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Total Phenolic Content and Antioxidant Activity of *Quercus infectoria* Galls Using Supercritical CO_2 Extraction Technique and Its Comparison with Soxhlet Extraction

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ABSTRACT

Quercus infectoria gall, which is known as manjakani in Malaysia, was traditionally used in treating diseases. The bioactive compounds from the galls can be extracted using various extraction methods. In this study, supercritical carbon dioxide (SC-CO_2) extraction was used to study the effects of CO_2 flow rate on the yield, total phenolic content and antioxidant activity of *Q. infectoria* extract by fixing the pressure and temperature at the highest density (P: 30 MPa, T: 40°C). The results were compared with those acquired from the Soxhlet extraction method. The results showed that the Soxhlet extraction had a higher percentage of extraction yield than SC-CO_2 extraction. The selectivity of *Q. infectoria* extracts using SC-CO_2 extraction was better than the Soxhlet extraction method. Meanwhile, the extraction efficiency using the SC-CO_2 extraction ranged from 46% to 53%. The SC-CO_2 extraction also yielded higher total phenolic content than using the Soxhlet extraction method when 2 mL/min of CO_2 flow rate was applied (203.53 mg GA/g sample). This study also revealed that the extracts from the SC-CO_2 extraction showed a better radical scavenging activity compared to the Soxhlet extraction when analysed using DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity assays.

Keywords: *Quercus infectoria*, radical scavenging activity, Soxhlet extraction, supercritical carbon dioxide extraction, radical scavenging activity, total phenolic content

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INTRODUCTION

Quercus infectoria, which is known as Manjakani in Malaysia, is a small tree native to Greece, Asia Minor and Iran (Basri & Fan, 2005). The attack by the gall-wasp *Adleria gallae-tinctoria* results in the galls arising on the young branches of this tree. The galls of *Q. infectoria* are usually used in herbal drink as a remedy for women after their childbirth and to restore the elasticity of the uterine wall. Besides, these galls are also traditionally used as dental powder and in the treatment of toothache and gingivitis. Inflammatory disease has traditionally been cured by using *Q. infectoria* extract. The potentials of *Q. infectoria* in medical and nutraceuticals areas as reported by Kaur *et al*. (2004) have encouraged other researchers to further study and investigate the usage and application of the galls of *Q. infectoria*.

In the galls of *Q. infectoria*, the bioactive compounds that have medicinal properties are tannin (50-70%), small amount of gallic acid, and ellagic acid (Wiart & Kumar, 2001). There are other constituents such as syringic acid, β-sitosterol, amentoflavone, hexamethyl ether, isocryptometrin, methyl betulate, methyl oleanate, and hexagalloyglucose (Hwang *et al*., 2000). Tannin, which is derived from phenolic compounds, has been found to have antioxidant activity and antimicrobial (Everest & Ozturk, 2005), antibacterial (Hamid *et al*., 2005), and antifungal (Yamunarani *et al*., 2005) properties.

Recently, supercritical fluid extraction has gained much interest as an alternative way to replace the conventional methods for the extraction of phytochemicals from plant matrix. Theoretically, Pourmortazavi and Hajimirsadeghi (2007) have reported that supercritical fluid extraction, which operates based on the utilisation of a fluid under supercritical conditions, is a technology suitable for extraction and purification of a variety of compounds especially those that have low volatility or that are susceptible to thermal degradation. This is because supercritical fluid has gas-like characteristics that help the fluid diffuse to the matrix and access the phytochemicals, while its liquid-like characteristics provide good salvation power. Furthermore, diffusivity, density, surface tension and viscosity of supercritical fluids can be varied by altering the operating conditions. Therefore, these properties of supercritical fluids can give advantages in controlling the extraction process. Additionally, using supercritical carbon dioxide (SC-CO_2) as a solvent gives advantages to the extraction process due to non-toxic and non-flammable characteristics of CO_2. This method is inexpensive and can be used under mild operating conditions, namely 7.4 MPa critical pressure and 31°C temperature of CO_2.

This study investigated the effects of CO_2 flow rate on the yield, total phenolic content, and antioxidant activity of *Q. infectoria* extract. Then, all the properties were compared with those acquired from the Soxhlet extraction method.

