Current Trends in Biotechnology and Pharmacy
Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.22

Antioxidant Potential of Optimized Aqueous Extract from *Garcinia Parvifolia (Miq.)* Dried Fruit Pericarp

Faizatul Isyraqiah[1,2]*, Nur Natasha Aza[3], Haryatie Yahya[3], Nurul Ain Syazwani Rosli[3], Johnathan Malagobadan[4]*, and Farnidah Jasnie[3]

[1]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, 47500 Subang Jaya, Selangor, Malaysia

[2]Faculty of Medicine, Manipal University College Malaysia, 75150 Bukit Baru, Melaka, Malaysia

[3]Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Sabah Branch, Kota Kinabalu Campus, 88997 Kota Kinabalu, Sabah, Malaysia

[4]Faculty of Medicine, University of Cyberjaya, 63000 Cyberjaya, Selangor, Malaysia

*Corresponding author: johnathan@cyberjaya.edu.my

Abstract

Introduction: *Garcinia parvifolia (Miq.)* or Takob-akob possesses various medicinal properties, including antioxidant, antibacterial, antiplasmodial, and antiviral effects. Previous studies have demonstrated the potent antioxidant and antibacterial activities of the plant's dried fruit pericarp extracted using organic and inorganic solvents. However, this extraction method is not suitable for direct consumption. To address this, a water-based extraction of *G. parvifolia (Miq.)* dried fruit pericarp was conducted to create a safe and halal product. Materials and methods: The extraction process involved varying parameters such as temperature (22.4°C, 60°C, and 85°C), solid-to-solvent ratio (1:4, 1:10, and 1:40 g/mL), and time (30 min and 120 min). The ideal temperature was used to determine the optimum solid-to-solvent ratio, and the optimum temperature and ratio were then used to determine the optimum time. Antioxidant activities were assessed using the DPPH radical scavenging assay and expressed as inhibition percentage. Ascorbic acid was used as the positive control. Results: Aqueous-based extraction of *G. parvifolia (Miq.)* dried fruit pericarp with a solid-to-solvent ratio of 1:40 g/mL at room temperature for 120 minutes showed the highest DPPH radical scavenging activity, with an inhibition percentage of 87.06%, equivalent to 142 µg/mL of ascorbic acid. Conclusion: This research has shown that the water-based extract of *G. parvifolia (Miq.)* dried fruit pericarp holds significant antioxidant capacity and has commercialization potential beyond just consumable products. Further exploration is needed to uncover additional health benefits of this fruit.

Keywords: Antioxidant, aqueous extract, Garcinia parvifolia, halal, takob akob

1. Introduction

Garcinia parvifolia (Miq.) is an indigenous tropical fruit found predominantly in Borneo, and widely known by the locals as “Takob Akob”, “Asam Kandis”, “Asam Aur-aur”, or “Asam Kedondong” (1). It belongs to the *Guttiferae* family which is the same family as mangosteen *(Garcinia mangostana)* (2). The fruit of *G. parvifolia (Miq.)* is small, rounded, and orange-coloured with small seeds wrapped in a thin-layer pericarp (2). The dried fruits have a sour taste and are used as additives in cooking (2). The natives often exploit parts of the plant as herbal medicines (1).

Strong antibacterial activities were identified in the twigs (3), stem bark (4), and dried fruit pericarp (5) of *G. parvifolia(Miq.)*. Its roots and stem bark showed anti-plasmodial activities (1), and recently it was discovered that its leaves contained anti-viral properties (6). The twigs (3), roots (1), and fruit peel and flesh (7) of *G. parvifolia (Miq.)* showed significant antioxidant capabilities. Our previous study showed that the crude extract of *G. parvifolia*

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(Miq.) dried fruit pericarp extracted successively using chloroform, hexane, ethyl acetate and methanol showed strong antioxidant properties comparable to ascorbic acid (8), possibly due to the high total flavonoid content (9).

With the purpose of establishing a safe consumable product, this study was then conducted using water as the extraction medium to isolate the antioxidants. Due to its high obtainability and sustainability, various health benefits of the water-based extract of *G. parvifolia (Miq.)* dried fruit pericarp can be explored. Therefore, this study was conducted to determine the antioxidant properties of *G. parvifolia (Miq.)* dried fruit pericarp in an optimized water-based extract.

