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# Comparison between Conventional Extraction and Ultrasound Assisted Extraction of Labisia Pumila Sp. in 25-L Mobile Extractor using Water as Solvent of Extraction

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Labisia pumila (Kacip Fatimah) is a medicinal plant enriches with functional compound and bioactive properties which can promote and provide a beneficial effect to human health. An antioxidant, gallic acid is widely obtained from natural sources such as Labisia pumila. To extract this bioactive compound, appropriate techniques of extraction are needed to fully utilise the resources without damaging the crude quality. Ultrasound technique is recognised for potential industrial application in the phytopharmaceutical extraction for a broad range of herbals. The aim of this study was to investigate the performance between conventional extraction and ultrasound-assisted extraction (UAE) in 25 L mobile extractor using four sonication duty cycle regimens; 40 %, 50 %, 80 % and 100 % in extraction bioactive compound from Labisia pumila. Other parameters such as temperature (50 ºC, 60 ºC, 80 ºC, and 100 ºC), the sample-to-water ratio (1 : 20 and 1 : 40) and duration of extraction (1 - 10 h) were also investigated. For the conventional method, the same parameters were conducted, but without the engagement of ultrasound irradiation. In all experiments, water was used as the solvent for the extraction. The yields of gallic acid from Labisia pumila was identified and quantify using HPLC-DAD. The optimum gallic acid, 133.25 ± 3.60 mg GAE/g dry weight was gained at conditions combination of 50 % duty cycle, the temperature at 60 ºC, the sample-to-water ratio at 1 : 20 (kg/L), and 8 h of extraction time. The study proved sonication regimes of ultrasound enhance extraction yield of gallic acid from Labisia pumila up to 1.23-fold compared to the conventional method.

## 1. Introduction

Plants are the primary source of phytochemical compounds that are utilised in various industrial branches such as the pharmaceutical products, food, cosmetics and agrochemical. In Malaysia, Labisia pumila locally known as Kacip Fatimah is widely scattered in rain forests of Southeast Asia such as Malaysia, Philippines, Indochina, New Guinea and Thailand (Christophe et al., 2002). This small herbaceous called Labisia pumila with creeping stem (Jaafar et al., 2008) and a few leaves pointing upward with the spike-like panicle of small clusters of white or pink flower (Pattiram et al., 2011). It contains flavonoid, phenolic and various bioactive volatile compounds (Ibrahim et al., 2014). For specific phenolic compound in Labisia pumila at several parts of the plant is studied in the previous report (Karimi and Jaafar, 2011). Several studies on antioxidative activities properties from Labisia pumila have been reported (Chua et al., 2011), anti-inflammatory activity (Vijayalakshmi et al., 2012) and antiproliferative properties reported by Nurhikmah (2015). In the past, Labisia pumila is popular amongst Malay woman for decoction drink to induce and facilitate childbirth, as well as being a postpartum medication to help contract the birth channel, to tone the abdominal muscles and to regain body strength. Commonly local people use conventional extraction to extract the beneficial compound from Labisia pumila which is time-consuming, produce a lower yield of active compound and the rawer material is used. The need for effective extraction of bioactive compounds from plants is very crucial now. Conventional extraction comprises solid-liquid technique depending organic solvents present many shortcomings such as toxic residues, chemical transformation of extract, use of a large quantity of organic solvents which are a risk to human and environment and long-term processing. In the modern industry, eco-friendly techniques such as

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ultrasound-assisted extraction (UAE) have gained high demand. As for ultrasound irradiation (20 - 100 kHz) that able to offer shorter extraction times, reduced solvent consumption, lower temperature and energy input and increasing the yield of the extraction in comparison with the conventional method (Pingret et al., 2013). During sonication, ultrasound produces cavitation bubbles from ultrasonic waves that permit penetration of the extraction solvent into the plant cell wall greater than conventional methods, effectively releasing the intracellular product of the plant (Lee and Lin, 2007). The aim of the current research is to examine the effect of ultrasound on Labisia pumila extraction in the 25 L mobile extractor. Different duty cycle was preliminary screened for their ability to extract phenolic compounds from Labisia pumila. The duty cycle with the most suitable properties was then used to perform a systematic study aimed at assessing the effect of the main process parameters on the extraction of phenolic compounds.