MATERIALS AND METHODS

Preparation of Material

The *Q. infectoria* galls were prepared by rinsing the galls with tap water in order to remove unwanted material from the samples. Then, the galls were subsequently dried overnight in an oven at 50°C. Before the extraction was done, the galls were crushed by using a mechanical mortar. The prepared galls were stored in a dark place at room temperature.

Supercritical Carbon Dioxide (SC-CO_2) Extraction

SC-CO_2 extraction was performed using the method proposed by Mandana *et al*. (2011a) with some modifications. The SC-CO_2 system comprised a 50-mL extraction vessel, a high-pressure pump, an automated back pressure regulator and an oven. CO_2 in liquid form was supplied from a gas cylinder. 15.00 ± 0.05 g of *Q. infectoria* was extracted using CO_2 flow rate of 2, 3, and 4 mL/min. The fractionation was done for every 10 min time interval of 2 h. The pressure and temperature were fixed at the highest possible density, which was 0.92 g/mL with the pressure of 30 MPa and the temperature of 40°C in order to determine the effect of CO_2 flow rate for the extraction process.

Soxhlet Extraction Method

Soxhlet extraction was carried out to compare the extraction performances with the SC-CO_2 extraction. In order to prepare the sample extract, 5.00 ± 0.05 g of powdered *Q. infectoria* galls was inserted in the thimble, while 150 mL of 100% methanol (boiling point, bp = 64.7°C) was placed in the flask of Soxhlet apparatus. The temperature of the process was corresponded to the boiling point of solvent used and the extraction time was set for 6 h. At the end of the process, the solvent was removed from the yield by using a rotary evaporator at 40°C. All the steps were repeated by using 100% ethanol (b_p = 78.37°C), acetone (b_p = 56.0°C) and water (b_p = 100°C) as the extraction solvents.

Yield Calculation

The yield of the extract defined on 1 g of *Q. infectoria* galls basis was calculated by using the following equation:

$$\text{Percentage extract yield} = \frac{m_1}{m_0} \times 100\% \qquad [1]$$

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

Total Phenolic Content Analysis

Total phenolic content (TPC) in the *Q. infectoria* extracts was analysed by using Folin-Ciocalteu (FC) reagent. The solution was prepared by mixing 20 µL of 1 mg/mL plant extract, 1.58 mL of distilled water, and 100 µL of FC reagent (diluted ten-fold) thoroughly in a test tube. The solution was left at room temperature for 7 min in order for the reaction to take place. Then, 300 µL of 75 g/L sodium carbonate (Na_2CO_3) solution was added into the sample solution and the tube was kept in a dark place for 30 min at room temperature. The absorbance of the solution was measured at 765 nm. The calculation of TPC was done on the basis of the gallic acid standard curve, which was constructed by using the same procedures and concentrations of 0, 50, 100, 150, 250, and 500 mg/mL. The results were expressed as gallic acid equivalents (mg GAE/g extract sample).

Antioxidant Activity Assay

Assay for antioxidant activity of the extract was done by dissolving 77 µL of 2.5 mg/mL extracts in 3 mL of 6×10^{-5} M methanolic DPPH solution. DPPH or 2,2-diphenyl-1-picrylhydrazyl is a stable free radical that forms a purple-coloured solution when dissolved in methanol. Antioxidant components can scavenge this stable free radical and therefore the purple colour will be bleached. The mixture was vortexed at room temperature for 30 s. The control sample absorbance ($A_{control}$), which contained methanolic solution of DPPH, was also carried out. All of the mixtures were placed in a dark place for 30 min at room temperature. The absorbance of all the sample solutions (A_{sample}) was measured at 517 nm using UV-Vis spectrophotometer. Antioxidant activity was calculated by using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \qquad [2]$$

Statistical Analysis

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

RESULTS AND DISCUSSION

Comparison of Extraction Yield from Soxhlet Extraction and Supercritical Carbon Dioxide Extraction

In order to get the percentage yield of *Q. infectoria* extracts using SFE, a set of extraction experiments was done at 30 MPa and 40°C, while the flow rate of CO_2 varied from 2 to 4 mL/min. Table 1 shows the effects of CO_2 flow rate on the extraction yield of the *Q. infectoria* extract.

