Jurnal Teknologi

Full paper

Effect of Supercritical Carbon Dioxide Flow Rate on Extraction Yield, Antioxidant Properties and Morphological Changes of *Quercus infectoria* Galls

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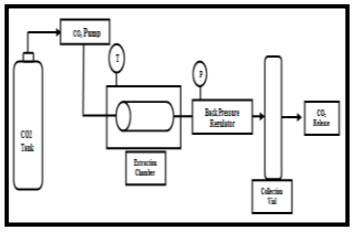
Article history

Received :4 October 2013
Received in revised form :
24 April 2014
Accepted :8 May 2014

Graphical abstract



Abstract

The extraction condition of supercritical carbon dioxide (SC-CO_2) extraction was used to extract *Quercus infectoria* galls, a medicinal plant which rich with bioactive compound, in order to maintain the green environment as well as the quality of the product. The study was performed to investigate the effect of extraction parameter (CO_2 flow rate) on *Quercus infectoria* galls extract using SC-CO_2 extraction. Then, the extract was analysed to determine their antioxidant activity and morphological changes of the *Quercus infectoria* galls before and after the extraction. Hence, three different CO_2 flow rate have been investigated which were 2, 3, and 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 showed the solvent flow rate of 2 mL/min give the highest percentage of extraction yield which is 0.37% compared to others. The extracts were screened for possible antioxidant activity by antioxidant activity 2,2-diphenyl-1-picryl hydrazyl (DPPH) assays. In this study, the best result obtained was at flow rate of 3 mL/min with inhibition percentage of 96.97% but it showed insignificant difference with other CO_2 flow rates. The change in morphology of the galls was significant when observed using scanning electron microscope (SEM). These results indicated that SC-CO_2 extraction could be an alternative method for extraction of antioxidative compound from *Q.infectoria* galls.

Keywords: *Quercus infectoria*; SC-CO_2 extraction; extraction yield; antioxidant activity; morphological changes

1.0 INTRODUCTION

In recent years, there has been increasing demand for functional ingredients obtained via natural processes as consumers are getting more interested in functional foods. New leads in pharmaceutical advances have always been served by natural product substances. In the early 20th century, medicines were mostly made from roots, barks and leaves as fluid extracts were in trend [1]. Besides, the reliance of early human evolution on medicinal plants and herbs for the use of curing the sick was well documented in the history of Egyptians, Chinese and Romans. In advance, the researchers would keep on using the medicinal herb and these should be incorporated into modern medicine [2].

Quercus infectoria (Q.infectoria) is a type of medicinal plant that can be used in treating diseases and it is already well-known since ancient times. Oak (*Quercus*) is part of Fagaceae family. The growth of the galls are stimulated by the reaction between plant hormones and powerful chemicals that regulate growth produced by an insect; known as *Cynips quercufolii* which lays its eggs in the bud in order to begin its life cycle [3-4]. As a consequence, the inner cells of the bud become big. During this time all starch will be converted into tannins, resulting in the galls containing tannins 60% from its total weight [5-6]. Besides, according to literature [7-8], the galls restrain tannin (50-70%) and small amount of free gallic acid and ellagic acid. Early studies shows *Q.infectoria* has been traditionally used after childbirth to strengthen the mother's womb. In addition, the galls of *Q.infectoria* are proven to possess antibacterial [9], larvicidal [10], anti-inflammatory [11], and antioxidant [12] properties.

Supercritical fluid extraction (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 since the system operates at low temperatures. Furthermore, supercritical fluids have higher diffusivity and lower density, surface tension and viscosity which

can be varied by changing the operating conditions, subsequently can give advantages to the extraction process. Besides, the absence of light and oxygen can prevent oxidation reactions and the product materials from SFE do not need to be further sterilized since bacteria present are already inactivated at mild temperature and high pressure gradient during pressure release, resulting longer shelf life of the extracts [13-14]. CO_2 is commonly used as the solvent due to its moderate critical conditions, nontoxic and easily removed from the products.

To date, less attention is given to the effect of solvent flow rate, antioxidant properties and morphological changes of the *Quercus infectoria* galls compared to other traditional plants such as tamarind seeds [15], pigeonpea leaves [16], and *Salvia miltiorrhiza* Bunge [17]. Hence, a study has been conducted to investigate the effect of supercritical carbon dioxide flow rate on extraction yield, antioxidant properties and morphological changes of *Q.infectoria* galls.

■2.0 MATERIAL AND METHODS

2.1 Raw Material

The galls of *Q.infectoria*, purchased from a local market (Kota Tinggi, Johor Malaysia), were first separated, rinsed using tap water to remove unwanted material and dried in an oven at 50℃ overnight. The galls were crushed with a grinder before analysis was done. The prepared seeds were stored in a dark place at room temperature.

