

Antioxidant Potential of Malaysian Herb *Centella Asiatica*

Rajesh Nithyanandam¹⁺, Mu'ammam Rusydi Shapheri¹, Mohamed H. Nassir¹

¹School of Engineering, Taylor's Lakeside Campus, Taylor's University, 47500, Subang Jaya, Selangor, Kuala Lumpur, Malaysia

Abstract. Westerners influenced by their life style have made many serious changes on their diets. One of the basic reasons for these dramatic changes was because of the nature of the corporate environment and subsequent time limitation for traditional food. To satisfy the western style, chemicals, as preservatives, are being added to the food where, later, found that these chemicals are the major source for cancer and similar diseases. Researchers have interfered trying to curb these serious diseases by implementing herbal-based food. *Centella Asiatica* was found to be an excellent antioxidant potential species which is widely available in Malaysia and hence bioactive antioxidant components could be extracted using various methods. Extraction techniques were carried out to determine and then to optimize the binary solvent ratio (ethanol and water), extraction time, and solid to solvent ratio. Out of the four common extraction techniques, this study suggests that the Ultrasonic-Assisted extraction method was the best method over other three techniques. The criterion for the optimization is based on the results of DPPH, TPC and TF. The use of Ultrasonic-Assisted extraction results in the following findings of DPPH, TPC and TF: 79% scavenging activity, 1350 mg GAE/100g DW, and 599 mg CE/100g DW.

Keywords: *Centella Asiatica*, extraction, ultrasonic assisted extraction, antioxidant potential

1. Introduction

It is until 1992, when the office of Alternative Medicine was established by the American National Institute of Health, the herbal medicine was in doubt. The office was boosted by endorsement of the World Health Organization (WHO) encouraging developing countries to use the traditional herbal medicine. The WHO estimated that approximately 80% of the world's population uses the traditional medicines as primary health care [1]. One of the basic reasons for using herbal medicine is the existence of antioxidant compounds as an ingredient. In his recent publication, [2] suggested that the antioxidant compounds have effectively counteract harmful cells –the ones that cause cellular injury such as cancer. For this reason and, of course others, it is found that the serious needs to extract these biological active components. During the last decade, many researchers are working to find out the best method for extraction and then how to optimize the method.

In this study, *Centella Asiatica*, known by local Malaysians as Pegaga, was selected due to its importance as a source for antioxidant benefits and for its vast availability locally. The importance of *Centella Asiatica* comes from two compounds: phenolic and flavonoid. Thus, researches, including this current study, aim at using *Centella Asiatica* as natural alternative to synthetic drugs for no side effects and its moderate cost. The criterion for determining the best extraction method is based on the quality of antioxidant properties of Pegaga. For extraction methods, researchers have been dealing with Conventional Solid-Liquid Extraction, Hot Water Extraction/Infusion, Ultrasonic Assisted Extraction, and Conventional Soxhlet Extraction. To evaluate the performance of each of the extraction methods, three parameters were taken into account: Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and free radical

⁺Corresponding author. Tel.: +60-356295000; fax: +60-356295477.
E-mail address: chemrajesh1982@gmail.com.

scavenging activity, 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The criteria among these techniques in resolving the best experimental approach are the highest recovery of phenolic and flavonoid compounds along with the highest antioxidant activity. For optimization, four parameters were experimented in this study in order to explore the best technique: the extract time, solvent concentrations, solid-to-solvent ratio, and extraction temperature. There is an optimistic hope to commercialize Pegaga soon to inspire the Malaysia's rapid growing economy and development [3].

2. Extraction Methodologies

All materials were purchased from very well-known companies locally and internationally. 2 gram of Pegaga powdered form was extracted with 30 ml of extraction solvent (40% ethanol; 60% water) in a 100 ml glass conical flask at 65 °C for 60 minutes. The extraction was done by using standard procedure [4] for solid-liquid extraction; [5] for Soxhlet extraction; [2] for hot-water extraction; and [5] for ultrasonic-assisted extraction. Pegaga was extracted and then the content of total phenolic content (TPC) was analyzed [4]; then the content of total flavonoid content was analyzed [4]; then the DPPH free radical was analyzed [4].

The optimization using Minitab software at 5% confidence was carried out taking the concentration of DPPH first, then the best DPPH concentration was tested for time optimization, then the sample of the minimum time was taken to maximize temperature of the solvent, and finally the sample was taken to optimize the solid-to-solvent ratio.

