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Comparison of Total Phenolic Contents and Antioxidant Activities of *Centella asiatica* Extracts Obtained by Three Extraction Techniques

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Abstract - Through different extraction techniques, the potential of *Centella asiatica* as a natural source of antioxidant was investigated. The *C. asiatica* aqueous extracts were obtained via infusion, decoction and ultrasound-assisted extraction techniques. The effects of different extraction techniques were studied on the extraction yield, total phenolic contents and antioxidant activity. The total phenolic contents of the extracts and antioxidant activity were examined using the Folin-Ciocalteu's reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay, respectively. Results indicated that the UAE exhibited the highest extraction yield, highest total phenolic contents, as well as highest antioxidant activity. The yield of the extracts increased in the order of infusion < decoction < UAE which were 18.2 %, 23.6% and 25.4 %, respectively. All extraction techniques had a significant effect (p <0.05) on the total phenolic contents and antioxidant activity of *C. asiatica* extracts. The total phenolic contents ranged from 3.42 ± 0.030 to 8.32 ± 0.105 mg GAE/g dry extract while the antioxidant activity was in the range of 75 to 86 %. This study confirms that *C. asiatica* has the potential to be a good resource for the future development of natural antioxidant. In addition, extraction via UAE can be an ideal technique to obtain phytochemical-rich extracts from medicinal plants.

Keywords - *C. asiatica*, total phenolic contents, extraction, antioxidant activity, ultrasound-assisted extraction

I. INTRODUCTION

The use of synthetic antioxidants compounds has been restricted by legislation due to doubts about their toxic and carcinogenic effects. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are reported to have toxic effects on the lungs and induce the formation of tumours [1]. Thus, increasing attention has been directed toward natural and safer antioxidants that could be isolated from natural sources and efficiently clear free radicals. Furthermore, the isolation of bioactive compounds from natural sources requires less volume of solvents and produces fewer toxic by-products. Plants have been reported as a potential source of natural antioxidant compounds. Phenolic and flavonoids are commonly known as the most common phytochemical molecules with antioxidant properties present in plants [2, 3]. A large number of plants like fruits and vegetables have been explored for their antioxidant potential such as bhut jolokia, peach, apples, cranberry, baby Chinese cabbage, broccoli, celery, chives, eggplant, mustard, onion, red pepper, spinach, and tomato.

Typically, the biologically active compounds in plants are present in low concentration. Prior to determining the bioactive compounds, extraction of compounds from plant materials is an important step. Extraction can be defined as a separation process where the distribution of analyte between two immiscible phases is based on the appropriate distribution coefficient [4]. Traditional extraction process using organic solvents often suffers from low extraction yields, long extraction times and has residual toxic organic solvents in final products. The residual of organic solvents in the extract can cause serious health problems if the extracts are taken into the human body [5]. Hence, it is more practical and safer

for the consumer if the plant material is extracted using water [6]. After extraction solvent has been selected, the most appropriate extraction method should be carefully selected considering its practicality, efficacy and time consumption that is appropriate to conduct the extraction of medicinal plants. There are various extraction techniques have been used, namely conventional techniques such as infusion, decoction and Soxhlet extraction, and non-conventional techniques such as ultrasound-assisted and microwave-assisted [7-10]. Some studies have reported variations in the number of biologically active compounds and their biological activity obtained by different extraction techniques [3,4,7,8]. Moreover, process variables also should be optimized for the most effective isolation of target constituents as they also influenced the number of biological compounds [10].

C. asiatica or pegaga is one of the medicinal herbs that have been used for thousands of years all over the world. It is easily grown and found in most tropical and subtropical countries. This medicinal plant has been used since ancient times for various medicinal and cosmetic purposes. Based on Ayurveda, *C. asiatica* is one of the main herbs used for revitalizing nerves and brain cells [11]. It has also been used to promote longevity, lower high blood pressure and improve memory. In addition to its use in the medicinal plant, it is also eaten raw as a salad, cooked as a dish, and blended as a drink. There are several important bioactive compounds present in *C. asiatica* such as triterpene saponins, phenolic compounds, vitamins, minerals, free amino acids, and polyacetylenic compounds [12]. All of these compounds are the major ingredients that are responsible for *C. asiatica* therapeutic effects. It has been denoted in many studies that *C. asiatica* promotes wound healing activity, antimicrobial activity, anticancer activity, antioxidant activity and antileprotic activity [13].