## 2. Experimental

### 2.1 Raw material

Chip leaves of Labisia pumila sp. were purchased from a local supplier (Delima Jelita Sdn Bhd) located in Kedah, Malaysia. The dry Labisia pumila sp leaves were kept in labelled capped plastic inside refrigerator at 4 ºC until use.

### 2.2 Material and chemical

An extraction vessel with a capacity of 25 L made of stainless steel 316 equipped with an ultrasonic homogeniser, 250 kHz working frequency, 500 W output amplitude, setting displayed in % on the scale 10 - 100 % from Bandelin (Berlin, Germany) was utilised for extraction experiments. Acetonitrile and sulphuric acid were HPLC grade. Gallic acid (3,4,5-trihydroxybenzoic acid) was purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA).

### 2.3 Experimental method

Dried Labisia pumila leaves were weighed and placed in an extraction chamber of the 25-L mobile extractor and were immersed in the ultrasonic bath. Then the mixture was sonicated at different duty cycles, time for extraction, temperature and the sample-to-solvent ratio (according to the experimental design) at constant power and frequency. After the extraction, the samples were centrifuged at 5,000 rpm for 10 min. Extracted was left to cool to room temperature and stored at -20 ºC before analysis.

### 2.4 Duty cycle screening

Duty screening was performed by extracting 0.952 kg of Labisa pumila leaves with 20 L of water in 25 L extractor chamber. Samples of the liquid were withdrawn for each 2 h, 4 h, 6 h and 8 h, passed through a 45 µm nylon fibre and assayed for determination of phenolic compound. The various value of duty cycle examined were 0 %, 40 %, 50 %, 80 % and 100 %.

### 2.5 Analytical methods

The phenolic compounds of Labisia pumila were quantitatively measured by High Performance Liquid Chromatography (HPLC) system Agilent Series 1100 based on the method described by Adilah et al. (2016) with some modifications. The phenolic standard used was gallic acid. An aliquot of sample extract was loaded on the HPLC equipped with diode array detection (DAD) and a column Phenomenex Prodigy 5µ (250 x 4.60 mm) analytical column. The phenolic was detected at 270 nm. Solvents comprising phosphoric acid (solvent A) and acetonitrile (solvent B) isocratic were used. This ratio was maintained for the next analysis with the flow rate of 1 mL/min. The results were expressed as gallic acid equivalents (GAE) per unit weight of dry solid using a calibration curve obtain with gallic acid standards.

### 2.6 Statistical analysis

All experiments and analyse of samples were run in triplicates. Experimental results were expressed as means ± standard deviation errors. Statistical analysis was performed and the results obtained were analysed using one-way analysis of variance (ANOVA) for mean differences among the samples. Differences were considered to statistically significant at $p < 0.05$.

## 3. Results and discussion

### 3.1 Yield of phenolic content from Labisia pumila

The maximum yield of phenolic content extracted from Labisia pumila for conventional extraction was 101.753 mg GAE/g dry weight and for UAE extraction was 134.368 mg GAE/g dry weight, these results were in line

with those reported in previous studies (Adilah et al., 2016). Based on this result shows that UAE gained higher phenolic content extract than conventional extraction same as Sharma and Gupta (2004) found that ultrasonication was a critical to obtain a high yield of extract. Of course, when considering the ultrasound extraction, the ultrasound will generate heat and it is important to control the extraction temperature (Salisova et al., 1997) accurately. Chemat et al. (2004) support the data recorded in this experiment said that extraction rates by UAE are 1.3 - 2 times more rapid than those of the conventional extraction depending on temperature. Furthermore, the yield and quality of active compound obtained by the UAE were better than those by a conventional method.

