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Determination of Supercritical Carbon Dioxide Extraction Parameters for *Quercus infectoria* Galls and the Effects on Extraction Yield and Antioxidant Activity

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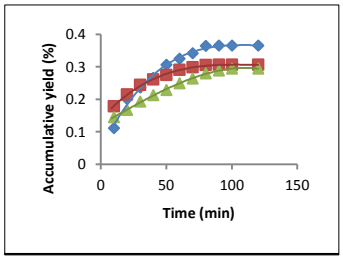
Graphical abstract



Abstract

Currently, finding alternative ways of extracting medicinal plant gain more interest from the researchers. *Quercus infectoria*, a medicinal plant, is rich with bioactive compound being extracted using supercritical carbon dioxide (SC-CO_2) extraction which helps to maintain the quality of the product as well as green environment. CO_2 is widely used as solvent due to moderate critical conditions, nontoxic and easily removed from the products. This work was performed to determine the optimum extraction parameters of SC-CO_2 extraction and their effects on the total phenolic content and antioxidant activity of *Q.infectoria* extract. Hence, two different parameters have been investigated which were extraction time and CO_2 flow rate (2, 3, 4 ml/min) while pressure (P) and temperature (T) were fixed at highest density (P = 30 MPa, T = 40°C). The results obtained from this study show that the solvent flow rate of 2 ml/min gives the highest percentage of yield (0.3652%) and the complete extraction of the sample was achieved at 80 minutes. Better quality of the extract was shown at 2 ml/min as resulted in high amount of phenolic compound in the extract presented as gallic acid equivalent (GAE) (2.04×10^2 mg GAE/g sample). The extracts were screened for possible antioxidant activity by 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assays. In this study, the best result obtained for antioxidant activity was at flow rate of 3 ml/min with inhibition percentage of 96.97%.

Keywords: *Quercus infectoria*; SC-CO_2 extraction; total phenolic content; antioxidant activity

1.0 INTRODUCTION

Healing powers in medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antioxidant, antimicrobial agent and more, and it is clinically proven. Generally, over three-quarters of the world population depends generally on plants and plants extract for health care purposes [1]. This is due to the fact that medicinal plants have the richest bio-resources of drugs in traditional medicinal systems, modern medicines, nutraceuticals, folk medicines, food supplements, intermediate and chemical entitled for synthetic drugs [2]. There are three basic parameters influencing the quality of extract which are the plant part used as starting material, extraction solvent and thirdly is extraction procedure [3]. The researchers would keep on using the medicinal herb and these should be incorporated into modern medicine.

Quercus infectoria, known as manjakani is a type of medicinal plant that can be used in treating diseases and it is already well-known since ancient time. Oak (*Quercus*) is part of Fagaceae family. *Q.infectoria* galls is stimulated by gall-wasp called *Cynips quercufolii*, through laying its eggs in the bark. As a result, the inner cells of the bud becomes bigger [4]. During this time all starch will be converted into tannins, resulting in the galls containing tannins 60% from its total weight [5-6]. Besides, the galls also reported to restrain small amount of free gallic acid (2-4%), ellagic acid, syringic acid, β-sitosterol, amentoflavone, hexametyhl ether, isocryptomerin, methyl oleanate, methyl betulate and hexagalloyl glucose [7-10]. *Q.infectoria* was claimed to be extremely valuable for the local Malay postpartum women and there are no risky effect reported until now.

Until today, the application of supercritical fluid extraction (SFE) technology in industry has been limited, however the use of this technique already grab much attention for removal of organic

compounds from different liquid and solid matrices. Examples of SFE technology that being used are extraction of oil from fig leaf gourd seeds, extraction of biaoactive compounds from *A.grossedentata* stems, extraction of essential oil from *Achillea santolina* and lycopene extraction from tomato peel [11-14]. SFE generally based on the utilization of a fluid under supercritical conditions, is a technology suitable for extraction and purification of a variety of compounds, especially for those that have low volatility or susceptible to thermal degradation. Supercritical fluids have higher diffusivity and lower density, surface tension and viscosity which can be varied by changing the operating conditions, subsequently give advantages to the extraction process. Furthermore, the use of CO_2 as a solvent in SFE can avoid the alteration of the product since the operating temperature used is near ambient temperature [15].

