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Ultrasonic Extraction of Ascorbic Acid from Indigenous Leaves Prior to Detection by Fluorescence Spectroscopy

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GRAPHICAL ABSTRACT

Ascorbic acid samples in volumetric flasks

ABSTRACT

Indigenous leaves are kind of vegetables with full of nutritional composition as compared to tropical vegetables, which are rich sources of essential vitamins and also rich in antioxidant. Ascorbic acid (vitamin C) is a well-known function as antioxidant which has the potential to inhibit the oxidative damage in body cells by trapping free radicals. In this study, the concentration of ascorbic acid in indigenous leaves was determined. Samples of indigenous leaves such as *tenggek burung (Euodia redlevi), ulam raja (Cosmos Caudatus)* and *pegaga (Centella asiatica)* were extracted by an ultrasonic extraction technique prior to the determination of ascorbic acid by fluorescence spectroscopy. The extraction parameters such as types of solvent, concentration of solvent, time of extraction, temperature of extraction and sample weight were optimized. The samples were extracted utilizing optimum parameters which were 1.4 g of the sample in 0.175 M sulphuric acid at 45 °C for 50 minutes. The result showed the ascorbic acid content for wet samples in *tenggek burung*, *pegaga* and *ulam raja* were 0.0145 mg/g, 0.0237 mg/g and 0.0309 mg/g while for dry samples were 0.0136 mg/g, 0.0214 mg/g and 0.0287 mg/g. It is proven that indigenous leaves were natural sources of ascorbic acid.

Keywords: indigenous leaves, ultrasonic extraction, ascorbic acid, fluorescence spectroscopy

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1. INTRODUCTION

Indigenous leaf is a fresh and natural plant that is very well-known in Malaysia. Another name for indigenous leaf known as *ulam. Ulam* is a green leafy vegetable that can be defined as part of plants that consist of leaves, stems, shoots and flowers. This kind of vegetables are full of nutritional composition as compared to the tropical vegetables, which are rich sources of essential carbohydrates, vitamins, proteins, minerals and dietary fibers [1]. They also help in the therapy of many serious illnesses in our body for examples low blood pressure, asthma and anti-cancer [1]. All types of indigenous vegetables can be found in both urban and rural locations. These plants can be used as a traditional treatment and food sources or ingredients especially in rural areas [2].

Based on the epidemiological test, the indigenous vegetables consist a lot of macronutrients and nutrients which is one of the effective ways to avoid long-term diseases especially vegetables that are abundant of natural antioxidant for example ascorbic acid [3]. Ascorbic acid or L-ascorbic acid is also called as Vitamin C. Vitamin is micronutrient which is the important element needed in small quantities only. All types of vitamins mostly can be found in food and plant sources such as in indigenous vegetables. It is required for the collagen's combination, bone, cartilage, carrier elements and substances of union muscle, skin and other tissues - examples for the substances and structures that consist of collagen [4]. Vitamin C also helps to protect our bodies from infection and prevents the cell damage. Ascorbic acid (vitamin C) is a well-known function as antioxidant. Different types of vegetables can produce different capacity of antioxidants [5]. Noteworthy, different instrumental methods have been used for the determination of vitamin C such as UV-vis spectroscopy, flow injection analysis (FIA), chemiluminescence, chemiluminescence flow injection analysis (FIA) and also fluorescence and kinetic fluorescence techniques, micro-fluorometric method, sorption–spectroscopy and high-performance liquid chromatography (HPLC) [15,16]. Indigenous leaves are largely used in the world. Regardless, more research into these indigenous leaves will help handle the problem especially any chronic illnesses due to the lack of nutrients and abundant of calories in the body. In that case, to reduce and protect from this trend, it is essential to determine the possible nutrient content especially ascorbic acid in indigenous leaves.

