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EFFECT OF TEMPERATURE AND SONICATION ON THE EXTRACTION OF GALLIC ACID FROM LABISIA PUMILA (KACIP FATIMAH)

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ABSTRACT

The increasing demand of herbal based product has created great opportunities for global marketing. *Labisia pumila* contains phenolic compounds and it has been proven to have multiple biological effects, such as high antioxidant properties and anti inflammatory activity. The gallic acid (3,4,5-Trihydroxybenzoic acid) is phenolic compounds that exist in *Labisia Pumila*. Therefore, it is vital to identify the best extraction technique to maximize the performance of the process. Recently, ultrasound-assisted extraction (UAE) widely reported for the extraction of medicinal plants and herbs due to its economic and green technology. The influence of several parameters on the extraction of *Labisia pumila* were investigated : extraction time (1-8 hours), temperature (40,50,60, and 80 °C) , and sonication (40% duty cycle and without sonication) with solvent-to-sample ratio (1:10). The power intensity at 8.66 W/cm^2 was implemented using ultrasonic processor Q700 (700 watts, 20kHz) provided by QSonica, Newtown, U.S. The study was found that, the gallic acid extract increased with increasing temperature up to 50 °C and 6 hours. Result indicated the extraction of gallic acid may occur to a certain level and then began to declined due to decomposition of the compound.The highest improvement by ultasound-assisted extraction was at 50 °C by 2.26 fold. It can be concluded that, sonication was improved the extraction of bioactive constituents yield without any chemical aid.

Keywords: ultrasound-assisted extraction, gallic acid, intensity.

INTRODUCTION

Labisia pumila,is a genus of small woody and leafy plants belonging to the Myrsinaceae family that can widely found in the tropical forest of South East Asian countries (Chua, Lee, Abdullah, & Sarmidi, 2012). Labisia pumila is a plant with creeping stems and is mainly found in the lowland and hill forests in Southeast Asia, particularly Malaysia, Indonesia, Thailand, Laos, Cambodia, and Vietnam (Farouk, Nawi, & Hassan, 2008) and mostly obtained from the natural tropical forest (Fazwa, Maideen, & Mohamad, 2013). It can be recognized as a small herbaceous under a shrub that roots from the stem with a few leaves pointing upwards with the spike like panicle of small clusters of white or pink flower (Pattiram, Olusegun, Tan, Sarker, & Islam, 2011). There are eight varieties of Labisia pumila (Sunarno, 2005) but only three of the varieties that widely found and studied are Labisia Pumila var. pumila, var. alata and var. Lanceolata (Chua *et al.* 2012). Varieties of Labisia pumila can be differentiated from each other by their petiole and leaf characteristics. Labisia pumila var. Alata has a winged petiole and red veins, while var. pumila has a marginate petiole and ovate leaf blade shape, and var.lanceolata has a long and non-winged petiole. The var. alata is widely used in traditional medicine preparation because it is the most commonly encountered variety in Malaysia. Labisia pumila having high potential in the management of chronic diseases (Nik Hussain & Kadir, 2013).

As mentioned in previous reports, Labisia pumila contains a phenolic compounds (Karimi & Jaafar, 2011) and it has been proven to have multiple biological effects, such as high antioxidant properties (Chua *et al.* 2011) and anti inflammatory activity (Vijayalakshmi & Ravindhran, 2012). The main functions of antioxidants function is to delay the oxidation processes of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals.Arguably this could at least in part be due to the presence of one of the important phytochemical, gallic acid (3,4,5-trihydroxybenzoic acid). The antioxidant activity of benzoic acids has been repoted higher than vitamin C and E agaist reactive oxygen species (Chua *et al.* 2012). The chemical formula of gallic acid is $C_7H_6O_5$ with an exact molecular mass of 170.12 g/mol. Their chemical structures are shown in Figure-1.

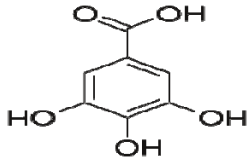


Figure-1. The chemical structures of gallic acid.