3. Results and Discussion

Fig. 1 shows clearly that yield of extraction was the best using the ultrasonic method as expected since the high frequency enhances the interaction between solvent and vegetal material [5], [6]. The other possible reason is due to high recovery amount of phenolic and flavonoid could be used as a reasonable justification on high DPPH % concentration.

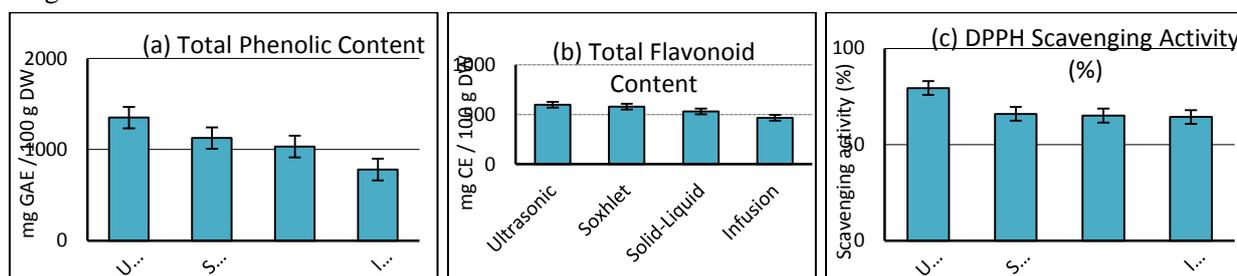


Fig. 1: Effects of methods of extraction on (a) TPC (b) TFC (c) DPPH.

The optimization procedure to determine the best extraction factors starts with considering the Total Flavonoid Content (TFC) assay and antioxidant activity assay (DPPH free radical scavenging activity) in Fig. 2. The concentration of ethanol had significant effect ($p < 0.05$) on the recovery of antioxidant compounds (TPC and TFC) and antioxidant activity (DPPH) on the extracts. Mono solvent (100% water or 100% ethanol) showed lower yield as compared to binary solvent (% water + % ethanol) in all the assays. Fig. 2(c) shows that the highest DPPH free radical scavenging activity was reached at 40% ethanol. Therefore, ethanol concentration of 40% was selected as the optimised solvent concentration for ultrasonic assisted extraction method of *Centella asiatica*.

Optimisation of extraction temperature is crucial in terms of minimizing cost of the process and energy used. The effects of extraction temperature on the recovery of antioxidant compounds and antioxidant activity are shown in Fig. 3 (a), (b) and (c) for Total Phenolic Content (TPC) assay, Total Flavonoid Content (TFC) assay and antioxidant activity assay (DPPH free radical scavenging activity). The results were expressed as mean \pm standard deviation.

From Fig. 3 (a), (b) and (c), it was observed that extraction temperature had significant effect ($p < 0.05$) on TPC and DPPH antioxidant activity except TFC. It was also observed that yield of TPC, TFC and DPPH antioxidant activity increased directly proportional to the temperature but only up to 45 °C. Beyond 45 °C, DPPH antioxidant activity was decreasing drastically while TPC and TFC were maintaining the yields

increment. TPC and TFC showed the highest values at 55 °C while DPPH antioxidant activity showed the highest value at 45 °C. In terms of antioxidant activity, based on Fig. 3(c), the highest DPPH free radical scavenging activity was reached at 45 °C. Therefore, extraction temperature of 45 °C was selected as the optimised extraction temperature for ultrasonic assisted extraction method of *Centella asiatica*.

