Extraction conditions on the DPPH scavenging ability of the extract of miracle fruit leaves

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Abstract: In the present work, the experimental parameters, including temperature, extraction time, and the ratio of solution to leaves on the DPPH scavenging ability of miracle fruit leaves extract were investigated. Results showed that the optimal conditions for DPPH scavenging were as follows: extracting 3 h at 60 °C with 70% methanol solution, and the solution to leaves ratio was 100:1. The research here was beneficial for improving the antioxidant activities of miracle fruit leaves. It could also promote the overall value of miracle fruit plant in natural antioxidants.

1 Introduction
Synsepalum dulcificum Daniell (Sapotaceae), also known as miracle fruit or mysterious fruit, is an evergreen tropical plant natively grown in West Africa. This plant is quite famous for the characteristic of remarkably altering the sour taste into sweet taste [8].

There has been a growing interest in the investigation of miracle fruit in recent years in its antioxidant activities and application. Studies suggested that miracle fruit contain high amounts of bioactive compounds, such as phenolic acids and flavonoids. Moreover, many functional components of it have been isolated and identified [4, 5, 7, 14].

DPPH is one of the most popular methods employed for the evaluation of antioxidant ability, especially in plant extracts [10]. Leaves are an important part of plants and they may also be rich in phytochemicals antioxidants. However, the DPPH scavenging ability of the leaves of miracle fruit has rarely been reported, especially the factors that show obvious effect on the ability.

In this paper, the experimental parameters, including temperature, time and extraction time and the ratio of solution to leaves on the DPPH scavenging ability of miracle fruit leaves were investigated. The optimal conditions for scavenging DPPH were obtained and this was quite useful for the usage of miracle fruit leaves in antioxidants.

2 Materials and Methods

2.1 Materials and reagents
The leaves used in this paper were collected just from the trees of miracle fruit planted in South Subtropical Crop Research Institute. Mature leaves were first cleared and dried at 50 °C, and then ground using a stainless-steel grinder. They were stored in vacuum-packaged polyethylene pouches at -20 °C until required for analysis.
The 2,2’-diphenyl-2-picrylhydrazyl (DPPH) radical was received from Sigma-Aldrich. All the other regents were of analytical grade and used without treatment.

2.2 Experiment design
Methanol solutions were used for the extraction of miracle fruit leaves. All the experiments were conducted by triplicate.

2.2.1 Extraction temperature
Dried powder sample (1.00 g) of miracle fruit leaves was weighed and refluxed with 30 ml of 70% methanol at different temperature (40, 50, 60, 70 and 80 °C) for 3 h under magnetic stirring. The filtrate was collected and used for DPPH scavenging test.

2.2.2 Extraction time
For extraction time assay, 1.00 g dried powder was refluxed with 30 ml of 70% methanol at 70 °C for different time(0.5, 1, 2, 3 and 4 h) under magnetic stirring.

2.2.3 Methanol concentration
To determine the effect of concentration, methanol solutions (50, 60, 70, 80, and 90%) were mixed with 1.00 g dried leaves powder and refluxed at 70 °C for 3 h.

2.2.4 Ratio of solution to leaves
The solution concentration, extraction time and temperature were set at 70%, 3 h and 70 °C when determining the effect of ratio of methanol solution to the mass of leaves. The ratios of 50:1, 75:1, 100:1, 150:1 and 200:1 were used in this assay.

2.3 DPPH radical scavenging activity test
The free radical scavenging activity of the extract was performed by measuring the decrease in absorbance of DPPH solution at 517 nm in the presence of the extracts by the method proposed by Liyana-Pathirana et al [9] with minor changes. The solution of 0.5 mM was prepared by dissolving DPPH in methanol. For the evaluation of free radical scavenging activity, 3 ml of DPPH was added into 0.5 ml of the extracts with different concentrations. The mixture was then allowed to stand at room temperature for 30 min in dark before the absorbance at 517 nm was read. The control was prepared as above without extract. The antioxidant activity could be expressed as the following equation:

\[
\text{Scavenging activity} = \frac{A_0 - A_s}{A_0} \times 100\%
\]

where \(A_0\) and \(A_s\) were the absorbance at 517 nm of the control and sample solution, respectively.