### 3.2 Effect of ultrasound exposure duty cycle

The effect of the duty cycle on the extraction efficiency was examined by fixing the temperature at 60 ºC and the material to solvent ratio at 1 : 20 while varying the duty cycle from 0 to 100 % as presented in Figure 1. Three set of experiments under the same experimental condition was conducted. There is a clear trend of increasing yield of gallic acid at various duty cycles as time increased. The comparison between 0 and 50 % duty cycles indicated that the mechanical effect of ultrasound induces a greater penetration of solvent (water) into the cellular material of Labisia pumila and improves mass transfer of active compound (gallic acid). The effect of different of duty cycles was demonstrated experimentally by Li et al. (2004). In his seminal study state that ultrasound waves will disrupt biological cell walls and facilitate the release of the content during extraction. Efficient cell disruption and efficient mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasound assisted (Mason et al., 1996) compared to conventional that are no ultrasound wave involved. For example, there is a significant change in the yield was observed between 0 and 50 % at 8 h; the extraction yields were 108.133 ± 2.128 and 133.250 ± 3.601 mg GAE/g dry weight. This study produced results which corroborate the findings of a great deal of the previous work in this field. The present findings seem to be consistent with Yu et al. (2015) research which found ultrasonic treatment could significantly enhance the extraction yield from Rabdosia rubescenes. 80 and 100 % of duty cycle at 8 h produced a lower yield of extraction (125.734 ± 2.795 and 121.412 ± 6.037 mg GAE/g dry weight) compared to an 8 h 50 % (133.250 ± 3.601 mg GAE/g dry weight). It is shown that 80 and 100 % duty cycle did not have a pronounced advantage over the 50 % duty cycle, as the ultrasonic irradiation in the latter treatment was sufficient to effect extraction. A 50 % duty cycles were chosen for succeeding experiments to replace continuous ultrasound irradiation and reduce the energy consumption. This finding supports previous research that the duty cycle was a significant factor in the UAE of natural dye from beetroot (Sivakumar et al., 2009) and phenolic compounds from strawberries (Herrera and De Castro, 2005).

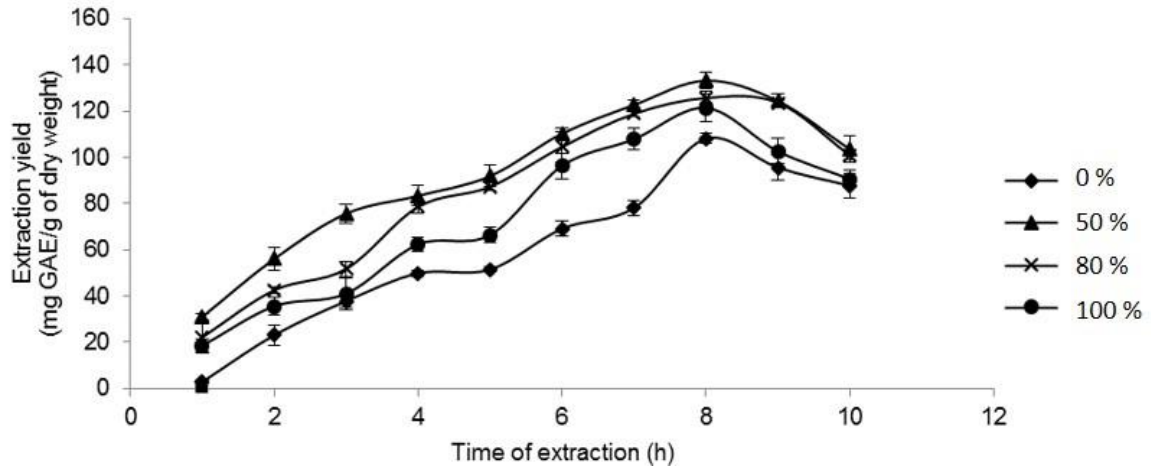


*Figure 1: Effect of duty cycle on the extraction yield*

### 3.3 Phenolic extraction and influential factor analysis

The ratio between material and solvent exerted a beneficial effect on gallic acid recovery from Labisia pumila. As can be seen from Figure 2, at a high material-to-solvent ratio (1 : 20) extract higher yield of gallic acid than low material-to-solvent ratio (1 : 40). The extraction yield in conventional and ultrasound assisted using 1 : 20 were 32.133 ± 8.263 and 105.731 ± 7.113 mg GAE/g dry weight, respectively compared to a 1 : 40 material to the solvent ratio were 19.285 ± 9.173 and 101.406 ± 10.263 mg GAE/g dry weight. This result explained by