2.2 Supercritical Carbon Dioxide (SC-CO_2) Extraction

The system consists of: 50 ml extraction vessel, high-pressure pump, automated back pressure regulator and oven as extraction chamber. Liquid CO_2 (99.9% purity; Kras Instrument, Johor Malaysia) was supplied from a gas cylinder (Figure 1). Extraction was conducted using CO_2 flow rate of 2, 3 and 4 mL/min and the fraction was taken every 10 minutes for 2 hours. In order to determine the effect of solvent flow rate, the pressure and temperature were fixed at 30 MPa and 40°C where at this condition, the solvent is at its highest density which is 0.919 g/mL.

The yield of the extract was calculated by using the following equation:

$$Extraction\ yield\ (\%) = m_1/m_o \times 100\% \qquad (1)$$

where m_1 is the mass of the extract in gram and m_0 is the mass of sample in gram.

2.3 Antioxidant Activity Assay

This assay was done according to previous work [18] with slight modifications as follows; 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 s. The control sample absorbance ($A_{control}$) which contains methanolic solution of DPPH was also carried out. 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 antioxidant activity was calculated using the following equation:

$$\%\ antioxidant\ activity = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\ \% \qquad (2)$$

2.4 Scanning Electron Microscopy

In order to clarify the effect of different solvent flow rate on gall's morphology, the dried gall's surface before and after SC-CO_2 extraction was examined with scanning electron microscopy (Tescan 3 Vega SEM-EDS) under high vacuum conditions and at accelerated voltage of 15 kV.

2.5 Statistical Analysis

Results are expressed as the mean ± *S.D.* of duplicate independent experiments. Data was analyzed using SPSS 16.00 for windows (SPSS Inc., Chicago, IL). The significance differences between the data were analyzed by using one-way analysis of variance (ANOVA) at 95% confidence level. P values < 0.05 were considered to be significant.

■3.0 RESULTS AND DISCUSSION

3.1 Effects of CO_2 Flow Rate on Extraction Yield

Figure 2 showed the plot of the effect of CO_2 flow rate on the extraction yield as a function of extraction time at operating pressure and temperature of 30 MPa and 40°C, respectively. The result shows the amount of yield extracted has significantly decreased as the CO_2 flow rate increased. The percentage yield of the extract at 2 mL/min showed the highest value compared to others which was 0.37% whereas the lowest percentage yield was given by 4 ml/min (0.29%). These results can be explained by when the low flow rate was implied, the solute-solvent saturation can be achieved [19], and therefore the solute solubility is increasing as the residence time per unit weight of solvent increased. In addition, more favourable diffusive behaviour through the galls' matrix can be found when using lower CO_2 flow rate [20]. Theoretically, when the flow rate of solvent increases, it will enhance the cumulative yield of extract, but further increase of flow rate will decrease the yield [21].

Similar result was observed when extracting amarath seed oil using supercritical carbon dioxide [22]. As the flow rate increased from 0.2 to 1.0 kg h^{-1}, the extraction rate was decreasing, per unit of solvent passed. The maximum yield was attained earlier for lower flow rate due to increase in contact time of solvent and matrix of seeds. However, the contradictive result was found by Machmudah *et al.*, where the amount of the total extract and astaxanthin extracted from *Haematococcus pluvialis* using supercritical carbon dioxide increased with increasing of CO_2 flow rate [23]. This condition happen due to the fact that the intraparticle diffusion resistance was dominant and mass transfer was highly influenced by increasing CO_2 flow rate [23].

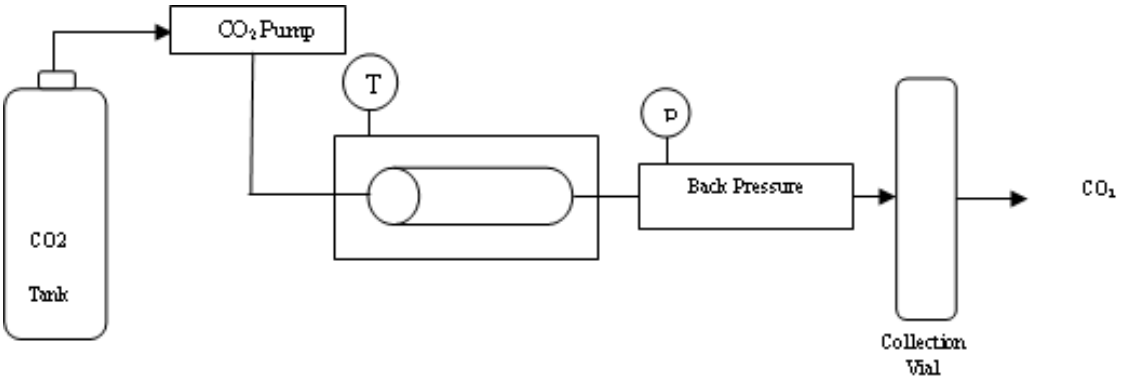


Figure 1 Schematic flow diagram of supercritical fluid extraction apparatus

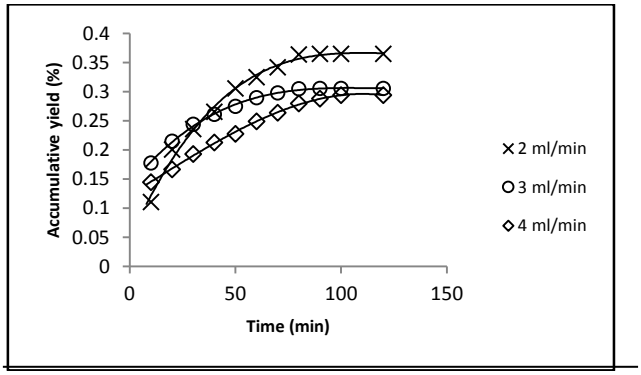


Figure 2 The effect of solvent flow rate on accumulative yield (%) of the extracts at 30 MPa and 40°C