2. EXPERIMENTAL

2.1. Sample Collection

Samples used in this study were indigenous leaves such as *tenggek burung (Euodiaredlevi)*, *ulam raja* leaf (*Cosmos Caudatus)* and *pegaga* leaf (*Centella asiatica).* All the leaves obtained from a supermarket in Skudai, Johor. The leaves were washed by using deionized water to remove soil and dust while the inedible parts also were removed. Then, the leaves cut into small pieces and ground by using a cold mortar to form fine powder and placed in a container for further use. The container was also wrapped with aluminum foil to protect it from light in order to avoid the ascorbic acid from being reduced. One of the three samples were selected and dried at 50 °C for overnight.

2.2 Sample Preparation

An amount of 1.0 g powder of leaves was weighed exactly in a beaker. Then, 5 mL of 0.15 M sulphuric acid added into beaker and the solution stirred for 10 minutes. After that, the leaves solution was placed inside an ultrasonic bath. Then, the ultrasonic bath was set up to ultrasound energy at 40 kHz and power of 180 W for 30 minutes at 45℃. After 30 minutes, the solution could cool to room temperature. Then, by using deionized water, the solution was filtered by using a filter paper to obtain the clear solution. The solution was diluted up to 50 mL in volumetric flask with deionized water.

2.3 Optimization of Type of Solvent

The sample used for the optimization analysis was *tenggek burung*. The samples were digested in two types of solvents which were 0.15 M acetic acid and 0.15 M sulphuric acid. The samples solution was stirred using a magnetic stirrer for 10 minutes and extracted by an ultrasonic cleaner for extraction process for 30 minutes at 45 °C. Then, the solutions were filtered into a 50 mL volumetric flask and the intensity of ascorbic acid was analyzed by fluorescence spectrometer. The best solvent that gave the highest intensity was selected for the next parameter.

2.4 Optimization of Concentration of Solvent

The best solvent was prepared in five different concentrations which were 0.075 M, 0.100 M, 0.125 M, 0.150 M and 0.175 M. After that, the samples were dissolved in the solvent. Then, the intensity of ascorbic acid was measured using a fluorescence spectrometer. The best solvent concentration that gave the highest intensity was selected for the next parameter.

2.5 Optimization of Time of Extraction

An amount of 1.0 g of samples powder were weighed into the beakers. The samples were dissolved in the best solvent and best concentration with different times of extraction which were 10, 20, 30, 40 and 50 minutes. The power set up on the ultrasonic bath was 180 W and frequency was 40 kHz. The intensity of ascorbic acid analyzed using a fluorescence spectrometer. The best time for extraction that gave the highest intensity was selected for the next parameter.

2.6 Optimization of Temperature of Extraction

An amount of 1.0 g of samples powder were weighed into beakers. The samples were dissolved into the chosen solvent and concentration with the best time of extraction. The power set up on the ultrasonic bath was 180 W and frequency was 40 kHz. The different temperature also set up which were 25, 35, 45, 55 and 65 °C. The intensity of ascorbic acid was analyzed using a fluorescence spectrometer. The best temperature for extraction that gave the highest intensity was selected for the next parameter.

2.7 Optimization of Weight of Sample

The different weight of samples were 0.6 g, 0.8 g, 1.0 g, 1.2 g and 1.4 g. The leaf samples were dissolved in the best solvent with best concentration followed by the best time and temperature for extraction. Then, the intensity

of ascorbic acid analyzed using a fluorescence spectrometer. The best weight for extraction that gave the highest intensity was selected in this parameter.

2.8 Analysis of Ascorbic Acid between Wet and Dry Samples

The leaf sample was dried at the oven for 50 °C for overnight. The sample was grounded to form fine powder and stored in a container for further use. The container was wrapped with aluminum foil to protect it from light. The optimum extraction parameters were applied for the extraction of ascorbic acid from the samples. The concentration of ascorbic acid for dry leaf sample analyzed using a fluorescence spectrometer and compared with the concentration of wet leaf sample.

2.9 Method Validation

Method validation is the process used to confirm that the analytical procedures applied for a specific experiment is acceptable for its intended purpose. In this study, two types of methods of validation were used which included repeatability and percentage recovery.