3 Result and Discussion

3.1 Effect of extraction temperature on DPPH scavenging abilities
The effect of extraction temperature on DPPH scavenging abilities of the extract of miracle fruit leaves was given in Figure 1. It can be seen that the scavenging ability first increased and then decreased with the increase of temperature from 40 to 80 °C, and reached a maximum at 60 °C. This may because the mass transfer rates can be accelerated at high temperature during extraction, thus making more antioxidants into extract [6]. However, higher temperature may cause the decomposition of antioxidants. Similar results have been reported on green tea and papaya leaves [12, 13].

3.2 Effect of extraction time on DPPH scavenging abilities

The time of extraction also showed obvious effect on the DPPH scavenging ability of miracle fruit leaves (Figure 2). The percentage of DPPH scavenging of miracle fruit leaves extracts increased from 0.5 to 3 h. However, the percentage decreased when the extraction time was more than 3 h. Therefore, 3 h was chosen as the optimum of extraction time. Alu’datt et al found that the antioxidant activity of the extracts from olive seeds was greatly improved with a longer extraction time [2]. This may be ascribed to that longer extraction time would result in more antioxidants into extraction. However, the antioxidants may be decomposed then the time was further lengthened. Similar results had been reported by Liyana-Pathirana and Shahidi [9].
3.3 Effect of methanol concentration on DPPH scavenging abilities
The DPPH scavenging abilities of miracle fruit leaves extracts were increased when the concentration of methanol was between 50 to 70 (Figure 3). However, the abilities began to decrease when the concentration was over 70%. At higher concentration, the dissolution of impurity like pigments increased, which would in turn decrease the connection of antioxidants with methanol and water [11]. Besides, higher concentration would lead to the leakage of antioxidants, thus decreasing the DPPH scavenging ability. So the optimum of methanol concentration was 70%.

![Figure 3. The effect of methanol concentration on the DPPH scavenging abilities of the extract of miracle fruit leaves.](image)

3.4 Effect of the ratio of solution to leaves on DPPH scavenging abilities
The effect of solution to leaves ratio on the scavenging activity was shown in Figure 4. It can be seen that the extract of miracle fruit leaves possessed highest DPPH scavenging capacity when the ratio of 100:1 was used.

It is generally considered that extraction is based on mass transfer principles. The driving force during mass transfer is a concentration gradient between the solid and the liquid, and the gradient is greater when a higher solvent to solid ratio is used [1]. A low solution to leaves ratio would result in incomplete extraction of antioxidants. While a higher solution to leaves would provide more dissolved oxygen in the reaction mixture, which may lead to the oxidation of antioxidants [3]. Therefore, a suitable ratio of solution to leaves was quite important to the DPPH scavenging abilities of miracle fruit leaves, and a value of 100:1 was chosen in this research.

![Figure 4. The effect of solution to leaves ratio on the DPPH scavenging abilities of the extract of miracle fruit leaves.](image)
4 Conclusions
In this research, the extraction conditions of miracle fruit leaves extracts in DPPH scavenging were investigated, including extraction temperature, time, solution concentration and the ratio of solution to leaves. The results showed that the optimization conditions for DPPH scavenging were as follows: extracting 3 h at 60 °C with 70% methanol solution, and the solution to leaves ratio was 100:1. Since DPPH is one of the most popular methods employed for the evaluation of antioxidant ability, the results here were quite useful for improving the antioxidant capacity of miracle fruit leaves. The research here indicated that miracle fruit leaves possessed giant potential in the synthesis of natural antioxidants, and this also improved the overall usage of miracle fruits.

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