3.2 Antioxidant Properties

Referring to Table 1, it shows that all of the extract using various CO_2 flow rate give significantly higher activity (%) than BHT (positive control) since $p > 0.05$. These results may be described by the fact that these extract are enriched with phenolic compounds which always play an important role in the antioxidant activity of the plant [24]. The figure clearly shows that the highest antioxidant activity was attained by using 3 mL/min (96.965%) but the difference between of the activity is low when comparing these flow rates. According to the preceding finding [22], CO_2 flow rate does not give any significant result in astaxanthin content as the various flow rates shows almost same contents. Hence, low CO_2 flow rate is can be considered to be more favourable flow rate due to better percentage of extraction yield and antioxidant activity of SC-CO_2 extracts as well as low cost since less CO_2 was used.

Table 1 The effect of CO_2 flow rate on the antioxidant activity compared to positive control (BHT)

CO_2 flow rate (mL/min)	Antioxidant activity ± SD (%)
2	96.93 ± 0.93[a]
3	96.97 ± 0.01[a]
4	95.84 ± 0.16[a]
Positive control	90.74 ± 2.49

SD: standard deviation; [a] $p<0.05$ compared with control.

3.3 Changes in Morphology of the Galls

Morphological changes of *Q.infectoria* galls which were analyzed using Tescan 3 Vega SEM–EDS before and after SC-CO_2 extraction were shown in Figure 3. Basically, all *Q.infectoria* samples showed unstructured, porous and not smooth. A few slight rupture was observed for the SEM image before extraction of the galls (Figure 3(a)) may be due to the grinding procedure during pre-treatment process. In addition, the significant interconnection also formed between the fibril structures of the galls. However, the galls formed a compact structure after the extraction process. The compaction of the samples become obvious as the solvent flow rate increases from 2 to 4 mL/min as shown in Figure 3(b–d). This observation suggests when using high solvent flow rate, it will caused compaction in the SFE vessel, hence may obstruct complete extraction of the oil [21].

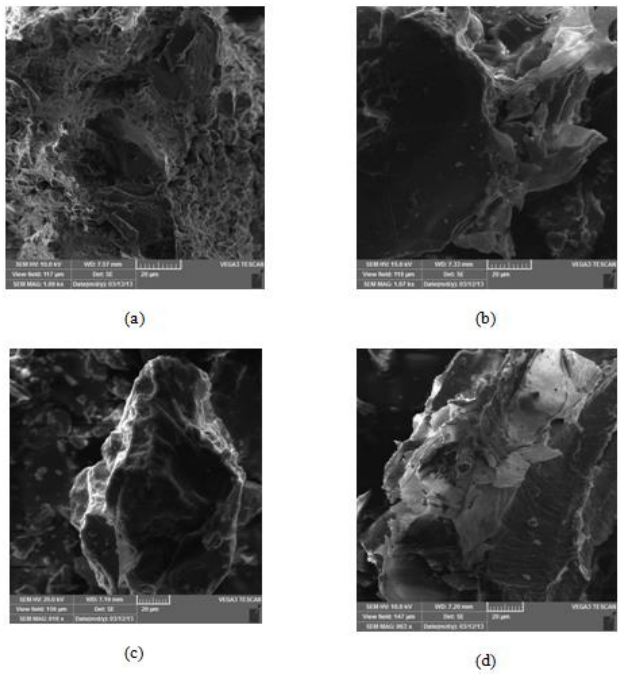


Figure 3 Scanning electron micrographs for *Quercus infectoria* galls a) before and after the supercritical fluid extraction at CO_2 flow rate of b) 2 ml/min, c) 3 ml/min, d) 4 ml/min

4.0 CONCLUSION

Based on the yields of the *Q.infectoria* extracts using SC-CO_2 extraction, the highest yield was at 2 mL/min (0.37%) followed by 3 and 4 mL/min. However, the CO_2 flow rate does not give significant effect on antioxidant activity and all of the extracts using different flow rates showed high scavenging effect compared to positive control. Changes in morphological of the galls were significant when analyzed using scanning electron microscope. Therefore, it is recommended to use low flow rate of carbon dioxide to extract biological active compounds from *Quercus infectoria* galls.

Acknowledgement

The authors gratefully acknowledge the financial supports from Ministry of Higher Education (MOHE) and Universiti Teknologi Malaysia for research grant during this study.

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