Thus, the aim of this work was to evaluate the effect of various extraction processes, including conventional infusion and decoction and non-conventional ultrasound-assisted extraction (UAE) on the yield and total phenolic content (TPC) of *C. asiatica* aqueous extracts. The antioxidant activity of *C. asiatica* obtained by each extraction technique was also determined.

II. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

C. asiatica plants were purchased from local market in Kuantan, Pahang. The plants were washed with running tap water and oven-dried at 50 ºC for three days. The dried plants were ground using a blender to a smaller size before kept in air-tight containers and stored at room temperature until further process. Gallic acid, Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, sodium nitrate, aluminium chloride, sodium hydroxide, ethanol and methanol were obtained from Sigma Aldrich (St. Louis, US).

2.2 Extraction Procedure

Three extraction techniques were studied, which were infusion, decoction and UAE. Infusion was carried out by adding 10 g of dried *C. asiatica* to 100 mL of boiled distilled water and then the mixture was left to stand at room temperature for 30 minutes. While for decoction, 10 g of dried *C. asiatica* was boiled with 100 mL of distilled water for 30 min. The decoction temperature was controlled and maintained at 100 °C. For UAE, 10 g of dried *C. asiatica* was mixed with 100 ml of distilled water in a beaker. Extraction was carried out by placing the beaker in an ultrasonic bath (Bandelin Sonorex Digitec, Germany) for 30 min under the high sonicating setting. Once completed, all the extracts were filtered through Whatman No. 1 filter paper. The filtrates were evaporated by a rotary evaporator (RV8 IKA, Germany) at 40 °C under reduced pressure for 30 min. The liquid extract was frozen at -80 °C for

four days before proceeded to freeze drying to obtain the powder extract. The samples were stored in a chiller until further analysis.

2.3 Yield of C. asiatica extract

The powder obtained from the freeze-drying process was weighed to determine the yield. The percent yield of dried extract was determined by calculating mass of dried extract obtained per mass of propolis used. It was expressed in percent mass/mass.

2.4 Determination of Total Phenolic Contents

The total phenolic contents (TPC) of the extracts were determined using Folin-Ciocalteu's reagent, following the method described by Chandra et al. [14] with some modifications. For each sample, 0.5 mL of the sample was mixed with 3 mL of distilled water and 0.5 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 1.5 mL of sodium carbonate solution (20 % w/v) was added to the mixture. The mixture was incubated for 2 hours to allow the reaction within the mixture. The absorbance of the samples was measured at 765 nm using an ultraviolet-visible spectrophotometer (UV-1800 Shimadzu, Japan). The TPC of the extract was calculated based on a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalent (GAE)/g of dry extract. Standard calibration was constructed from different concentrations of gallic acid varying from 25 to 400 μg/mL, and its data on absorbance based on calibration equation: (Absorbance) = 0.0005 [GAE] + 0.0093 with $R^2 = 0.97$.
All the determinations were carried out in triplicate.

2.5 Determination of DPPH Free Radical Scavenging Activity

Free radical scavenging activity of the extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Zhao et al. [15] with slight modification. The propolis extracts were dissolved in distilled water to a series of concentration of 25, 50, and 100 μg/mL. Briefly, 2 mL of each sample solution was mixed with 2 mL of 0.004 % methanolic DPPH solution and incubated in dark for 30 min at room temperature. Radical scavenging activities were examined by measuring the absorbance of the samples at 517 nm using an ultraviolet-visible spectrophotometer. The mixture of methanol and DPPH solution was used as a control. All the tests were performed in triplicate and the percentage of scavenging of DPPH free radical was calculated using Equation 1:

$$\text{Percentage of scavenging of DPPH free radical} = \frac{A_{control} - A_{sample}}{A_{control}} x100\% \quad (1)$$

where A_{sample} is the absorbance of sample and $A_{control}$ is the absorbance of control (DPPH solution without sample).

2.6 Statistical Analysis

All the measurements were performed in triplicate. The results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way of analysis of variance (ANOVA) by Microsoft Excel Data Analysis Tool 2016. A *p*-value lower than 0.05 was considered to be statistically significant.

