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Extraction and Identification of Bioactive Compounds from *L. pumila*

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Abstract: This paper presents the comparison of extraction methods between maceration extraction (ME) and ultrasonic assisted extarction (UAE) to the bioactive compounds yield (gallic acid, protocatechuic acid, epigallocatechin and rutin) of *Labisia pumila.* A grinded dried plant material with size ranging 246.58µm to 257.72µm was performed throughout this work. The gallic acid, protoctechuic acid, epigallocatechin and rutin qualification and quantification were performed using an ultra-performance liquid chromatography coupled photodiode array (UPLC-PDA). Exact match between the residence time from the plant extract and external standard was found indicating a presence of these four targeted bioactive compounds. It was found that UAE method has the highest extraction yield; gallic acid (0.0293 mg GA/g DW), protocatechuic acid (0.0081 mg PCA/g DW), epigallocatechin (0.0057 mg EGC/g DW) and rutin (0.0038 mg Rutin/g DW) compared to ME. The findings in this work may serve as a useful guide to obtain a highest extraction yield of these four targeted bioactive compounds from *L. pumila.*

Keywords: *Labisia pumila, maceration extraction, ultrasonic assisted extraction*

INTRODUCTION

Labisia pumila, (vernacular name: Kacip Fatimah) is a small herbaceous plant that is grows wild all over in South East Asian countries especially in Malaysia. Generally, *L. pumila* is widely used in traditional and medical treatment especially for women with premenstrual symptomps or menopausal symptoms. Recently, many researchers reported about the health benefits of *L. pumila* which used to induce and facilitate childbirth and reduce the risk of cardiovascular disease [1], relief menstrual cramps and treat menstrual irregularities [2-3]. *L. pumila* also provides phytoestrogenic property which is natural plant estrogen that can act as an estrogen replacement therapy agent related to menopausal symptoms [4]. The study of bioactive compounds in *L. pumila* is very important in discovering of the numerous biological effects such as antioxidant, anticancer, antimicrobial, anti-inflammatory and antiobesity [5-7]. Furthermore, the bioactive compounds which existed in *L. pumila* are phenolics compound; gallic acid, pyrogallol, caffeic acid, protocatechuic acid, flavonoids; kaempferol, myricetin, naringin, rutin, epigallocatechin, quercetin and phytochemical; ascorbic acid, beta carotene [8-9].

Generally, the bioactive compounds based on its yield were extracted from the plant materials. Previously, the traditional and conventional methods which are maceration extraction (ME) and soxhlet extraction are used at small research setting area under the high temperature, longer extraction time and use of large volume of solvent. For instance, maceration extraction

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was performed within 3-5 days which is time consuming process and decoction method used high temperature that can affect the active compound of the plant [10]. In addition, Dukić et al. [11] reported that maceration process took seven days to extract from *Thymus serpyllum* L but the result was in lower yield. Apart from aforementioned methods, the bioactive compounds will denatured by high processing temperatures and prolonged extraction time. However, the latest and more advanced technology extraction methods such as ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE) and others have been developed to overcome this problem. Innovative extraction method such as ultrasonic assisted extraction (UAE) is more enviromentally method has been developed for improving efficiency and selectivity. Recently, Yeop et al. [12] reported that ultrasonic wave from the UAE probe can break the cell membrane of the plant material which enhances the inner mass transport. Furthermore, the cavitation bubble generated will direct contact with *L. pumila* surface in resulting destroy the cell walls of the plant and the bioactive compounds are released into the medium of solvent. Therefore, this work aims to study the effect of extraction methods on the yield of gallic acid, protocatechuic acid, epigallocatechin and rutin extraction from *L. pumila* using UAE and ME method.

