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COMPARISON OF SUPERCRITICAL CO_2 EXTRACTION AND SOXHLET EXTRACTION OF BIOACTIVE COMPOUND FROM *QUERCUS INFECTORIA*

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ABSTRACT

Quercus infectoria also known as manjakani, can be used in treating diseases and it has been traditionally used after childbirth to strengthen the mother's womb. The bioactive compound in *Q.infectoria* can be extracted using various extraction methods. Supercritical fluid extraction, which 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. 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. Supercritical carbon dioxide (SC-CO_2) extraction was conducted to study the effect of CO_2 flow rate on the yield, total phenolic content and antioxidant activity of *Quercus infectoria* extract by fixing the pressure and temperature at highest density (P: 30 MPa, T: 40^{o}C). The results were compared with those acquired from Soxhlet extraction method. The results from SC-CO_2 extraction showed that the increases of CO_2 flow rate will reduce the extraction yield. The selectivity of *Q.infectoria* extracts using SC-CO_2 extraction is better which in contrast with Soxhlet extraction method since it shows higher total phenolic content (2.04×10^2 mg GA/g sample). This study also revealed that the extracts from both extraction methods can posses antioxidant activity when analyzed by DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical scavenging activity assays.

Keywords: *Antioxidant activity, extraction method, Q.infectoria, SC-CO_2, Soxhlet, total phenolic content.*

INTRODUCTION

Quercus infectoria (manjakani) plant, known as oak tree, is a small tree native to Greece, Asia Minor and Iran [1]. The attack by the gall-wasp *Adleria gallae-tinctoria* results in the galls arises on young branches of this tree. In Malaysia, the galls are used as herbal drink to remedy the women after their childbirth to restore the elasticity of the uterine wall. While in India, it is well known as *Majuphal* and often used as dental powder and in the treatment of toothache and gingivitis. In Asian, it has traditionally been used for centuries for treating inflammatory disease [2]. In addition, by using the hot water extract of *Q.infectoria* galls as a mouth antiseptic, it can control the inflammation of tonsils, while the direct application of it onto the skin cures swelling or inflammation [3]. Hemorrhoids caused by inflammation of the skin can also be treated by applying the powdered *Q.infectoria* in the form of ointment to the skin. The potential of *Q.infectoria* in medical and nutraceutical areas have induced the researcher to study and investigate the further details about its usage and application.

Q.infectoria is greatly used as medicinal plant since ancient time because it was reported contains large amount of bioactive constituents such as tannins, gallic acid, syringic acid, ellagic acid, β-sitosterol, amentoflavone, hexamethyl ether, isocryptometrin, methyl betulate, methyl oleanate, hexagalloyglucose and others [4,5,6]. The main constituents found in the galls of *Q.infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid [5,7]. Tannin which is derived from phenolic compounds has been reported to

have antioxidant activity and has the ability to be antimicrobial [8], antibacterial [9] and the antifungal agent [10].

Recently, extraction of phytochemicals from plant matrix with supercritical fluid extraction has gain many interests as it is priority in finding alternative way to replace conventional extraction methods [11]. Theoretically, supercritical fluid extraction 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. It is widely used for the extraction of essential oil, metal cation extraction, and polymer synthesis and particle nucleation [12]. Furthermore, supercritical fluids have higher diffusivity and lower density, surface tension and viscosity which can be varied by altering the operating conditions, subsequently can give advantages to the extraction process.
A study had been performed in order to investigate the effect of CO_2 flow rate on the yield, total phenolic content and antioxidant activity of *Quercus infectoria* extract. Then, all of these properties were compared with those acquired from Soxhlet extraction method.

MATERIALS AND METHODS

The *Q.infectoria* galls were prepared by separating them first, and then the galls were rinsed with tap water in order to remove unwanted material from the samples. The galls were subsequently dried in an oven at 60℃ overnight. Before the extraction done, they were crushed by mechanical mortar. The prepared galls were stored in dark place at room temperature.

Supercritical carbon dioxide (SC-CO_2) extraction was performed using the method done by Mandana *et al.* [13] with some modifications. The SC-CO_2 system comprises of 50 ml extraction vessel, high-pressure pump, automated back pressure regulator and oven. Liquid CO_2 was supplied from a gas cylinder. The plant was extracted using CO_2 flow rate of 2, 3 and 4 ml/min and the fractionation was done for every 10 minutes time interval. In order to determine the effect of solvent flow rate, the pressure and temperature were fixed at highest possible density which is 0.92 g/ml with pressure of 30 MPa and temperature of 40^{o}C.

Soxhlet extraction was carried out to compare the extraction performances with SC-CO_2 extraction. To prepare the sample extract, 5.00±0.05 g of powdered Que*ercus infectoria* gall was inserted in the thimble while 150 ml of methanol (100 %) placed in the flask of soxhlet apparatus. The temperature of the process was corresponded to boiling point of solvent used and the extraction time was set for 6 hours. Lastly, the solvent was removed from the yield by using rotary evaporator at temperature of 40℃. All of the steps were repeated by using 70% methanol, 100% ethanol, 70% ethanol, water, hexane, petroleum ether and acetone as the extraction solvent. The yield of the extract was calculated by using following equation:

$$\text{Percentage extract yield} = \frac{m_1}{m_0} \times 100 \quad (1)$$

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

Total phenolic content (TPC) in *Q.infectoria* extracts was analyzed by using Folin-Ciocalteu (FC) reagent. The solution was prepared by mixed thoroughly 20 µl of 1 mg/ml plant extract, 1.58 ml of distilled water and 100 µl of FC reagent (diluted ten-fold) in a test tube. The solution was left at room temperature for 7 minutes in order for the reaction to take place. Then, 300 µl of 75 g/l sodium carbonate (Na_2CO_3) solution was added to the sample solution and the tube was kept in a dark place for 30 minutes 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 construct by using same procedure 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).

