18.56±0.74	AGJ3	42.50±0.34
				AGBG16	13.12±0.84	AGJ25	41.59±0.26
				AGBG26	11.28±0.27	AGJ6	39.85±0.51
				AGBG18	10.88±0.12	AGJ19	31.65±0.03
				AGBG27	9.07±0.15		
				AGBG7	8.58±0.03		
				AGBG4	7.97±4.43		
				AGBG5	7.55±2.08		
				AGBG1	6.45±2.42		
				AGBG2	5.65±0.02		
				AGBG9	5.35±0.07		

than other parts such as seed (61.56±3.92), stem barks (55.68±2.41) and leaves (75.44±4.46).

From the total of 69 genotypes evaluated for HCA content, only ten genotypes of *G. atroviridis* from the Jeli, Kelantan population were selected for future clone development. The amounts of HCA in these ten genotypes were higher compared to the other genotypes (>50% w/w) as shown in Table 4. The HPLC chromatogram for ten desirable genotypes of *G. atroviridis* within 4.15 mins retention times and UV spectra detection at 199.4 were shown in Figure 2. A study by Antony *et al.* (2004) revealed the quantification by the HPLC method is more accurate and specific

Table 4. Ten selected genotypes of *Garcinia atroviridis* with a high concentration of hydroxy citric acid (HCA) content

Genotype	Average of hydroxycitric acid±RSD (% w/w)
AGJ15	57.21±0.85
AGJ14	56.75±0.38
AGJ31	56.32±0.09
AGJ16	55.05±0.72
AGJ12	54.71±0.45
AGJ11	53.46±0.14
AGJ28	53.19±0.15
AGJ24	53.12±0.44
AGJ20	52.71±0.19
AGJ30	52.61±0.42

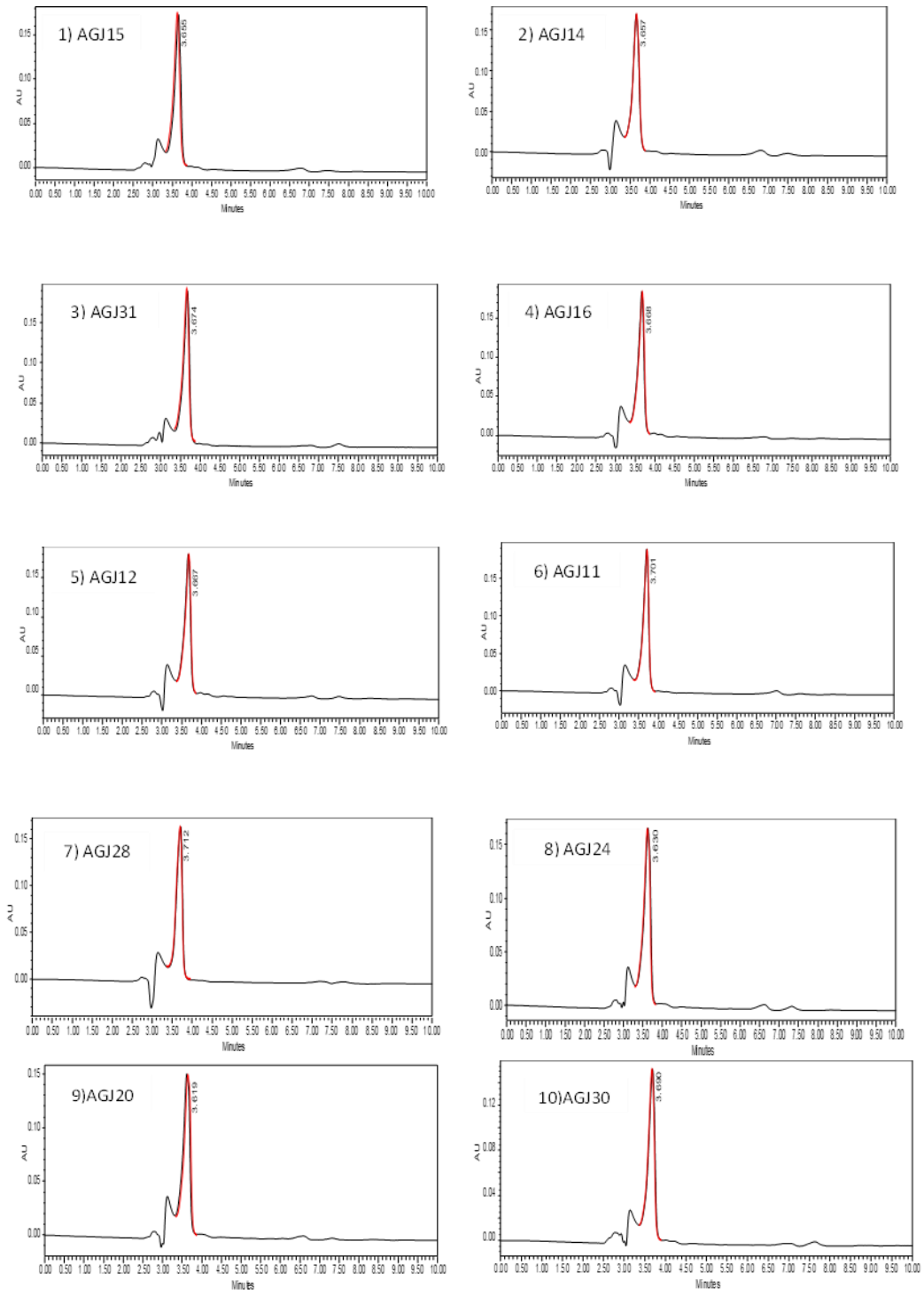

Figure 2. High-performance liquid chromatogram (HPLC) at 210 nm of ten selected genotypes of *Garcinia atroviridis.*

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compared to the spectrophotometric method and the value of HCA obtained was also higher using HPLC. In the future, these selected genotypes will be used for clonal propagation.

4. Conclusion

Quantification of hydroxycitric acid (HCA) among 69 genotypes using the HPLC method was possible and effective in identifying and screening the superior genotypes of *G. atroviridis*. Genotype screening is the first phase in the breeding program for the improvement of the yield and quality of *G. atroviridis.* This will be followed by other breeding strategies for further development of superior clones for large-scale production of quality raw materials.

Conflict of interest

There is no conflict of interest.

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