2. Methodology

Sample Collection and Optimization of Extraction Parameters

Dried fruit pericarp from *G. parvifolia* was sourced from the Dongongan local market in the Penampang District of Sabah, Malaysia. The sample was coarsely grounded using a conventional food blender. The sample was then soaked in reverse-osmosis water and was subjected to three extraction parameters (temperature, ratio, time) based on (10, 11) with some modifications. The optimum extraction method was determined by alternating and assessing the extraction parameters in the following chronological order: extraction temperatures, solid-to-solvent extraction ratios, and extraction time (Figure 1). The ideal extraction temperature was used to determine the optimum solid-to-solvent extraction ratio. The optimum temperature and ratio were then subsequently used to determine the optimum extraction time.

i) Extraction Temperature

Five grams of grounded sample was mixed with 100 mL of distilled water in a conical flask (1:20 g/mL solid-to-solvent ratio). The extraction was performed using a water bath at 60°C (EC60) for 120 minutes. The extraction was repeated by replacing the extraction temperature to 85°C (EC85) and 22.4°C (ECRT).

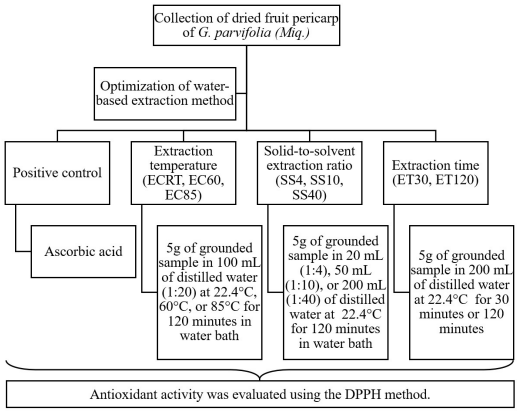

Fig. 1: Optimization of water-based extraction of dried fruit pericarp of *G. parvifolia (Miq.).*

ii) Solid-to-Solvent Extraction Ratio

Five grams of grounded sample was added in each conical flask containing 20 mL (1:4, SS4), 50 mL (1:10, SS10), and 200 mL (1:40, SS40) of distilled water. The extraction was performed using a water bath set at 22.4°C for 120 minutes.

iii) Extraction Time

Five grams of grounded sample was mixed with 200 mL of distilled water in a conical flask (1:40 g/mL solid-to-solvent ratio). The extraction was performed using a water bath set at 22.4°C for 30 minutes (ET30). The extraction was repeated by replacing the extraction time to 120 minutes (ET120).

The crude extract of each parameter was filtered using Whatman No. 1 filter paper. Sample was evaporated by a double boiling method using a hot plate until the weight of the sample was constant. The crude extract was transferred into a dark bottle and stored in the refrigerator. The effect of different extraction parameters on its antioxidant activities of *G. parvifolia (Miq.)* extract was tested using DPPH assay.

Preparation of DPPH methanol solution

2, 2-diphenyl-1-picrylhydrazyl (DPPH) powder was obtained (Sigma Aldrich, USA), and fresh DPPH solution was prepared on analysis day. A concentration of 0.2 mM DPPH in methanol solution was prepared by

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dissolving 0.002 g of DPPH powder with 30 mL absolute methanol in a conical flask wrapped with aluminium foil. The flask is sealed with a rubber stopper and kept in a 4°C refrigerator for 2 hours before use (12).

Scavenging activity of *G. parvifolia (Miq.)* extract

DPPH assay was performed according to the procedure by (11). Eight groups with varying parameters of extraction (ECRT, EC60, EC85, SS4, SS10, SS40, ET30, ET120) were used in this study, and ascorbic acid was used as positive control (7). A volume of 0.6 mL of control or crude extract group was mixed with 2.34 mL of 0.2 mM DPPH solution. The solution was vortexed for 20 seconds before incubating in a dark environment in an ice bath for 30 minutes. The change of DPPH solution from purple to yellow indicated a reduced reaction exhibited by the antioxidant molecules (13). The sample was then filtered with a syringe filter and transferred into a UV-cuvette. The absorbance of the sample was measured using a UV-vis spectrophotometer (517 nm) in triplicates. Distilled water and methanol were used as blank for the sample and control respectively (14). The negative control is a mixture of DPPH and water. The inhibition percentage was calculated based on the following formula:

$$\text{Scavenging effect / inhibition percentage (\%)} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \times 100\%$$

Equation 1

The DPPH scavenging activity of each *G. parvifolia (Miq.)* crude extract group was expressed in inhibition percentage. The optimum extraction parameters were determined based on the groups that showed the highest inhibition percentage.

Standard curve of Ascorbic Acid

A standard curve of ascorbic acid in various concentrations was determined by performing serial dilutions at 60, 80, 100, 150 and 200 µg/mL (15). The graph of ascorbic acid standard curve was plotted with inhibition percentage against concentration of ascorbic acid (µg/mL). The DPPH scavenging activity of the optimized sample was compared with ascorbic acid using the standard curve.