III. RESULTS AND DISCUSSION

3.1 Effects of Different Extraction Techniques on Yield and Total Phenolic Contents in C. asiatica Extracts

The effects of different extraction techniques on the yield and TPC of *C. asiatica* extracts are summarized in Table 1. The extraction yields obtained were as varied as the extraction techniques applied. The highest extraction yield was obtained by UAE (25.4%), followed by decoction (23.6%) and infusion (18.2%). Overall, decoction and UAE techniques resulted in higher yield as compared to the infusion technique. For TPC, the amount obtained in *C. asiatica* extracts was between 3.42 ± 0.030 to 8.32 ± 0.105 mg GAE/g dry extract. The highest amounts of TPC was obtained by UAE (8.32 ± 0.105 mg GAE/g dry extract), followed by decoction (4.77 ± 0.006 mg GAE/g dry extract) and the lowest TPC was shown by infusion (3.42 ± 0.030 mg GAE/g dry extract). As reported by Das and Eun [16], extraction via ultrasound-assisted had enhanced the extraction of phytochemicals as compared to conventional extraction techniques. Overall, the TPC obtained is within the range as reported previously [17].

The highest extraction yield and TPC exhibited by the UAE was attributed to the technique that used high intensity and high-frequency sound waves. Propagation and interaction of sound waves to the plant materials disrupted the plant cell walls and enhanced the mass transport of solvent from continuous phase to the plant cells, thus facilitating the release of extractable compounds [18]. Drying and grinding of samples before extraction also assisted in the destruction of cell wall which contributed to higher extraction yield and phytochemicals [19, 20].

For conventional extraction techniques like infusion and decoction, the techniques were dependent on the thermal energy. During the extraction process, heat is transferred through convection and conduction [7]. Soaking plant materials in the solvent at elevated temperatures for a specified time could break down the cell walls of the plant. Therefore, a solvent can penetrate through the matrix and the extraction of targeted solute occurred at a more rapid rate. In this study, the time contact between the solvent and plant materials at 100 ºC for the decoction technique was much longer than an infusion. This explained why the decoction technique possesses higher yield and TPC than infusion.

In this work, it can be concluded that the extraction of *C. asiatica* via non-conventional technique, UAE is more efficient than the conventional techniques applied, decoction and infusion. UAE can be used as a plant extraction technique as it has good potential with the highest yield and TPC that provide more elevated health benefits.

Table 1: Extraction yield and TPC of *C. asiatica* extracts obtained using different extraction techniques

Extraction method	Extraction yield (%)	TPC (mg GAE/ g dry extract)
Infusion	18.2	3.42 ± 0.030
Decoction	23.6	4.77 ± 0.006
UAE	25.4	8.32 ± 0.105

Note: GAE = gallic acid equivalent; TPC = total phenolic contents

3.2 DPPH Radical Scavenging Activity

The antioxidant activities of the *C. asiatica* extracts were evaluated based on the free radical scavenging capacity using DPPH assay as shown in Figure 1. The percentage of DPPH free radical

inhibition ranged from 75% to 86%, which varies according to the concentration of the extract. The extract obtained by the UAE technique exhibited the highest radical scavenging activity followed by decoction and infusion at all concentrations of the extract. This result correlated with the TPC evaluated which follows the order of UAE > decoction > infusion. The regression analysis in Figure 2 shows the correlation between antioxidant activity and TPC in *C. asiatica* extracts. The results indicated a strong association between antioxidant activity and TPC (R^2 = 0.9809), suggesting that the TPC is probably responsible for the antioxidant activity of *C. asiatica* extracts.

In addition, it was observed that there was a gradual increase in the scavenging effect on the DPPH radical with the increase of extract concentrations from 25 to 100 μg/mL. By increasing the concentration of the extracts, it is believed that the phenolic compounds also increased. As reported by Castro-López et al. [9], the amounts of phenolic compounds are correlated with the number of hydroxyl groups available in the reaction medium. Therefore, the possibility of hydrogen donation to free radicals increased. A similar study was reported by Turrini et al. [21], whereby the antioxidant activity exhibited by pomegranate peel extracts correlates with the TPC obtained.

In general, these findings demonstrate that *C. asiatica* extract obtained by UAE possesses the highest free radical scavenging activity. It shows the potential of *C. asiatica* as a source of natural antioxidant. Compared to the conventional methods, UAE is considered as much more promising and has shown a greater potential and better efficiency for the extraction of TPC. Moreover, UAE is cheaper, easy to use and environmental friendly and is not only suitable for laboratory extraction but also pilot and industrial scale.