To the best of our knowledge, there is no research on the extraction of *Quercus infectoria* by supercritical fluid extraction using carbon dioxide (CO_2) as solvent. Hence, a study has been conducted to determine the optimum extraction parameter (CO_2 flow rate, extraction time), total phenolic content and its antioxidant activity.

2.0 MATERIALS AND METHODS

2.1 Preparation of Plant Material

The galls of *Q.infectoria*, purchased from local market (Kota Tinggi, Johor Malaysia), were first separated, rinsed using tap water to remove unwanted material and dried in an oven at 60°C overnight. They were crushed by blender before extraction was done. The prepared seeds were stored in dark place at room temperature.

2.2 Supercritical Carbon Dioxide Extraction

The extraction of *Q.infectoria* galls was done using Supercritical Fluid Extraction Unit [16-17]. The system consists 50 ml extraction vessel, a high-pressure pump, an automated back pressure regulator and an oven. Liquid CO_2 was supplied from a gas cylinder.

The extraction was conducted using CO_2 flow rate of 2, 3 and 4 ml/min and the samples were taken for every 10 minutes. The pressure and temperature were fixed at 30 MPa and 40°C. At these conditions, the solvent is at highest density which is 0.919 g/ml.

2.3 DPPH Radical Scavenging Activity Assay

The assay of antioxidant activity was done using 2,2-diphenyl-1-picryl hydrazyl (DPPH) methanolic solution [18]. In this method, 77 µl of 2.5 mg/ml extracts and positive control (BHT) in methanol were added into 3 ml of methanolic DPPH solution. The mixture was vortex at room temperature for 30 seconds. The control sample absorbance ($A_{control}$) which contains methanolic solution of DPPH was also measured. All of the mixtures were allowed to stand in a dark place for 30 minutes. The absorbance of all sample solutions was measured at 517 nm using UV-Vis spectrophotometer. The percentage of scavenging effect was calculated using Equation (1).

$$\% \text{ scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

2.4 TPC Determination

Total phenolic content (TPC) in the extracts was determined using Folin-Ciocalteu (FC) reagent [19]. Twenty µl of 1 mg/ml plant extract, 1.58 ml of distilled water and 100 µl of FC reagent (diluted ten-fold) were mixed in a test tube. The mixture was left at room temperature in order to allow the reaction take place. After 7 minutes, 300 µl of 75 g/l sodium carbonate (Na_2CO_3) solution was added to the sample solution. The tube was kept in a dark place for 30 minutes at room temperature. The absorbance of the colour changes was measured at 765 nm. The calculation of TPC was done on the basis of the gallic acid standard curve which constructs using the same procedure and at concentrations of 0, 50, 100, 150, 250 and 500 mg/ ml. The results were expressed as gallic acid equivalents (mg GAE/g extract sample).

3.0 RESULTS AND DISCUSSION

3.1 Effects of CO_2 Flow Rate and Extraction Time on the Extraction Yield

Figure 1 shows the effect of CO_2 flow rate at respective extraction time at pressure of 30 MPa and temperature of 40°C. The accumulative yield of the extract at CO_2 flow rate of 2 ml/min showed the highest value (0.37%) whereas the lowest percentage yield was given by 4 ml/min (0.29%).

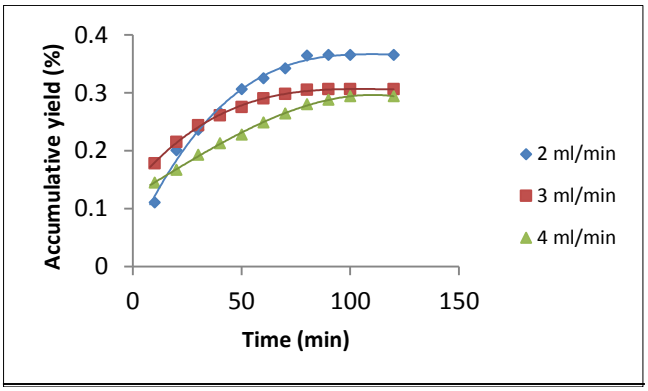


Figure 1 The effects of CO_2 flow rate and extraction time on the accumulative yield (%) of the extracts at P = 30 MPa and T = 40°C

At extraction time of 2 hours, the solute-solvent saturation was achieved. When the saturation occurs, the solubility is increasing as the residence time increased. Besides, extracting seeds with high oil content is best when lower solvent flow rate is used in order to hinder compaction of the sample in the vessel that may obstruct complete extraction of the oil [20]. In addition, when the flow rate of solvent increases, it enhance the cumulative yield of extract, but further increase of flow rate decrease the yield [21].