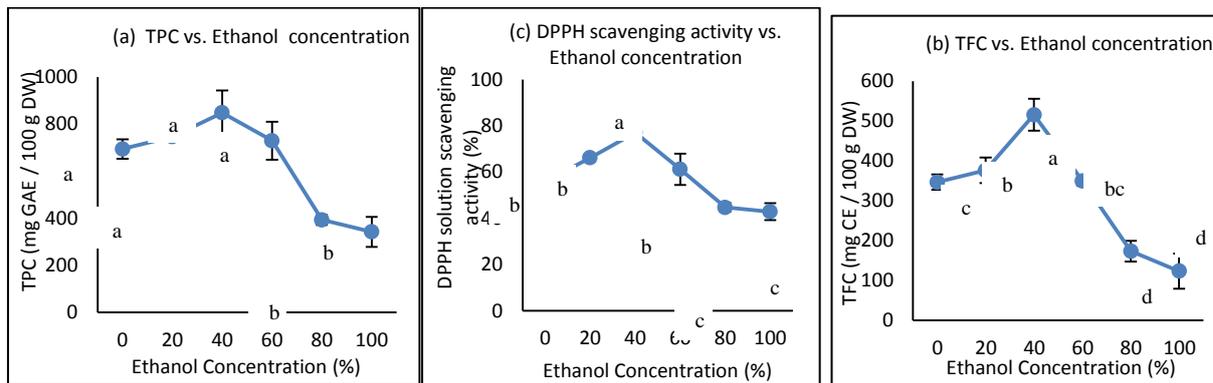


Fig. 2: Effects of extraction solvent concentrations on (a) TPC (b) TFC (c) DPPH. Values marked by different letter are significantly different ($p < 0.05$).

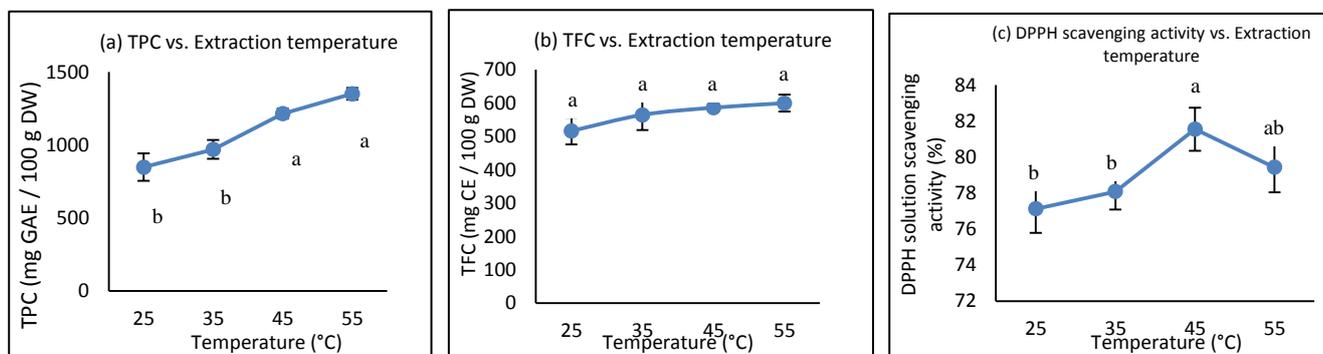


Fig. 3: Effects of extraction temperatures on (a) TPC (b) TFC (c) DPPH. Values marked by different letter are significantly different ($p < 0.05$).

Fig. 4 shows that the optimisation of solid to solvent is crucial in terms of determining an efficient usage solid to solvent mixtures to extract antioxidant compounds from *Centella asiatica*. From Fig. 4 (a), (b) and (c), it was observed that concentration of ethanol had significant effect ($p < 0.05$) on the recovery of antioxidant compounds (TPC and TFC) and antioxidant activity (DPPH) on the extracts. It was also observed that yield of TPC, TFC and DPPH antioxidant activity increased directly proportional to solid to solvent ratio.