Ultrasound-assisted extraction (UAE) has been recognised for potential industrial application in the phyto-pharmaceutical extraction industry for a wide range of herbal extracts. UAE process enhancement for food and allied industries includes herbal, oil, protein and bioactives from plant and animal materials. UAE method able to increase yield of extracted components, increase rate of extraction, achieve reduction in extraction time and higher processing throughput. Vilkhu, Mawson, Simons, and Bates (2008) were reported that, ultrasound can enhanced existing extraction processes and enable new commercial extraction opportunities and processes.

Living tissues where the desired components are localized in surface glands can be stimulated to release the components by relatively mild ultrasonic stressing (Toma, Vinatoru, Paniwnyk, & Mason, 2001). In tissues where the desired components are located within cells, pre-ultrasound treatment by size reduction to maximise surface area is critical for achieving rapid and complete extraction (Balachandran, Kentish, Mawson, & Ashokkumar, 2006). Devgun, Nanda, and Ansari (2012) reported that ultrasonic-assisted extraction technique enable automation, shortened extraction time and reduce organic solvent consumption. The UAE performance is contributed by the factors of intensity, time, solvent,temperature, pulsation and matrix. Besides that, UAE involve mechanical vibrations which is sound waves with high frequency. Ultrasound can increase in the permeability of the cell wall, mechanical stressing and cavitation effect during the extraction process.

This work focused on examining the effects of ultrasound on the Labisia pumila extraction. Sonication regimens which could influence a process relative to control were identified (model system are using a water). Attempts were made to understand the possible causes of ultrasound-induced enhancement in diverse model of extraction situations. The model processes investigated included: 1) an extraction involving water-based system with the heat; 2) the effect of sonication on the bioactive constituents yield (gallic acid) and to observe the solid-liquid mass transfer limitations.

MATERIALS AND METHODS

Plant Materials

The plant material, Labisia pumila (Kacip Fatimah) were purchased locally from Delima Jelita,Simpang Empat, Alor Setar, Kedah. Prior experiment, the Labisia pumila was grounded and sieved with particle size ~0.3 mm and then stored in the fridge at 4 ºC until it used for the experiments.

Conventional Extraction

In this study, sample Labisia pumila were used in powder forms. The particle size was determined in the range of 0.15 to 0.3 mm by sieving using a standard sample sieve and a sieve shaker. Ground Labisia pumila leaves were immersed in the extraction solvent and the mixture was heated on a hotplate with continuous stirring for 8 hours. Four different extraction temperature were applied in this study (40,50,60, and 80 °C) and the sample-to-solvent ratio of each mixture was set at 1: 10 (sample : water) with the volume of infusion was set at 300 ml. The mixture was covered with aluminium foil throughout the extraction to minimize the evaporation in order to maintain the sample-to-water ratio. Then, the sample is centrifuged with speeds 5000 rpm for 10 minutes to separate a heterogeneous mixture of solid and liquid after extraction process. Extracted product was left to cool at room temperature and then was kept at 4 ˚C prior to analysis for determination of active compounds by using HPLC. The temperature was measured with an external temperature probe. Each experiment was performed in triplicate.

Ultrasound-Assisted Extraction

Ultrasound-assisted extractions of gallic acid from Labisia pumila was conducted by using ultrasonic processor Q700 (700 watts, 20kHz) from QSonica, Newtown, U.S.A with a replaceable flat tip ultrasonic probe (sonotrode) made of titanium alloy that had a tip diameter of 12.7 mm and 127 mm length. The ultrasonic probe was immersed in the extraction medium and the energy is transmitted via the sonotrode directly into the sample. The ultrasound power level was fixed by setting the amplitude of the sonotrode and the cumulative average ultrasound dose by adjusting the duty cycle. The sonication intensity was calculated using the following equation :

$$I = \frac{P}{A}$$

where A (cm^2) was the area of the sonotrode tip. The A value was 1.27 cm^2. The control (conventional) experiments did not use sonication although the sonotrode was installed as in Figure-2. The amplitude was set at position 1 to correspond to a power input P of 11W, and of 8.66 W/cm^2 sonication intensity,I using 40% duty cycles (A duty cycle of 40%, for example,was obtained by sonicating for 4 s followed by a rest period of 6s).

Eight different extraction processes were applied in this study: four conventional processes (at four difference temperature;40,50,60,80 ºC) and four innovative ultrasound-assisted extraction process (at four difference temperature;40,50,60,80 ºC with 40% duty cycle of sonication).