Statistical Analysis

The absorbance reading of each sample was performed in triplicates. Data was expressed as mean ± standard deviation. The mean differences were analysed using one-way ANOVA with Scheffé post-hoc test, while the student's T-test was done to compare the mean differences between two groups. A 95% confidence interval was taken as statistically significant at $p<0.05$. Linear regression was performed on the standard curve. Data was analysed using Microsoft Excel.

3. Results and Discussion

Optimization of Water-based Extraction Parameters

i) Extraction Temperature

Table 1 shows the mean absorbance reading for samples extracted with different extraction temperatures and the inhibition percentage. The absorbance of samples was significantly different between the groups

Table 1: DPPH radical scavenging activity of *G. parvifolia (Miq.)* dried fruit pericarp at different extraction temperatures (°C)

Sample	Absorbance Reading (Au)				Inhibition Percentage (%)
	R1	R2	R3	Mean ± SD	
ECRT	0.6601	0.6596	0.6595	0.6597 ± 0.0003***	74.65
EC60	0.9453	0.9458	0.9461	0.9457 ± 0.0004***	63.66
EC85	0.9327	0.9326	0.9336	0.9330 ± 0.0006***	64.15
Control	2.6031	2.6162	2.5884	2.6026 ± 0.0139	-
***p value <0.001 between the groups					

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($p<0.001$). The DPPH scavenging effect of *G. parvifolia (Miq.)* extract at room temperature is the highest (74.65%) compared to EC60 and EC85 (Table 1).

This study has found that the optimum aqueous-based extraction method of dried fruit pericarp of *G. parvifolia (Miq.)* is a solid-to-solvent ratio of 1:40 extracted at room temperature for 120 minutes, which resulted in a DPPH scavenging activity of 87.06%. In comparison to ascorbic acid as the positive control, the DPPH scavenging activity of *G. parvifolia (Miq.)* dried fruit pericarp is equivalent to the antioxidant activity of 142 µg/mL ascorbic acid. The lowest extraction temperature (ECRT) of *G. parvifolia (Miq.)* fruit pericarp exhibited the highest antioxidant activity. This method was similarly applied in previous studies on *G. parvifolia (Miq.)* fruit peel and flesh (7) and dried leaves (6). The fruit peel and flesh of *G. parvifolia (Miq.)* are rich in total phenolic content (7); however, most phenolic compounds are heat-sensitive and prone to degradation (16). Raising the extraction temperature to 80°C notably decreased the yield of compounds from the leaves of *Clinacanthus nutans* Lindau *(C. nutans)*, likely due to oxidation and degradation of the desired chemicals (11). This was similarly observed in our study, whereby extractions at both 65°C and 85°C significantly lowered the scavenging activity of antioxidants from the fruit pericarp (Table 1). Given the significant positive correlation between total phenolic content and antioxidant activity (17), there might be degradation of bio-active phenolic compounds in the *G. parvifolia (Miq.)* extracted at high temperatures resulting in significant reduction of antioxidant effects (Figure 1).

ii) Solid-to-Solvent Extraction Ratio

Table 2 shows the mean absorbance reading for samples extracted with different solid-to-solvent extraction ratio and its inhibition percentage. The absorbance of samples was significantly different between the groups ($p<0.001$). A solid-to-solvent ratio of 1:40 exhibited the highest scavenging activity (87.06%) compared to SS4 and SS10 (Table 2).

The optimum aqueous-based extraction method of dried fruit pericarp of *G. parvifolia (Miq.)* at solid-to-solvent ratio of 1:40 extracted at room temperature for 120 minutes, which resulted in a DPPH scavenging activity of 87.06%. In comparison to ascorbic acid as the positive control, the DPPH scavenging activity of *G. parvifolia (Miq.)* dried fruit pericarp is equivalent to the antioxidant activity of 142 µg/mL ascorbic acid. The lowest extraction temperature (ECRT) of *G. parvifolia (Miq.)* fruit pericarp exhibited the highest antioxidant activity. This method was similarly applied in previous studies on *G. parvifolia (Miq.)* fruit peel and flesh (7) and dried leaves (6). The fruit peel and flesh of *G. parvifolia (Miq.)* are rich in total phenolic content (7); however, most phenolic compounds are heat-sensitive and prone to degradation (16). Raising the extraction temperature to 80 °C notably decreased the yield of compounds from the leaves of *Clinacanthus nutans* Lindau (*C. nutans*), likely due to oxidation and degradation of the desired chemicals (11). This was similarly observed in our study, whereby extractions at both 65°C and 85°C significantly lowered the scavenging activity of antioxidants from the fruit pericarp (Table 1). Given the significant positive