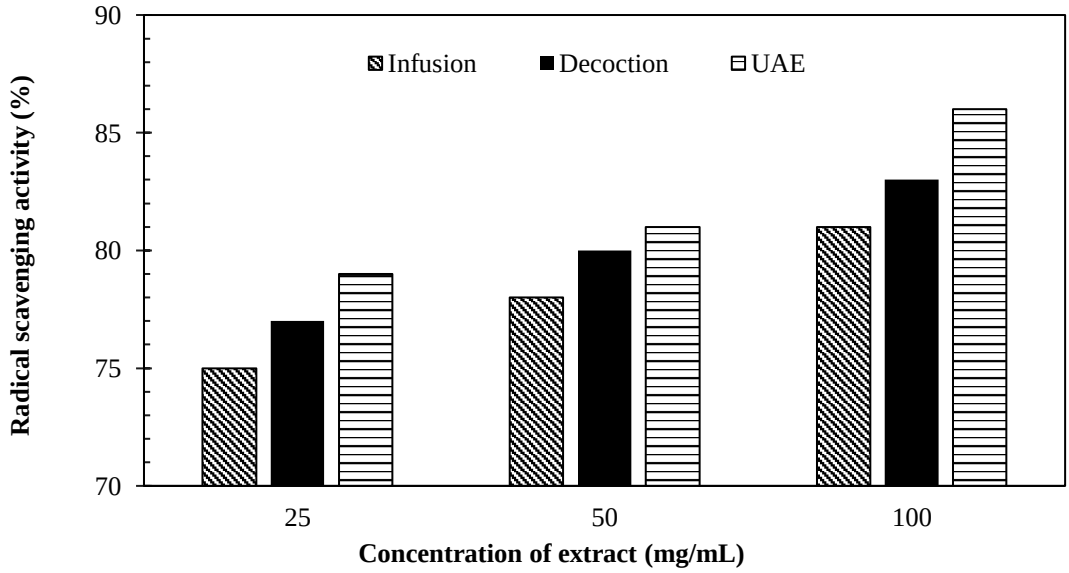

Figure 1: Radical scavenging activity of *C. asiatica* extract. Error bars indicates the standard deviation of means

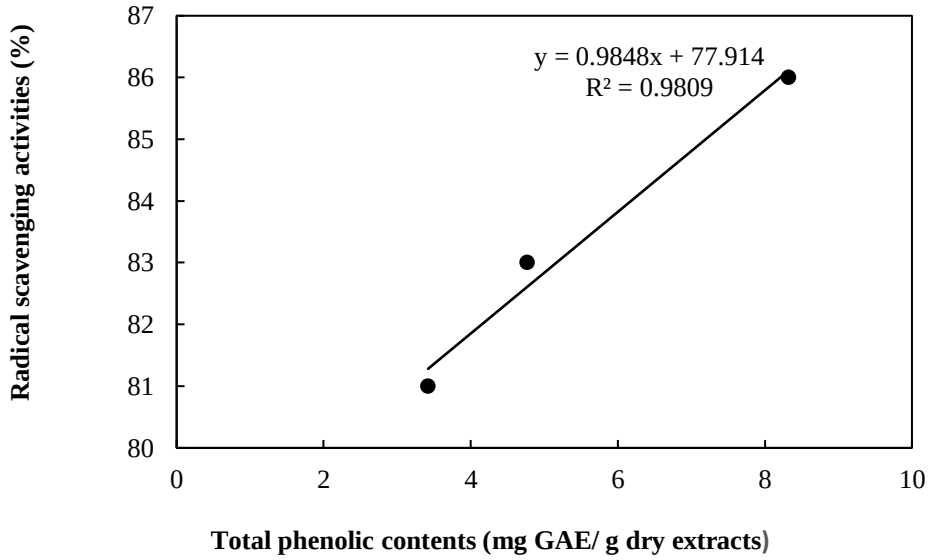

Figure 2: Relationship between total phenolic contents and antioxidant activity of *C. asiatica* extract

IV. CONCLUSIONS

In the present study, three extraction techniques, namely infusion, decoction and UAE, were studied and compared to obtain the aqueous *C. asiatica* extracts. The results concluded that extraction via UAE is superior to the other extraction techniques studied. The UAE technique has succeeded in enhancing the extraction yield (25.4 %) compared to infusion (18.2 %) and decoction (23.6 %). Meanwhile, the DPPH assay showed that the antioxidant activities were positively correlated with the TPC obtained. The extract obtained by UAE exhibited the highest TPC (8.32 ± 0.105 mg GAE/g dry extract) which contributed to the antioxidant activity, while the infusion (3.42 ± 0.030 mg GAE/g dry extract) and decoction (4.77 ± 0.006 mg GAE/g dry extract) techniques resulted in lower TPC value. This study showed that *C. asiatica* has the potential to be a source of natural antioxidant. Also, UAE is shown to be a simple, viable technique and useful for commercialization of plant extracts. However, comprehensive optimization studies are required for cost reduction, extraction time, energy usage, raw materials and impacts towards the environment.

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