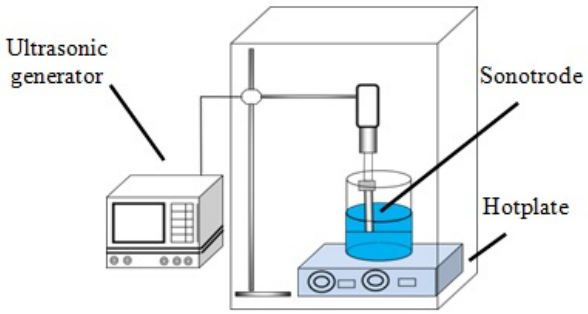


Figure-2. Schematic diagram of ultrasound-assisted extraction.

Analysis of Sample

The measurements of separation and determination of gallic acid were performed using an High Performance Liquid Chromatography (HPLC) system Agilent Series 1100 equipped with diode array detection (DAD) and a column Phenomenex Prodigy 5µ (250 X 4.60 mm). The wavelength for detection of gallic acid was set at 270 nm. Separation was achieved by flow rate of 1

ml/min with 3.0% Phosphoric acid (90%) / Acetonitrile(10%), in an isocratic programme. The injection volume was 10 µl. Each sample and standard were filtered with nylon syringe filter (pore size of 0.45 µm). For standard preparation,, the mobile phase of phosphoric acid and acetonitrile were prepared, degassed in an ultrasonic bath and will be injected through the chromatographic column.

RESULT AND DISCUSSION

The Chromatographic Separation and Detection of Gallic Acid

Minor modification were made to the chromatographic protocol proposed by Malaysian Standard (2013) for the analysis of gallic acid. The calibration curve for gallic acid was plotted based on the peak areas of chromatograms obtained for various concentrations of standard solutions, prepared from the stock solutions.

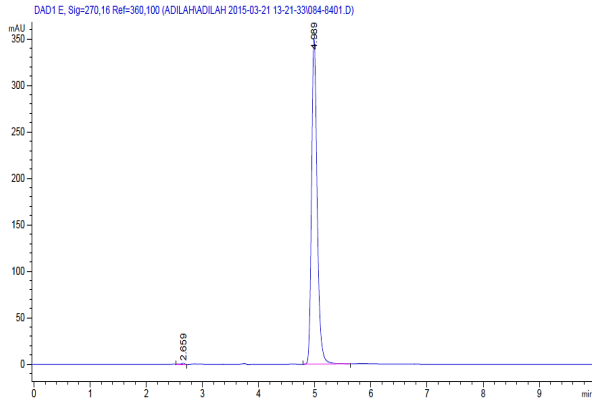


Figure-3. Chromatographic analysis of a gallic acid standard solution(injection volume 10µl).

From the characteristics, HPLC chromatogram of gallic acid (illustrated in Figure-3), it can be observed that a good resolution of the chromatographic peak is obtained. The retention time was 4.9 ± 0.2 min. The calibration curve was obtained by plotting a straight line based on the concentration of gallic acid at 5 differences concentration of standard solution and it shows in Figure-3. An excellent linearity (R^2 ≈1) for gallic acid in the range of 5-80 mg/L concentrations is obtained, with an excellent regression factor.

Figure-4 and Figure-5 shows the yield of gallic acid extracted from Labisia pumila by conventional extraction for 1-4hours and 5-8 hours at different temperature with fixed water-sample ratio. From the data obtained, at 40, 50, 60 and 80oC the extraction yield was gradually increased from 873.365±7.203 to 1026.793±4.538 mg gallic acid/kg dry sample, 640.578±3.293 to 1062.211±2.065 mg gallic acid/kg dry sample, 742.492±7.827 to 1015.490±3.530 mg gallic acid/kg dry sample and 666.350±3.857 to 1086.076±6.910 mg gallic acid/kg dry sample respectively.For the first 5 hours, 40 ºC gives the higher yield compared to the other temperature but after 6 hours of extraction the performance for all temperature was approximately stay at the same level. During the extraction process, the molecular diffusion occured once the solvent penetrates the plant matrix. Then solvent will dissolve the solute and brings out the target compound from the matrix. The higher temperature can produce the more energy to enhance the extraction process but, certain antioxidant which extracted at lower temperature can decomposed at higher temperature.Certain antioxidants may mobilize and decomposed at higher temperature(Liyana-Pathirana & Shahidi, 2005).