Table 2: DPPH radical scavenging activity of *G. parvifolia (Miq.)* dried fruit pericarp at different solid-to-solvent extraction ratios (g/mL)

Sample	Absorbance Reading (Au)				Inhibition Percentage (%)
	R1	R2	R3	Mean ± SD	
SS4	1.3220	1.3244	1.3185	1.3216 ± 0.0030***	83.32
SS10	1.1673	1.1681	1.1668	1.1674 ± 0.0007***	85.26
SS40	1.0254	1.0252	1.0251	1.0252 ± 0.0002***	87.06
Control	3.7640	10.000	10.000	7.9213 ± 3.6004	-
***p value <0.001 between the groups					

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correlation between total phenolic content and antioxidant activity (17), there might be degradation of bio-active phenolic compounds in the *G. parvifolia (Miq.)* extracted at high temperatures resulting in significant reduction of antioxidant effects (Figure 1).

The antioxidant activity of *G. parvifolia (Miq.)* fruit pericarp showed the highest DPPH scavenging activity at a solid-to-solvent ratio of 1:40 g/mL (Table 2). Aqueous extract of mulberry leaf at varying solid-to-solvent extraction ratios of 1:10 until 1:100 g/mL found that 1:40 g/mL was the optimum ratio, and the extraction yield reached plateau at 1:90 g/mL (18). Compared to solid-to-solvent ratio of 1:5 (w:v), extraction of phenolic compounds in dog-rose, sea buckthorn and hawthorn fruits was higher in 1:10 (w:v) ratio (19). Our study showed the same increasing pattern as the extraction ratio of solid-to-solvent increases. However, the plateau trend was not observed in this study but may likely be seen if the ratio of solid-to-solvent is further increased. A higher solid-to-solvent ratio widens the concentration gradient and increases the compound diffusion rate (19). It is possible that more antioxidant compounds were extracted into the solvent, and that the extraction yield may reach a plateau once the solid-to-solvent extraction ratio reaches equilibrium. Interestingly, a similar effect is also reported in different solvent ratios. Decreasing the water-ethanol ratio from 90:10 to 70:30 caused a small decrease in scavenging activity of *C. nutans* leaves. This could be attributed to the polarity of the extraction medium, where a solvent system rich in water enhanced the antioxidant activity (11).

iii) Extraction Time

Table 3 shows the mean absorbance reading for samples extracted at different extraction times and its inhibition percentage. The absorbance of samples was significantly different between the groups ($p<0.001$). An extraction time of 120 minutes (ET120) showed higher DPPH scavenging activity compared to ET30 (Table 3).

Exposure to a solvent for an extended period allows the desired chemicals to diffuse into the solvent (11). Our study showed that extracting the fruit pericarp of *G. parvifolia (Miq.)* for 120 minutes increases the antioxidant effects compared to 30 minutes (Table 3). The same extraction time was used in extracting *G. parvifolia (Miq.)* fruit peel and flesh (7). Extraction of *Garcinia quaesita* leaves for 6 hours at 60°C yielded higher polyphenolics and antioxidant contents compared to 1 hour (20). However, a prolonged extraction time may cause decomposition and oxidation of phenolics, greatly reducing the extraction yield (21). It was reported that extraction of mangosteen peel (*Garcinia mangostana* L.) with acetone for 36 and 48 hours showed significantly reduced antioxidant level, and inferred that 24 hours was the optimum extraction time (22). Other studies also reportedly utilized 24-hour extraction time to maximize the yield (4, 6). Our study did not extend the extraction time more than 2 hours, as a shorter extraction time is more favourable in terms of manufacturing to increase production.

Figure 2 shows the inhibition percentages of all groups. The optimum water-based extraction method of dried fruit pericarp of *G. parvifolia (Miq.)* with maximum DPPH scavenging activity is a solid-to-solvent

Table 3: DPPH radical scavenging activity of *G. parvifolia (Miq.)* dried fruit pericarp at different extraction time (minutes)

Sample	Absorbance Reading (Au)				Inhibition Percentage (%)
	R1	R2	R3	Mean ± SD	
ET30	1.0445	1.0439	1.0439	1.0441 ± 0.0004***	86.82
ET120	1.0266	1.0274	1.0284	1.0275 ± 0.0009***	87.03
Control	3.7640	10.000	10.000	7.9213 ± 3.6004	-
***p value <0.001 between the groups					

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Table 4: DPPH radical scavenging activity of different concentrations of ascorbic acid