3. RESULTS AND DISCUSSION

3.1 Type of solvents

One of the objectives of this study is to find the best solvent used to determine the ascorbic acid content in indigenous leaf samples. The best solvent was selected based on the extraction that can give the highest intensity by spectrofluorometer. Based on previous report, ascorbic acid is more stable in acidic medium compared to basic medium [29]. Hence, two types of solvents were used to extract ascorbic acid from indigenous leaves sample which are acetic acid, CH_3COOH and sulphuric acid, H_2SO_4 with the concentration 0.15 M. Figure 1 showed that sulphuric acid gave the higher fluorescence intensity of solvent compared to acetic acid. Different acid may have different influences in extracting. Sulphuric acid is a strong acid so it could dissolve more ascorbic acid while acetic acid is a weak acid. Therefore, the more suitable extracting solvent is sulphuric acid. From this study, sulphuric acid was selected as the best solvent used in extracting ascorbic acid.

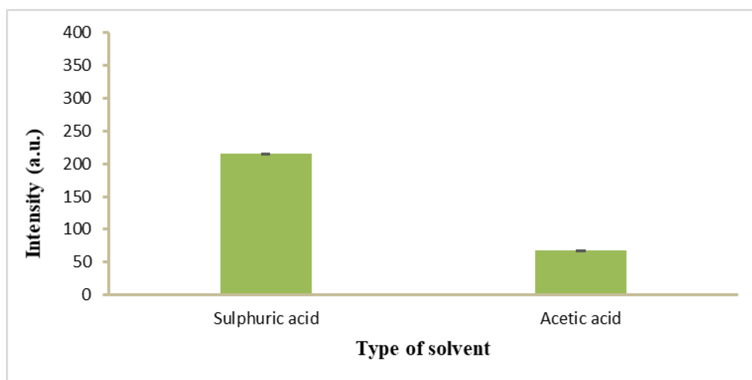

Figure 1 Intensity of ascorbic acid in several types of concentration.

3.2 Concentration of Solvents

Based on the first parameter, sulphuric acid was selected as the optimum solvent in extracting ascorbic acid from the indigenous leaf samples. The concentration of sulphuric acid was assorted from 0.075 M, 01.00 M, 0.125 M, 0.150 M and 0.175 M. The best concentration was selected based on the extraction that can give the highest intensity of fluorescence spectroscopy. As shown in Figure 2, the intensity of ascorbic acid increased when the sulphuric acid concentration increased. 0.175 M of sulphuric acid was gave the best result for the extraction of

ascorbic acid. This is due to the concentration-dependent manner. The acidity of sulphuric acid was affected so the more concentrated sulphuric acid, the higher total of extraction. As a result, 0.175 M has the highest intensity that lead to highest effectiveness on extracting. In this study, the optimum parameter in term of concentration of solvent was 0.175 M. These results were in agreement according to the previous study. The total antioxidant activity was determined in spicy and medicinal herb¸ *Limnophila aromatica*. By using hydrochloric acid (HCl) as extraction solvent with varied concentration in 0.5, 1.0, 1.5 and 2.0 mol/L, resulting the concentration of ascorbic acid content in the sample was increased as concentration increased from 16.54 mg to 83.38 mg per 100 g [31].

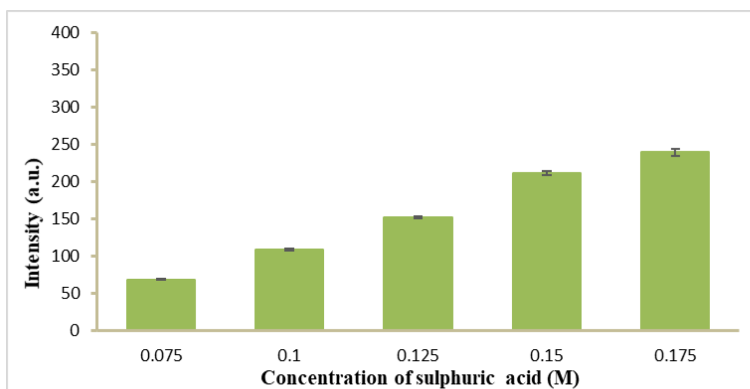

Figure 2 Intensity of ascorbic acid in several concentrations of sulphuric acid.