Table-1. Analytical data of gallic acid obtained from conventional extraction (CE) by high performance liquid chromatography (HPLC) system agilent series 1100.

Temperature (°C)	Time(h)	Gallic Acid Concentration (mg gallic acid/kg dry sample)	
40	1	873.365	±7.203
	2	934.813	±10.511
	3	898.677	±7.986
	4	960.569	±11.021
	5	933.486	±1.458
	6	953.624	±2.979
	7	972.411	±2.469
	8	1026.793	±4.538
50	1	640.578	±3.293
	2	740.658	±2.751
	3	803.378	±3.489
	4	847.354	±2.296
	5	884.251	±3.004
	6	958.821	±3.989
	7	987.265	±2.433
	8	1062.211	±2.065
60	1	742.492	±7.827
	2	797.686	±6.021
	3	833.268	±8.221
	4	846.360	±5.386
	5	867.568	±2.826
	6	930.738	±7.602
	7	993.831	±3.979
	8	1015.490	±3.530
80	1	666.350	±3.857
	2	697.500	±4.637
	3	904.777	±9.332
	4	927.991	±5.367
	5	924.408	±6.495
	6	1054.847	±2.631
	7	946.914	±2.388
	8	1086.076	±6.910

Table-2. Analytical data of gallic acid obtained from ultrasound-assisted extraction (UAE) by high performance liquid chromatography (HPLC) system agilent series 1100.

Temperature (°C)	Time(h)	Gallic Acid Concentration (mg gallic acid/kg dry sample)		Increament (fold)
40	1	898.162	±44.526	1.028
	2	979.340	±132.025	1.048
	3	1045.456	±52.872	1.163
	4	882.734	±13.334	0.919
	5	1003.622	±43.778	1.075
	6	965.584	±62.187	1.013
	7	985.749	±33.473	1.014
	8	1034.240	±41.996	1.007
50	1	1451.684	±110.469	2.266
	2	1529.734	±107.444	2.126
	3	1541.683	±125.889	1.769
	4	1657.058	±118.796	1.672
	5	1777.063	±42.229	2.010
	6	1845.863	±183.146	1.230
	7	1832.798	±17.082	1.631
	8	1603.540	±213.761	1.510
60	1	763.279	±63.073	1.028
	2	796.572	±23.683	0.999
	3	784.337	±50.574	0.941
	4	865.836	±109.655	1.023
	5	854.965	±74.823	0.985
	6	913.564	±71.504	0.982
	7	980.018	±67.426	0.986
	8	986.141	±47.636	0.971
80	1	668.149	±40.787	1.003
	2	775.011	±44.409	1.111
	3	828.776	±13.490	0.916
	4	903.888	±23.615	0.974
	5	1011.539	±50.522	1.094
	6	1071.917	±60.112	1.016
	7	1123.873	±77.379	1.187
	8	1235.110	±75.907	1.065

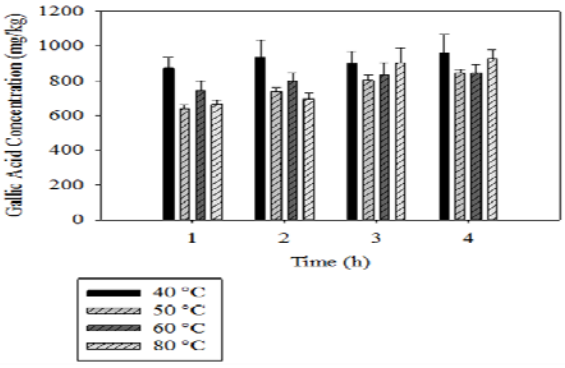


Figure-4. Extraction yield of gallic acid from conventional extraction method at different temperature with 10:1 water to sample ratio for 1-4 hours.