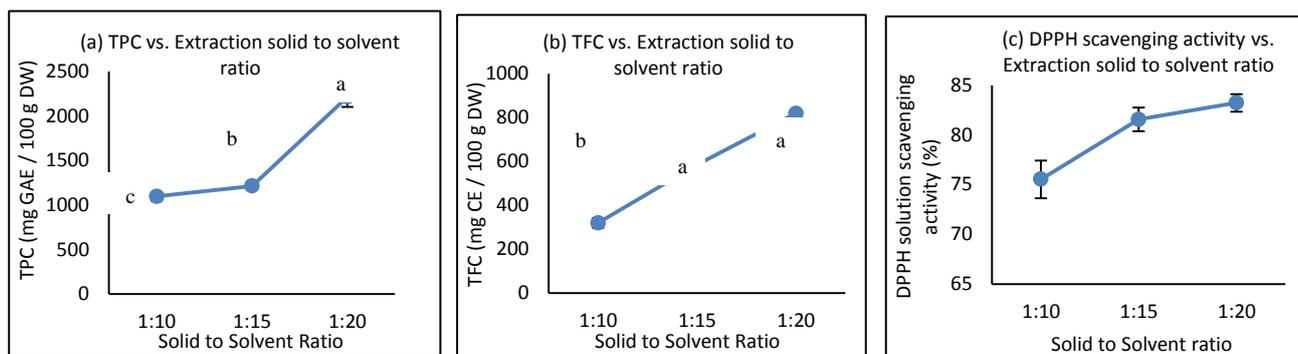


Fig. 4: Effects of extraction solid to solvent ratio on (a) TPC (b) TFC (c) DPPH. Values marked by different letter are significantly different ($p < 0.05$).

Based on Fig. 4 (c), the highest DPPH free radical scavenging activity was reached at solid to solvent ratio of 1:20. However, from economical perspective and point of view, solid to solvent ratio of 1:15 was selected as the optimised solid to solvent ratio for ultrasonic assisted extraction method of *Centella asiatica*. This was because there was no significant difference ($p > 0.05$) observed for DPPH antioxidant activity between solid to solvent ratio of 1:15 and 1:20.

Optimisation of extraction period is crucial in terms of minimizing cost of the process and energy used. The effects of extraction period on the recovery of antioxidant compounds and antioxidant activity are shown in Fig. 5 (a), (b) and (c) for Total Phenolic Content (TPC) assay, Total Flavonoid Content (TFC) assay and antioxidant activity assay (DPPH free radical scavenging activity). The results were expressed as mean \pm standard deviation.

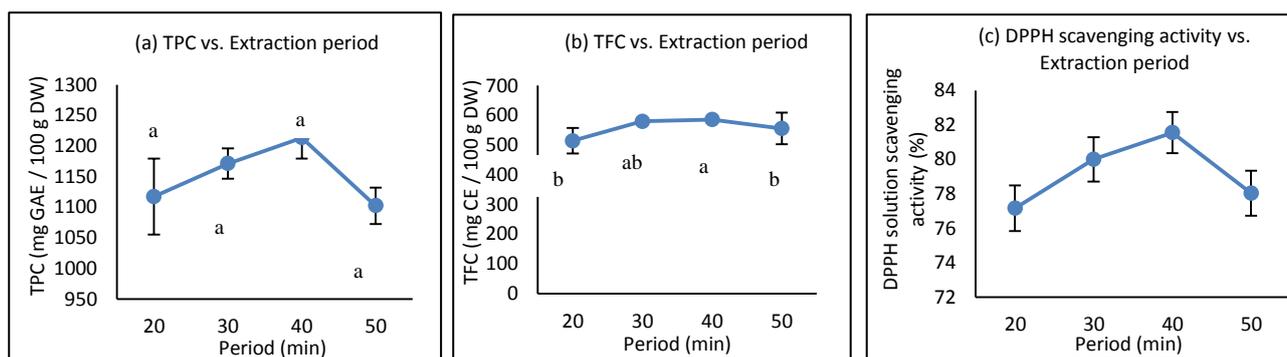


Fig. 5: Effects of extraction period on (a) TPC (b) TFC (c) DPPH. Values marked by different letter are significantly different ($p < 0.05$).

Based on Fig. 5(c), the highest DPPH free radical scavenging activity was reached at extraction period of 40 minutes. However, from economical perspective and point of view, extraction period of 30 minutes was selected as the optimised extraction period for ultrasonic assisted extraction method of *Centella asiatica*. This was because there was no significant difference ($p > 0.05$) observed for DPPH antioxidant activity between solid to extraction period of 30 minutes and 40 minutes.

4. Conclusion

It has been concluded that ultrasonic assisted extraction is selected as best method of extraction of *Centella asiatica* as it shows significance recovery of antioxidant compounds and shows the highest antioxidant activity with values of 1350 mg GAE/100 g DW, 599 mg CE/100 g DW and 79 % scavenging activity on Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and DPPH compared to the other method. Apart from that, optimisation of ultrasonic assisted reaction reveals the optimum parameters to extract *Centella asiatica* are at 40 % ethanol concentration for extraction solvent concentration, 45 °C of extraction temperature, 1:15 of extraction solid to solvent ratio and 30 minutes of extraction period yielding 1171 mg GAE, 580 mg CE and 80% scavenging activity on TPC, TFC and DPPH.

5. References

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