MATERIAL AND METHODS

Chemical and plant materials

The standards of gallic acid**,** protocatechuic acid, epigallocatechin and rutin were obtained from Sigma Aldrich (St Louis, MO) whereas HPLC grade trifluoroacetic acid (TFA) and acetonitrile (ACN) were purchased from Fisher Scientific (Leicestershire, UK) and Merck (Darmstadt, Germany), respectively. The dried plant of *L. pumila* var. alata were purchased from a local manufacturer. The leaves are then dried in oven at 35°C to eliminate the moisture content. Blender (Waring Commercial Blender) is used to grind it into fine powder. The powderized plant was preserved in an air-tight at room temperature prior to extraction.

Moisture content analysis

The moisture content from a grinded *L. pumila* was determined using a moisture analyzer (AND MS-70, Japan). 0.1 g of sample was placed on the heating pan and heated continuously at 110 °C. The moisture content evaporates and the heating stopped automatically once the mas of the sample attained a constant value.

Maceration extraction (ME)

L. pumila's extract was performed by soaking the *L. pumila* powdered in a tube made up from stainless steel to stand at 90 °C temperature for 120 min in Water Bath Memmert. A volume of 20 mL solvent (100% water) was added into weighted powder (dry weight) at the solid-to-solvent ratio of 0.05g/100 mL. The supernatant was then separated by centrifugation (Eppendorf 5810 R, Hamburg, Germany) at 10 000 rpm for 15 min to obtain a clear solution. The extracts were than stored at -80°C to avoid the degradation and prior to analysis.

Ultrasonic assisted extraction (UAE)

L. pumila's extract were prepared via Qsonica Q700 (Newton, USA) equipped with a standard probe. A volume of 100 mL solvent (100% water) was added into weighted powder (dry weight) at the solid-to-solvent ratio of 0.05g/100 mL. The mixture was then immediately sonicated at the extraction time of 10 min with the amplitude of 90%. The supernatant was then separated by centrifugation (Eppendorf 5810 R, Hamburg, Germany) at 10 000 rpm for 15 min to obtain a clear solution. The extracts were than stored at -80°C to avoid the degradation and prior to analysis.

Analysis of bioactive compounds content

Qualitative and quantitative determinations of *L. pumila* extracts of gallic acid, protocatechuic acid, epigallocatechin and rutin were performed on Waters Acquity UPLC H-Class (Milford, USA) fitted with Kinetex® 1.7 µm XB-C18 100 Å, Column 50 x 2.1 mm. The UPLC system is equipped with a photodiode array detector and controlled by Waters Empower 3 software. The mobile phase consists of solvent A: Trifluoroacetic acid (TFA) in water, B: Trifluoroacetic acid (TFA) in acetonitrile (ACN), C: 20% ACN and D: 100% ACN. The gradient elution: 0-0.8 min, 5-10% B; 0.8-1.8 min, 10-12% B; 1.8-3 min, 12-15% B; 3-4 min, 15-20% B; 4-5.3 min, 20-22% B; 5.3-6.1 min, 22-25% B; 6.1-7.3 min, 25-30% B; 7.3-8.5 min, 30-32% B; 8.5-9.7 min, 32-35% B; 9.7-10.5 min, 35-40% B; 10.5-11.5 min, 40-45% B; 11.5-12 min, 45-50% B; 12-15 min, 50-10% B: 15-20 min, 10-5% B and finally washing and reconditioning of the column with 5% B for 3 min. The column temperature was maintained at room temperature, 24°C with an injection volume of 3 µL and flow rate at 0.17 mL/min. Before the samples are injecting into the UPLC system, it was filtered with 0.22 µm nylon membrane.