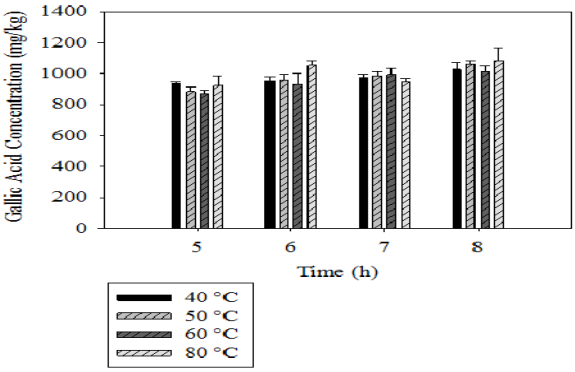


Figure-5. Extraction yield of gallic acid from from conventional extraction method at different temperature with 10:1 water to sample ratio for 5-8 hours.

Effect of sonication on the extraction yield of Labisia pumila at 40, 50, 60 and 80 °C with fixed duty cycle 40% showed in Figure-6 and Figure-7. In general, ultrasound-assited extraction was improved and accelerated the extraction processes. From the data obtained, the highest gallic acid yield is observed at 50 °C after 6 hours extraction with 1845.863±183.146 mg gallic acid/kg dry sample. The extraction process was improved due to sonochemistry effect on the molecular and microstructure of the cell wall. Comparing with the highest yield from conventional extraction, this method just need less than 1 hour to have the same amount of yield at 80 °C after 8 hours extraction process. It was proved that the ultrasound-assisted extraction has accelerated the extraction process and shorten the process time. Acoustic power produced by sonication provide the mechanical effects which results to distruption of cell wall and gives greater penetration of solvent into the cellular materials which leads to facilitate the gallic acid extraction from Labisia pumila.

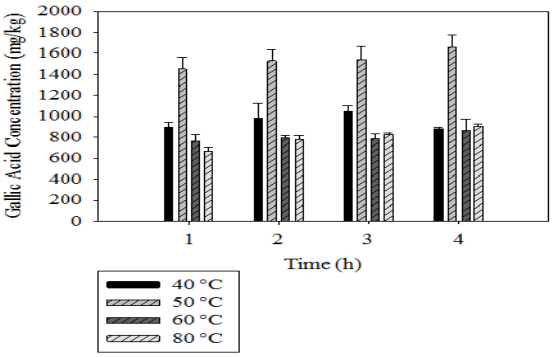


Figure-6. Extraction yield of gallic acid from ultrasound-assisted extraction method at different temperature with 10:1 water to sample ratio for 1-4 hours.

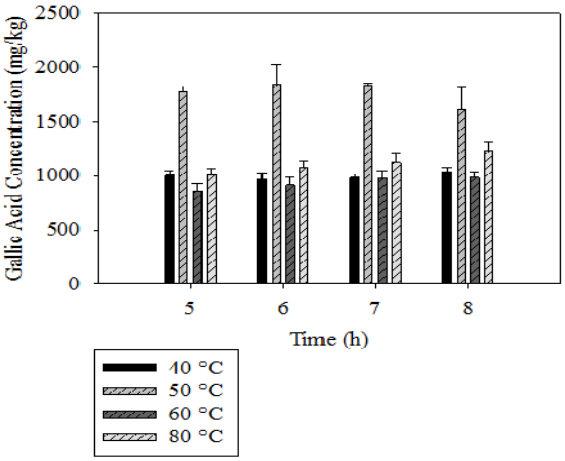


Figure-7. Extraction yield of gallic acid from from ultrasound-assisted extraction method at different temperature with 10:1 water to sample ratio for 5-8 hours.

Figure-8 indicated the highest improvement of gallic acid yield extracted from Labisia pumila by conventional and ultrasound-assisted extraction for each temperature. The highest improvement was at 50 ºC after 1 hour extraction process by 2.26 fold. Figure-9 showed the comparison of extraction yield for 8 hours extraction at 50 ºC . The target compund of gallic acid was a intracellular compund and it was not freely available. Hence, the improvement by the ultrasound-assisted extraction due to sonication was not only facilitate the process but the energy forms was loosen the matrix and the chemical bonds in the cell wall.