3.3 Time of Extraction

The optimum solvent with its concentration, sulphuric acid of 0.175 M was selected to optimize the third parameter. There were five different time 10, 20, 30, 40 and 50 minutes of extraction have been examined to extract the sample. The optimum time of extraction was selected based on highest intensity of ascorbic acid by fluorescence spectroscopy. As can be seen in Figure 3, the intensity of ascorbic acid increased as the time of extraction increased. 50 minutes of extraction time showed the best result for the extraction compared to the others. The indigenous leaf sample was extracted at the same condition but with different time for the extraction process. Consequently, time of extraction for 50 minutes was chosen as the optimized third parameter.

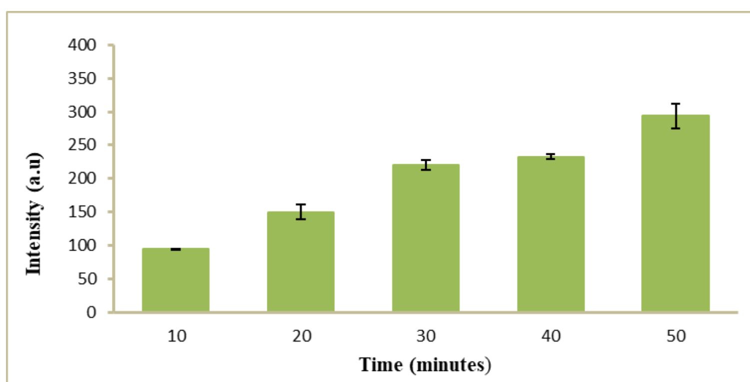

Figure 3 Intensity of ascorbic acid in several times of extraction.

3.4 Temperature of Extraction

Based on the previous optimum parameters obtained, the extraction was chosen to continue for selected fourth parameter, temperature of extraction. There were five condition of temperatures which were 25, 35, 45, 55 and 65 °C to extract the sample. Based on Figure 4, the data show that the intensity of ascorbic acid increased when the temperature increased from 25, 35 and 45 °C but the intensity decreased sharply from 55 to 65 °C. According to the previous study, the temperature and storage will affect the ascorbic acid content in vegetables and fruit juices. Ascorbic acid or vitamin C content can reduce by heat, water and air exposure. The longer the exposure, the greater the loss of vitamin C. The vitamin C is first leached out of the fruits and vegetables into the water and then degraded by the heat. High temperature has effects on vitamin C content cause a noticeable loss in vitamin C that is thermally labile. Other than that, this might be due to the evaporation of solvent at 55°C and 65°C during extraction occur which causes concentration of ascorbic acid loss by heat processing. Consequently, it is better to preserve or store vitamin C in a place below the room temperature. Thus, the optimum result was chosen at 45 °C for temperature of extraction ascorbic acid from indigenous leaves.

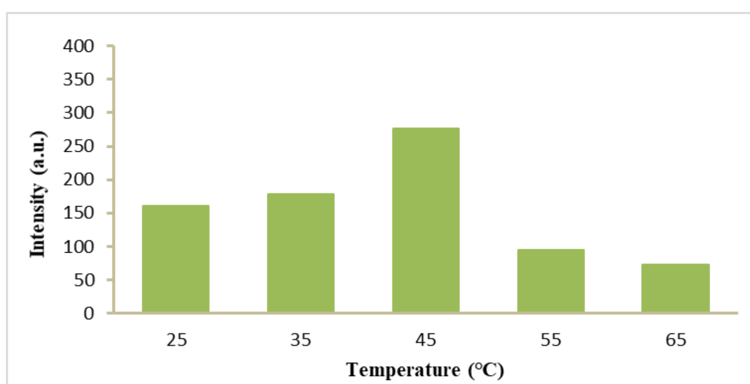

Figure 4 Intensity of ascorbic acid in several temperatures of extraction.