the fact that at low material to solvent ratio produce a low yield of gallic acid because the mass transfer from tissue-bound phenolic into the solvent was reduced and parallel with (Cussler et al., 2009). In Figure 2, there is clear evidence that extraction yield using ultrasound assisted is high for both materials to solvent ratio. There are several possible explanations for this result. Firstly, it is because of intensification of extraction process using ultrasound has attributed to the cavitation phenomena. Secondly, ultrasound also exerts a mechanical effect that has a strong impact on the solid surface, therefore increasing solvent penetration into the cell and increasing the contact surface area between solid and liquid phase (Luque Gracia et al., 2004). According to Vinatoru et al. (1997), to improve the diffusion process, the ultrasound wave will facilitate swelling and hydration, so that the pores of the cell wall become large. This effect of ultrasound wave is enhancing the mass transfer.

Zhang et al. (2009) published a paper in which they described the extraction temperature is an important parameter in UAE because of largely determines the solvent power, analyse physical properties and ultrasound cavitation effects, which in turn highly influence the extraction efficiency. Figure 2 presents the yield of gallic acid extracted from Labisia pumila by conventional extraction and UAE 50 % duty cycle at four different temperatures. The data was taken after 8 h extraction with fixed material-to-solvent ratio 1 : 20. Among this four temperature (50, 60, 80 and 100 ºC), the amount of extracted in both conventional and UAE shows the highest value at 60 ºC. The value then decreased by 36.048 ± 2.128 and 50.1 ± 5.601 mg GAE/g dry weight from 60 to 80 ºC in conventional and UAE respectively and 18.632 ± 3.931 and 3.611 ± 1.243 mg GAE/g dry weight from 80 to 100 ºC in conventional and UAE respectively. The value of extraction yield decreased as the temperature increase. This finding is in agreement with Mohd et al. (2016) finding which showed the value of vitexin from Ficus deltoidea is inversely proportional to temperature because of during UAE, few cavitational bubbles were produced as a result of high acoustic cavitation threshold. In the same vein, Yu-Chiong et al. (2015) in his paper notes at a higher temperature, the solvent viscosity decreased, which may help the solvent diffuse into the cells and enhance the target compound desorption from the cells and solubility which ultimately increases the extraction efficiency. Equally important, increasing the extraction temperature might increase the energy costs and extract impurities. Taken together these results suggest that there is an association between temperature and extraction yield. 60 ºC was selected as the optimal extraction temperature to maintain the best extract quality and lower cost.

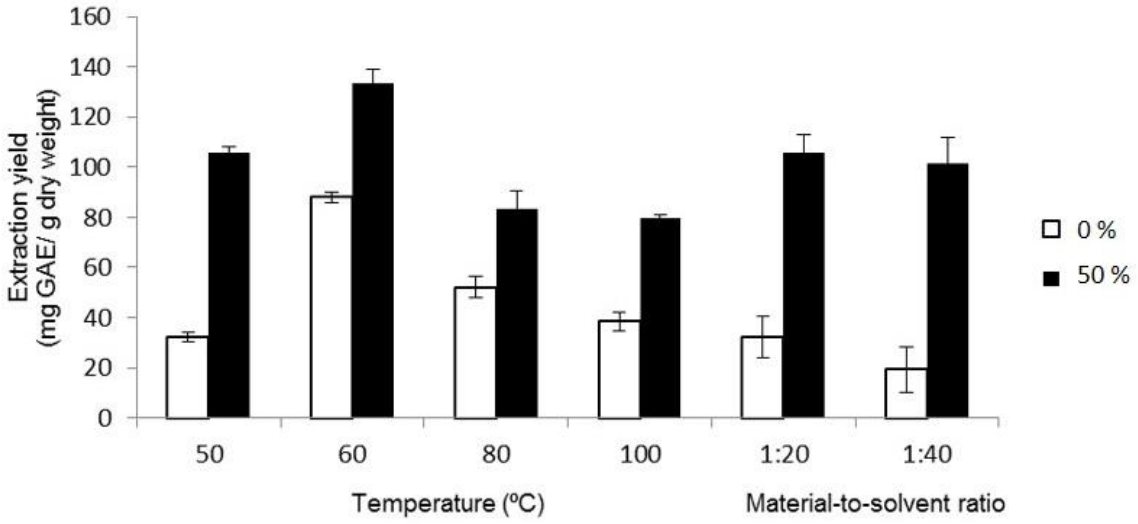


*Figure 2: Effect of temperature and material-to-solvent ratio on the extraction yield*