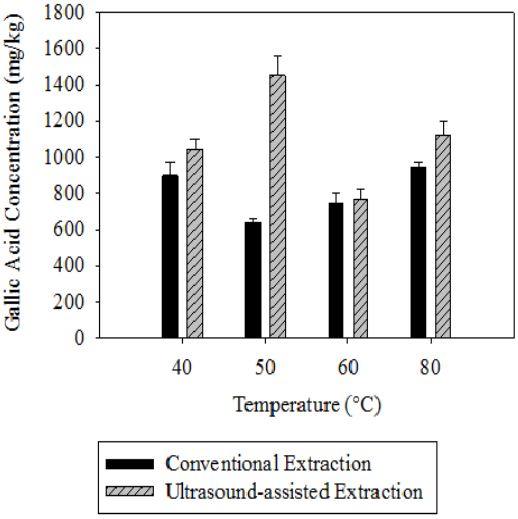


Figure-8. Comparison performance of conventional and ulrasound assisted extraction at 40,50,60 and 80 ºC by 8.66 W/cm² ultrasound intensity.

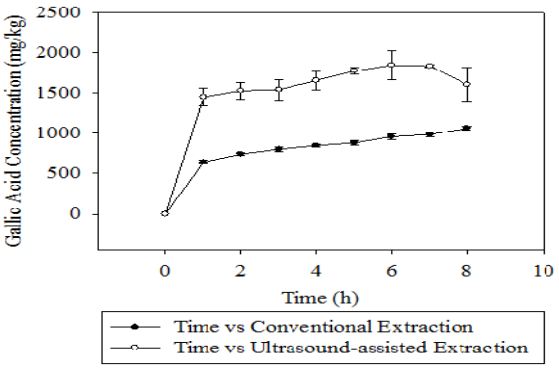
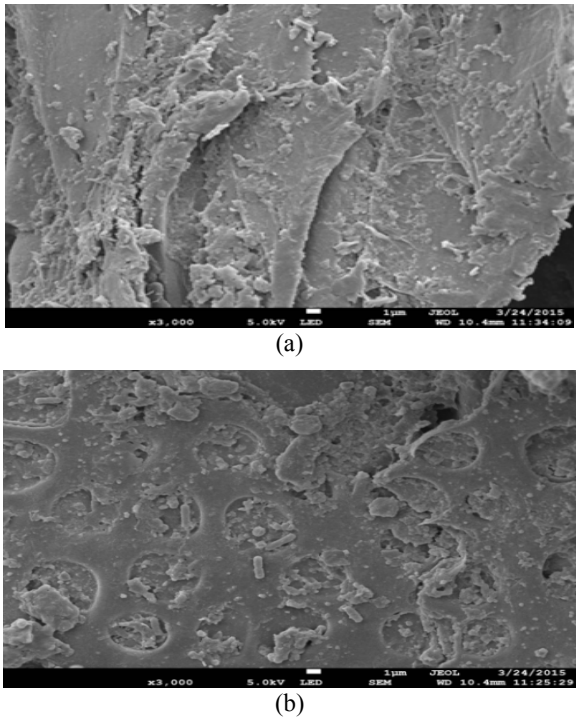


Figure-9. Comparison performance of conventional and ulrasound assisted extraction at 50 ºC by 8.66 W/cm² ultrasound intensity for 8 hours.

The extracted Labisia pumila was examined by FESEM to investigate the effect of the different extraction methods on the physical structure of the fine powder. Based on Figure-10, there is no severe fracture was observed during the conventional extraction except few slight ruptures on the surface of the sample. In case of ultrasound –assisted extraction, the swelling and softening process of the cell wall was observed. The effect of acoustic power produced by sonication clearly seen on the Figure-10(a). This, helping in permeation processes of the desired compound out of the matrix.



(a)

(b)

Figure-10. The structures of extracted L.pumila a) FESEM images by conventional extraction with 3,000x magnification; b) FESEM images by ultrasound-assited extraction with 3,000x magnification.

CONCLUSIONS

This research was carried out to determine the performance of ultrasound-assisted extraction method in extraction of gallic acid from Labisia pumila. The maximum gallic acid yield extracted was at temperature 50 ^{o}C with sonication intensity 8.66 W/cm^2 and 40% duty cycle by 2.26 fold increment.The efficiency of the ultrasound-assisted extraction procedure exceed the conventional extraction by improving the yield
and shorten the extraction time.

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