TABLE 1 : Comparison of percentage yield of Q. infectoria by using supercritical CO_2 extraction

Type of plant	CO_2 Flow Rate (mL/min)		
	2	3	4
Q. infectoria	0.3652*	0.3060	0.2940

*Extraction yield expressed as % dry weight.

The percentage yield of the *Q. infectoria* extract at 2 mL/min (0.37%) is the highest value compared to others, whereas the lowest percentage yield is 4 mL/min (0.29%). This finding revealed that the solute-solvent saturation was achieved when lower flow rate was applied (Ana Najwa, 2008). When this happened, the increase in residence time would increase the solubility of the solute in the solvent. Moreover, King (1997) mentioned that extracting seeds with high oil content is best when a low solvent flow rate is used in order to hinder compaction of the

sample in the vessel that may obstruct complete extraction of the oil. In addition, Kumoro and Hasan (2007) stated that the cumulative yield of the extract is improved as the flow rate of the solvent increases.

The results obtained by using the Soxhlet extraction method are shown in Fig.1. Generally, each solvent used gave high percentage yield in the range between 45.71% and 80.03%. Based on the findings, the use of 100% aqueous as a solvent showed the highest percentage yield for the extraction of *Q. infectoria* by using the Soxhlet extraction method. On the other hand, the lowest extraction yield for *Q. infectoria* was found when 100% acetone was used, suggesting that polar compounds in biological plant are easier to extract with more polar solvents, while the less polar solvent allows the extraction of the less polar compounds (Mandana *et al*., 2011b). This is based on the theory of 'likes dissolve likes'. The results showed that pure aqueous gave higher yield compared to others because of the higher polarity of the solvent. The preceding findings were opposite to the results by Wang *et al*. (2011) who found that pomegrated peel was extracted effectively using methanol, followed by water, ethanol, and acetone. This contrary finding might be caused by the different characteristics of the desired compound, extraction method and the origin of the raw material used (Caldera *et al*., 2012).

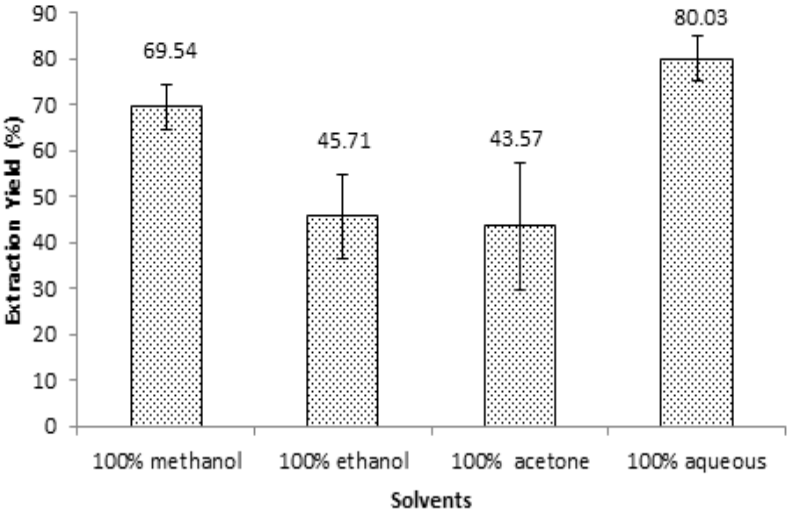

Fig.1: Percentage yield of Q. infectoria by using the Soxhlet extraction method

From these results, the performance of SC-CO_2 extraction was low as it obviously showed that the yield obtained from the Soxhlet extraction was higher than the SC-CO_2 extraction with $P<0.05$. Nevertheless, the selectivity of the extracts using SC-CO_2 extraction offers considerable advantages such as clear colour of the extracts attained from the SC-CO_2 extraction process. This observation was due to the less impurity or organic matrices which were extracted during the process. However, the extracts of the Soxhlet method were dark in colour and highly turbid. Similar findings were found by Berglö *et al*. (1999) and Hawthorne *et al*. (2000). The performance of the SC-CO_2 extraction could also be explained by several analyses done on the *Q. infectoria* extract, which were total phenolic content and antioxidant analysis.

Total Phenolic Content

Total phenolic content (TPC), as determined by using the Folin Ciocalteu method, was reported as gallic acid equivalents (mg GAE/g sample). This analysis was used to examine its contribution in the antioxidant activity of the plant extracts. The total phenolic content of the extract is shown in Table 2.