In order to determine the optimum extraction time, the experiment was done until the constant yield was obtained. Figure 1 shows that after 80 minutes, the accumulative yield was performed to be constant. Thus, the extraction time of 80 minutes was chosen to be optimum parameter due to less CO_2 consumption was utilized. Besides, time has no significant effect on extraction yield after maximum conversion was obtained [22].

3.2 Total Phenolic Content

Total phenolic content (TPC) of extracts were measured to investigate its contribution in antioxidant activity and to support the choice of parameters in term of solvent flow rate and extraction time. The phenol content in the extracts at 2, 3 and 4 ml/min was found to be 203.54 ± 10.56, 186.14 ± 7.46 and 193.61 ±9.88 mg

GAE/g sample, respectively as shown in Table 1. Amount of phenol content when using CO_2 flow rate of 2 ml/min suggested that higher solubility of the sample in the solvent. When the solubility is high, the amount of desired compound extracted is increased and it can prevent impurities to be extracted.

Table 1 Total phenolic content shows at different CO_2 flow rate at P = 30 MPa and T = 40°C

CO_2 flow rate (ml/min)	Total phenolic content (mg GAE/g sample)±SD
2	203.54 ± 10.56
3	186.14 ± 7.46
4	193.61 ± 9.88

SD: Standard deviation

3.3 Antioxidant activity of extract

The antioxidant activity assay was carried out to investigate the ability of *Q.infectoria* to scavenge free radicals in vitro by increasing the scavenging activity. Based on Figure 2, it shows that all of the extract obtained at the specified CO_2 flow rate give higher activity (%) than butylated hydroxyanisole (positive control) but the difference was insignificant (P>0.05). These results may explained by the fact that these extract enriched with phenolic compounds and comparable to positive control, which always play an important role in the antioxidant activity of the plant [23].

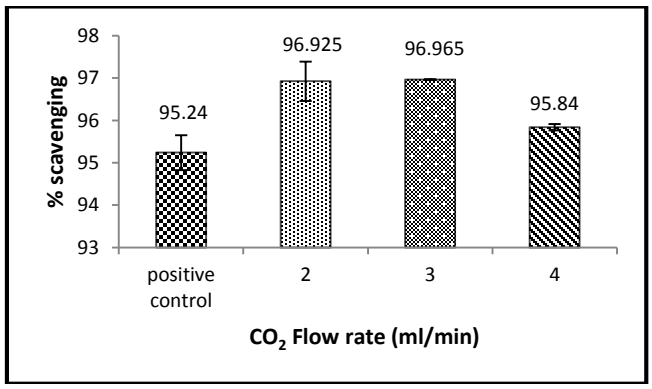


Figure 2 The effect of CO_2 flow rate on the DPPH radical scavenging activity compared to positive control (BHT) at P = 30 MPa and T = 40°C

Figure 2 clearly demonstrate that the highest antioxidant activity was attained at CO_2 flow rate of 3 ml/min (96.965%) but the difference between flow rate of 3 ml/min and 2 ml/min is too low (0.04%). Hence, 2 ml/min of CO_2 flow rate is more favourable to be used as optimum extraction parameter due to positive results obtained from the analysis as well as low cost since less CO_2 was used.

4.0 CONCLUSION

Based on the yields of the *Q.infectoria* extracts using SC-CO_2 extraction at 30 MPa and 40°C, the highest yield was at CO_2 flow rate of 2 ml/min (0.37%) and the extraction time of 80 minutes. The total phenolic compound at the CO_2 flow rate is 2.04×10^2 mg GAE/g sample. All of the extracts posses high antioxidant activity and could act as natural antioxidants for human body.

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