3.5 Weight of Samples

Four optimum parameters for extracting ascorbic acid from sulphuric acid of 0.175 M at 45 °C for 50 minutes were selected to optimize last parameter. Five different weights of the indigenous leaf samples have been used which were 0.6 g, 0.8 g, 1.0 g, 1.2 g and 1.4 g. Figure 6 shows the highest intensity of ascorbic acid have been detected from the sample 1.4 g. This might due to the amount of ascorbic acid extracted from the samples were increased when the higher samples weight was used. Therefore, 1.4 g was the best sample weight and selected as the optimum condition for the fifth parameter.

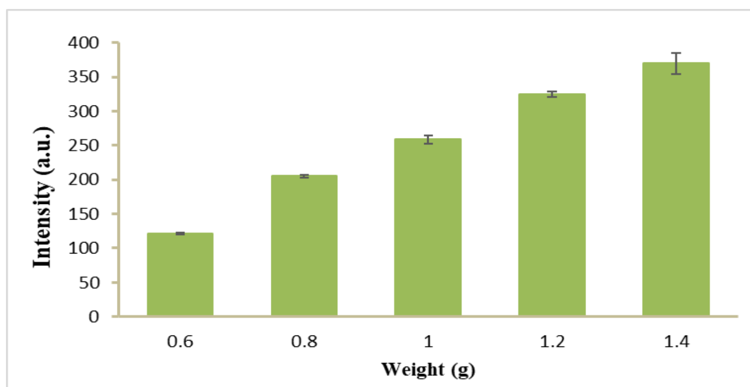

Figure 6 Intensity of ascorbic acid in several weights of extraction.

Based on Table 1, showed the overall result for five parameter extractions. The fluorescence intensity for parameter concentration of solvent increased when then concentration increased, same as time of extraction and weight of sample. However, fluorescence intensity for temperature of extraction increased to 45 °C then decreased sharply at 55 °C and 65 °C.

Table 1 Final value for optimized parameter

Type of parameters	Optimized parameter
Type of solvent	Sulphuric acid
Concentration of solvent	0.175 M
Time of extraction	50 minutes
Temperature of extraction	45 ℃
Weight of sample	1.4 g

3.6 Analysis of Ascorbic Acid

In this study, five optimum parameters for determination of ascorbic acid in the indigenous leaves were studied. 1.4 g of samples were extracted in 0.175 M of sulphuric acid at 45°C for 50 minutes. Ascorbic acid content was being analyzed using a fluorescence spectroscopy. The concentration of ascorbic acid in three samples were stated in Table 2 and Figure 7 for wet samples and Table 3 and Figure 8 for dried samples. The results showed that *ulam raja* had the highest ascorbic acid content followed by *pegaga* and *tenggek burung* for both conditions. Apparently, the ascorbic acid content for dried condition become lower compared to wet condition. This might be happened due to the prolonged exposure to heat by drying process of samples at 50 °C overnight and continued with extracting process at 50°C for 50 minutes. Increasing the temperature cause loss of the analyte in the samples. Other than that, the samples have been exposed to the light and water during the cleaning process occurred.

Table 2 Concentration of ascorbic acid in three different wet samples

Indigenous Leaves	Concentration of ascorbic acid (mg/g ± s.d.)
Tenggek Burung	0.0145 ± 0.0003
Pegaga	0.0237 ± 0.0003
Ulam Raja	0.0309 ± 0.0002

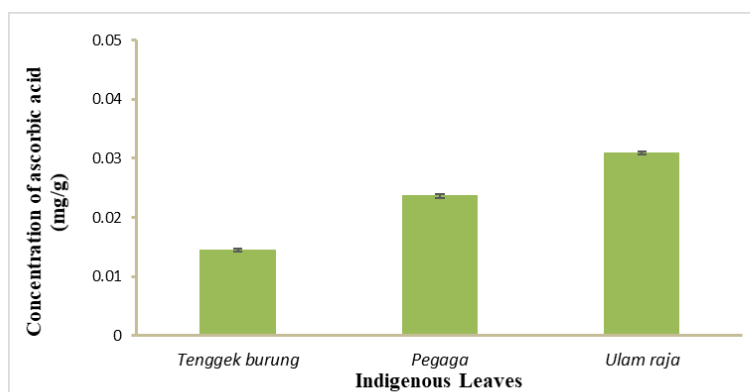

Figure 7 Concentration of ascorbic acid in three different samples of wet condition.