Concentration of Ascorbic Acid (µg/mL)	Absorbance Reading (Au)				Inhibition Percentage (%)
	R1	R2	R3	Mean ± SD	
200	0.6709	0.6893	0.7047	0.6883 ± 0.0169	91.31
150	0.8117	0.8216	0.8299	0.8211 ± 0.0091	89.63
100	2.1445	2.1669	2.1761	2.1625 ± 0.0163	72.70
80	3.2999	3.8226	3.4765	3.5330 ± 0.2659	55.40
60	10.0000	3.6413	3.9852	5.8755 ± 3.5761	25.83
Control	3.7640	10.000	10.000	7.9213 ± 3.6004	-

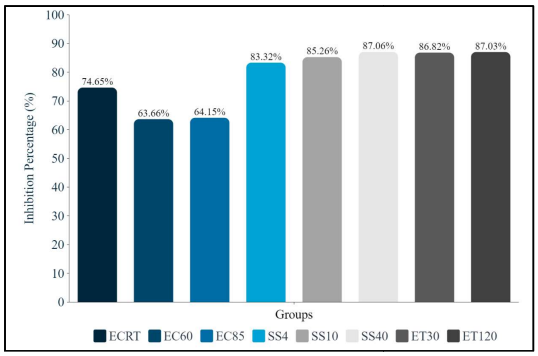

Fig. 2: DPPH radical scavenging activity of *G. parvifolia (Miq.)* dried fruit pericarp of all groups. Water-based extraction of *G. parvifolia (Miq.)* dried fruit pericarp at room temperature (ECRT) with solid-to-solvent ratio of 1:40 (SS40) for 120 minutes (ET120) exhibited the highest inhibition percentage.

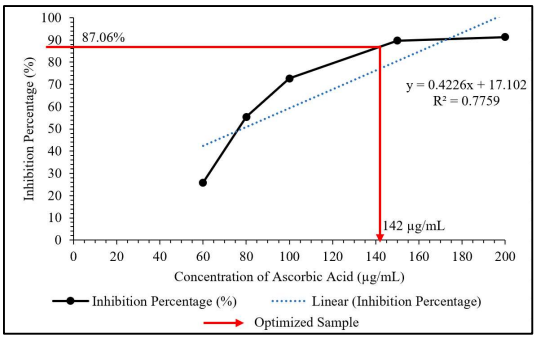

Fig. 3: Standard curve of ascorbic acid. The DPPH scavenging activity of *G. parvifolia (Miq.)* was equivalent to 142 µg/mL ascorbic acid.

ratio of 1:40 at room temperature for 120 minutes (Figure 2). Since the optimum extraction method is similar to the SS40 sample, the maximum DPPH scavenging activity is 87.06%.

Comparison of *G. parvifolia (Miq.)* Scavenging Activity with Ascorbic Acid

Table 4 shows the absorbance reading for different concentrations of ascorbic acid (µg/mL). The inhibition percentage increases as the concentration of ascorbic acid increases. Based on the standard curve of ascorbic acid, the optimized extraction sample of *G. parvifolia (Miq.)* dried fruit pericarp with DPPH scavenging activity of 87.06% is equivalent to 142 µg/mL of ascorbic acid (Figure 3).

4. Conclusion

G. parvifolia (Miq.) fruit pericarp has been traditionally used as "flavour enhancers" in cooking. Its antibacterial effects (5) may be applicable to treat small wounds. It was also previously incorporated with other nutraceutical compounds to create halal natural product-based cosmetics (23). Its high antioxidant effects reported in this preliminary study may be consumed as a drink or ice-cream. These findings suggest that *G. parvifolia (Miq.)* has a promising commercialization in the future, and it is likely that there are more health benefits in this plant currently undiscovered.

The maximum DPPH scavenging activity of aqueous-based extraction method of dried fruit pericarp of *G. parvifolia (Miq.)* is a solid-to-solvent ratio of 1:40 extracted at

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room temperature for 120 minutes, which showed 87.06% of scavenging effects. The DPPH radical scavenging activity of *G. parvifolia (Miq.)* was equivalent to 142 µg/mL ascorbic acid. Water continues to be the most affordable and safest solvent for extracting bioactive compounds. In line with its extractive capacity, the optimized method is cost-effective in terms of reducing time and consumables as well as being respectful to the environment.

Acknowledgement

This research was funded by Manipal University College Malaysia grant number [MUCM/001/2021]. The authors would like to thank Universiti Teknologi MARA, Kota Kinabalu campus for providing the facilities and technical support to conduct this study.

Conflict of Interest

The authors declare no conflict of interest.

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