UAE is a potential alternative to conventional extraction method that can achieve higher extraction rate and yield at lower temperatures, substantially decreasing the overall operating cost. Despite this, the time of extraction must consider it as one of a factor that contributes to the value of extraction yield. Ma et al. (2008) claim ultrasonic irradiation at a long time may damage the quality of heat-sensitive materials. As well as costing aspect, long extraction times are not cost-effective due to their high-energy consumption. The correlation between extraction time and value of extraction yield was tested. From Figure 3, we can observe the performance of UAE is better than conventional. The extraction yield increases gradually from 1 to 8 h and decreasing from 8 to 10 h in both methods of extraction. The maximum yield of extraction at 8 h were 108.1 ± 2.128 and 133.2 ± 9.601 mg GAE/g dry weight in conventional and UAE, respectively. It is shown that from the

data that there is 1.23-fold increment yield of extraction using of UAE instead of using conventional extraction. A possible explanation for these results might be related to 50 % of duty cycle was not only facilitating the process, but the energy forms were loosening the matrix and the chemical bond in the cell wall. Eventually, it will increase extraction kinetics even improve the quality of extract. The finding of the current study is consistent with those of Luque-Garcia et al. (2004) who claim the use of ultrasound reduced the extraction duration at least half of the time needed by conventional extraction methods. This finding further supports the idea of Wu et al. (2001) found the extraction of ginseng saponins is about three times faster using UAE than conventional extraction. The preferred extraction time was 8 h, corresponding to the maximum extraction yield. This duration was used as a constant parameter for all further experiments.

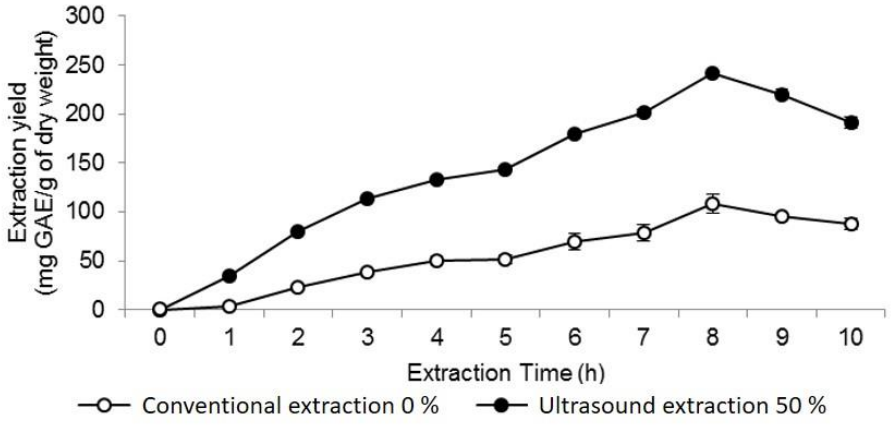


*Figure 3: Effect of extraction time on the extraction yield*

## 4. Conclusion

The combination of these factors is used to gain maximum extraction yield of gallic acid in a short period of extraction. The recovery of natural antioxidant or other health-promoting compounds from herb extraction is a topic growing interested nowadays. This study report comparison yield of gallic acid in Labisia pumila using conventional and ultrasound assisted extraction in the 25 L mobile extractor. The yield of gallic acid can be easily extract and recovery by using an appropriate duty cycle of ultrasound during the extraction process. The results obtained indicate that the extraction efficiency is affected by duty cycle and the process conditions (temperature, material-to-solvent ratio and extraction time). This research also has shown that an analysis of influential factors can provide useful indications of the effect of process variables on gallic acid recovery from Labisia pumila. The efficiency of ultrasound-assisted extraction procedure exceeds the conventional extraction by improving the yield and shorten the extraction time is presented in this paper.

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