RESULT AND DISCUSSION

UPLC Quantification in *L. pumila* extracts

The gallic acid, protocatechuic acid, epigallocatechin and rutin were identified by means of the retention time of the standard and the plant extract. The six point

calibration curve in the concentration range 0.5-0.005 mg/ml showed a good linearity; gallic acid (R^2=0.9996), protocatechuic acid (R^2=0.9995), epigallocatechin (R^2=0.9994) and rutin (R^2=0.9999). A total of 10 min is sufficient to separate the bioactive compounds evenly and gallic acid was spotted at the retention time of 2.71 min, protocatechuic acid at 4.62 min whereas epigallocatechin at 5.66 min and 8.65 min for rutin (Figure 1). The spectrum of gallic acid, protocatechuic acid, epigallocatechin and rutin were detected at 280, 260, 210 and 355 nm. UPLC enhances mainly in speed, sensitivity and resolution and allow better separation of bioactive compounds compared to HPLC which less sensitivity and requires longer time about 60 min of running time [13]. Therefore, this method is capable for an accurate qualitative and quantitative analysis for these four targeted bioactive compounds (gallic acid, protocatechuic acid, epigallocatechin and rutin) from *L. pumila* extracts thus, similar method were used throughout this work.

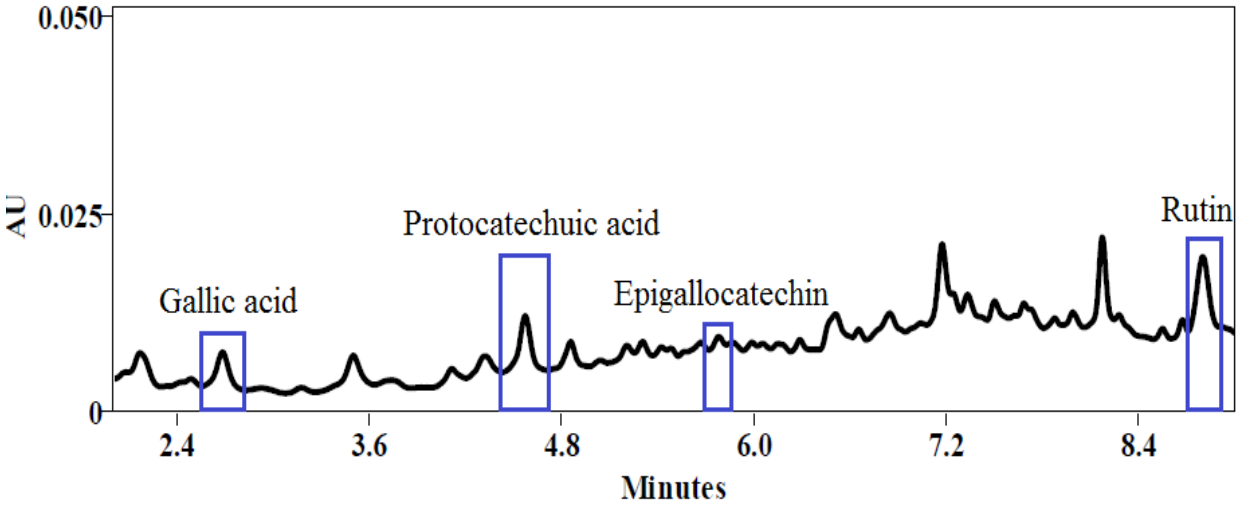

Fig 1 UPLC chromatogram of *L. pumila* extract

Comparison between ME and UAE method

Bioactive compounds from *L. pumila* is often extracted traditionally using a conventional method which is maceration. However, due to the lowest yield and also the longer time extraction as well as high temperature ME cannot perform an effective extraction of these targeted bioactive compounds (gallic acid, protocatechuic acid, epigallocatechin and rutin) compared to the UAE. According to [14], UAE causes rapid extraction due to increase in the permeability of the cell wall, cavitation effect due to the dynamic stressing and increase internal friction of the cells. These factors affect the yield of *L. pumila* during the extraction process. In this work, UAE is the better extraction method from *L. pumila* that gave the highest yield with only 10 min compared with ME which took 120 min.