TABLE 2 : Total Phenolic Content of *Q. infectoria* for different extraction methods

Extraction Method	Total Phenolic Content ± SD (mg GA/g sample)
SC-CO_2 extraction	
Flow rate: 2 ml/min	203.53 ± 10.56[a]
Flow rate: 3 ml/min	186.13 ± 7.46[a]
Flow rate: 4 ml/min	193.60 ± 9.88[a]
Soxhlet extraction	
100% methanol	95.86 ± 2.02[b]
100% ethanol	109.78 ± 6.57[b]
100% acetone	107.64 ± 0.50[b]
100% aqueous	95.86 ± 1.01[b]

SD: standard deviation

a,b shows significantly different ($P<0.05$)

The total phenolic content in the extracts when 2, 3, and 4 mL/min of CO_2 flow rate were used was found out to be 203.53, 186.13, and 193.60 mg GAE/g sample, respectively. The CO_2 flow rate of 2 mL/min showed the highest phenolic content, suggesting the highest solubility of the sample in the solvent. The solvent can easily access the solute when the solubility is high; hence, the amount of the desired compound extracted increases and the extraction of impurities can be avoided.

As for the Soxhlet extraction method, the best content of phenolic compound was found in 100% ethanol extract (109.78 mg GAE/g sample), but the difference with the other solvents was shown to be insignificant ($P>0.05$). These findings clarified that the amount of yield extracted did not affect the content of phenolic compound in the extract.

Based on the findings, the SC-CO_2 extraction gave significantly higher total phenolic content ($P<0.05$) in the *Q. infectoria* extract as the efficiency of SC-CO_2 extraction ranged from 46% to 53% compared to the extracts of Soxhlet extraction method. Basically, a higher amount of phenolic compound is useful for the prevention of oxidative activities of the plant's extract.

DPPH Radical Scavenging Activity of Q. infectoria

The DPPH radical scavenging activity analysis was accomplished to investigate the ability of *Q. infectoria* to scavenge free radicals in vitro by improving the percentage of the scavenging activity. For the extraction using SC-CO_2, all of the extracts using various CO_2 flow rates gave significantly high activity, as established in Table 3. The table clearly shows that the highest

DPPH radical scavenging activity was obtained by using 3 mL/min (96.96%), but the difference with the other CO_2 flow rate was very low (P>0.05). The extracts from the Soxhlet extraction showed that the water extract (94.55%) gave the highest antioxidant activity, but with a slight or no significant difference with the others (P>0.05). These findings revealed that the types of solvent used in the Soxhlet extraction method did not give significant effect on DPPH radical scavenging activity of the extracts. In addition, the results from these two extraction methods could be explained by the fact that the extract is rich in phenolic compounds which always play an important role in the radical scavenging activity of the plant (Poumorad *et al*., 2006).

TABLE 3: DPPH radical scavenging activity of *Q. infectoria* for different extraction methods

Extraction Method	Antioxidant activity ± SD (%)
SC-CO_2 extraction	
Flow rate 2 mL/min	96.93 ± 0.92[a]
Flow rate 3 mL/min	96.96 ± 0.01[a]
Flow rate 4 mL/min	95.84 ± 0.15[a,b]
Soxhlet extraction	
100% methanol	93.38 ± 0.18[b]
100% ethanol	92.60 ± 1.28[b]
100% acetone	92.83 ± 1.61[b]
100% aqueous	94.55 ± 0.37[a,b]

SD: standard deviation

a,b shows significantly different (P<0.05)

CONCLUSION

In conclusion, the *Q. infectoria* extracts from supercritical carbon dioxide extraction undoubtedly gave better total phenolic content and DPPH radical scavenging activity compared to the Soxhlet extraction method. The findings showed that there was a significant difference between these two extraction methods even though the Soxhlet extraction showed better result in terms of extraction yield. Furthermore, supercritical carbon dioxide extraction was found to be more efficient than the Soxhlet extraction method as the supercritical carbon dioxide extraction took lesser time consumption and amount of solvent. Thus, supercritical carbon dioxide extraction is more suitable for extracting food material than the Soxhlet extraction method.

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