Table 3 Concentration of ascorbic acid in three different dry samples

Indigenous Leaves	Concentration of ascorbic acid (mg/g ± s.d.)
Tenggek Burung	0.0136 ± 0.0003
Pegaga	0.0214 ± 0.0010
Ulam Raja	0.0287 ± 0.0004

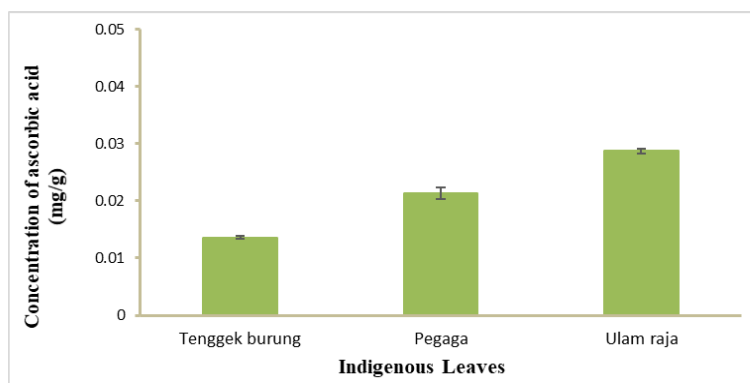

Figure 8 Concentration of ascorbic acid in three different samples of dried condition.

3.7 Method Validation

In this study, method of validation was studied by repeatability and percentage of recovery.

3.7.1 Repeatability

In this experiment, repeatability was done to measure the closeness of the datas obtained from the same sample using the same procedures. Results were obtained in intraday and interday. As can be seen in Tables 4, there was minimal change in concentration of ascorbic acid in the indigenous leaves' samples. Hence, the result can be considered as close to each other and thus has good precision.

Table 4 Concentration of ascorbic acid in *pegaga* leaf for intraday measurement

Day	Concentration of ascorbic acid (mg/g ± s.d.)	Relative standard deviation (%)
1	0.0238 ± 0.0002	0.8403
3	0.0230 ± 0.0003	1.0304

3.7.2 Percentage of Recovery

The percentage value achieved was 36.36% ± 0.0421, showing that the experimental has very poor recovery. The smaller the percent of recovery, the larger the bias that is affecting the method and thus the lower the trueness. This might be caused due to the incomplete reaction during the extraction process so that the reactants are not completely converted to products, maximal losses of analyte when the sample was prolonged exposed to the high temperature, light and water. Furthermore, due to the low value of percent recovery, this method not suitable for low concentration of analyte in a sample. Due to matrix effect and poor alignment between spiking and calibration solution also effected the method recovery [10].

4. CONCLUSION

The analysis of ascorbic acid in the indigenous leaves sample was carried out using fluorescence spectroscopy. Sulphuric acid with concentration of 0.175 M was the most suitable solvent used to obtained efficient extraction. A 50-minute time of extraction with temperature of extraction at 45 °C and 1.4 g of sample were the optimum condition to extract ascorbic acid. Using the optimum condition, two type of samples were analyzed for ascorbic acid which were wet and dry samples. For wet samples, the concentration of ascorbic acid in *tenggek burung*, *pegaga* and *ulam raja* were 0.0145 mg/g, 0.0237 mg/g and 0.0309 mg/g while for dried condition were 0.0136 mg/g, 0.0214 mg/g and 0.0287 mg/g. There were slightly different between both conditions. *Ulam raja* showed the highest ascorbic acid content, followed by *pegaga* and finally *tenggek burung*. The procedure for determination of ascorbic acid content in the indigenous leave samples gave a good precision but poor recovery. Thus, the procedure should be re-optimized for future study.

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