Comparison of gallic acid, protocatechuic acid, epigallocatechin and rutin yield

The yield of targeted bioactive compounds; gallic acid, protocatechuic acid, epigallocatechin and rutin by using UAE are higher than that ME as shown in Figure. 2. The results showed that the extraction using UAE yielded about 5.02% higher gallic acid content (0.0293 mg GA/g DW) than that of ME (0.0279 mg GA/g DW). Meanwhile the yield of protocatechuic acid; UAE (0.0081 mg PCA/g DW) and ME (0.0051 mg PCA/g DW) which is the difference is about 58.82%. Similarly for epigallocatechin the yield from UAE (0.0057 mg EGC/g DW) is higher than ME (0.0045 mg EGC/g DW) about 26.67% and for rutin UAE yielded about 15.15% higher rutin content (0.0038 mg Rutin/g DW) compared to ME (0.0033 mg Rutin/g DW). From this result, it show that UAE can conduct an effective extraction of these four targeted bioactive compounds compared to conventional method ME. During ME, the heat was fluctuated in the water bath which make the bioactive compounds in the *L. pumila* are not consistently heated and low yield as the result. Other than that, ME is time consuming which took 120 min compared to 10 min for UAE. As mentioned, the cell wall of the plant materials will broken down via sonication of UAE that induces cavitation and penetrate into the plant matrix. As result, the highest yield of gallic acid, protocatechuic acid, epigallocatechin and rutin obtained via UAE. In this work, UAE is the best method of *L. pumila* extraction that harvested highest yield of gallic acid, protocatechuic acid, epigallocatechin and rutin.

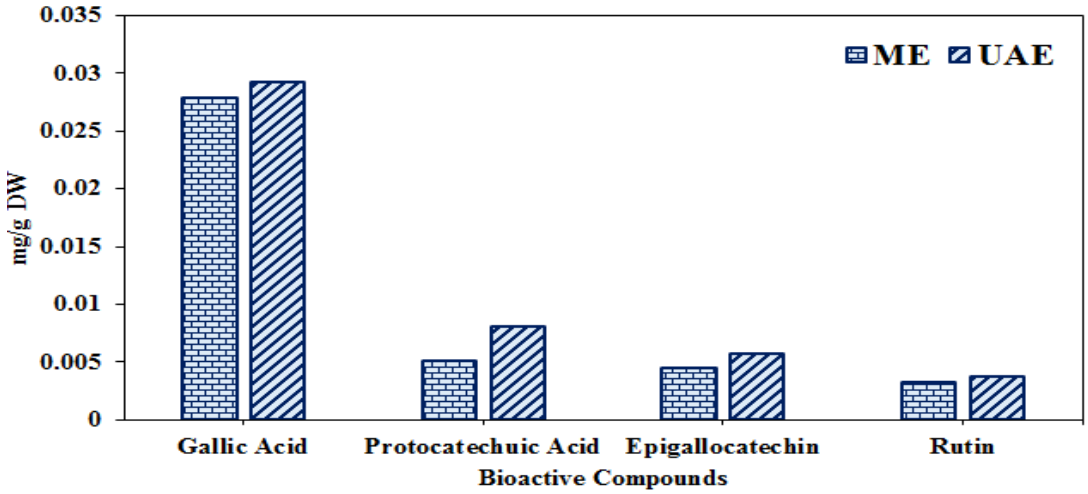

Fig 2 Quantification of bioactive compounds of *L. pumila*

CONCLUSION

The highest yield of gallic acid, protocatechuic acid, epigallocatechin and rutin; 0.0293, 0.0081, 0.0057 and 0.0038 (mg/g DW) respectively was obtained using UAE. The UPLC separation method for *L. pumila* extracts developed in this work is capable to perform faster analysis within 10 min to identify and confirm the presence of gallic acid, protocatechuic acid, epigallocatechin and rutin in *L. pumila* extract. The results indicate that UAE is better method of extraction as the time of extraction, yield of harvested and efficiency of extraction are concerned as compared to the conventional method, ME. The findings in this work may serve useful guide to maximise these targeted bioactive compound from *L. pumila* via UAE.

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