Assay for antioxidant activity of the extract was done by dissolving 77 µl of 2.5 mg/ml extracts in 3 ml of 6 x 10^{-5} M methanolic DPPH solution. DPPH or 2, 2-diphenyl-1-picrylhydrazyl is a stable free radical, which 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 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 placed in a dark place for 30 minutes at room temperature. The absorbance of all sample solutions was measured at 517 nm using UV-Vis spectrophotometer. Radical scavenging activity was calculated by using the following equation:

$$DPPH\ quenched\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where A_{blank} is the absorbance of the blank; A_{sample} is the absorbance of the sample.

RESULTS AND DISCUSSIONS

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

Figure 1 shows the results of percentage extraction yield for *Q.infectoria*. Generally, every solvent used give high percentage yield in the range between 45.71% and 76.34%. Based on the findings, the used of 70% methanol as a solvent shows the highest percentage yield for the extraction of *Q.infectoria* by using Soxhlet extraction method. On the other hand, the lowest extraction yield for *Q.infectoria* resulting from 100% acetone suggesting that polar compounds in biological plant is easier to extract with more polar solvents while the less polar solvent allow the extraction of the polar bioactive compounds. It is based on the theory of 'likes dissolve likes'. The results specify that the mixture of the organic solvents give higher extraction yield than the pure solvent while the pure solvent of methanol gives higher yield compare to ethanol because of the higher polarity of the solvents. The preceding findings was opposite with the result above where they found that pomegrated peel was extracted effectively using methanol followed water, ethanol and acetone [14]. This contrary finding might be caused by the different extraction method and different raw material used.

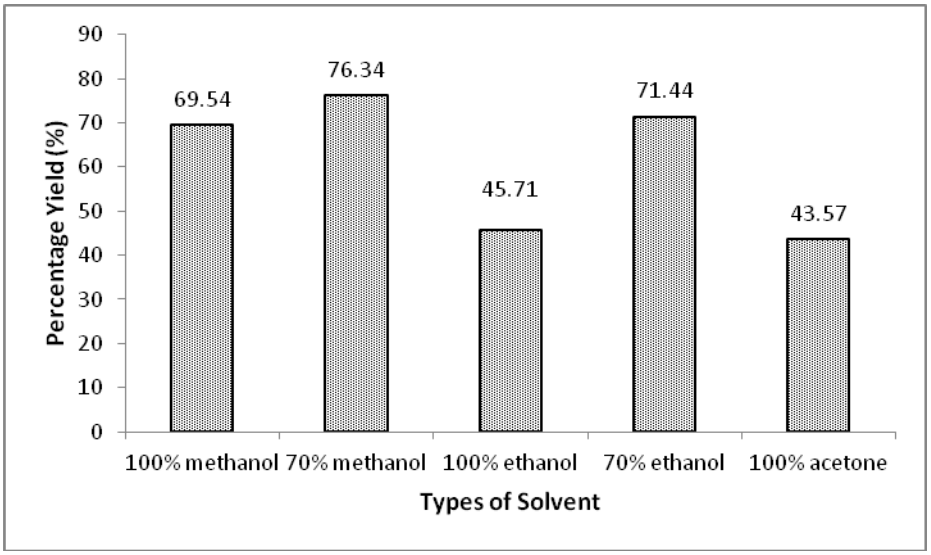

Fig. 1: Percentage yield of Q.infectoria by using Soxhlet extraction

The choice of suitable solvent to extract the interest compounds need to be considered thoroughly since the extracted compound will be depends on the type of solvent used [15]. The differences in solvents will defer the extracts and its composition since a polar solvent will favour polar compound more and vice versa. The used of methanol, ethanol and the mixture of them with water usually give high yield of the extracts. However, the commonly used solvents are ethanol and water due to their low toxicity and high extraction yield. Furthermore the polarity of these solvents can be altered by mixing the solvents at selected ratio [16]. This theory was proved by the finding where found that 70% solvent-water mixture show more effective isolation of phenolics compound from cherry liqueur pomace compared to the pure solvents [17].

In order to get the percentage yield of *Q.infectoria* extracts using SFE, a set of experiments was done at 30 MPa and 40^{o}C, while the flow rate of CO_2 was varied from 2 to 4 ml/min. Table 1 was constructed to show the effect of CO_2 flow rate on the extraction yield of the plant extract.

Table 1: Comparison of percentage yield of S.mahagoni and 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 shows the highest value compared to others which was 0.37% whereas the lowest percentage yield was given by 4 ml/min (0.29%). This result is obtained based on the solute-solvent saturation that has been achieved when lower flow rate was applied [18]. When this happen, the increased in residence time will increased the solubility of the solute in the solvent. Moreover, 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 [19]. Other than that, the cumulative yield of the extract was improved as the flow rate of solvent increases, but as further increase of flow rate will reduce the yield [20].

From these results, it obviously shows that the yield obtained from Soxhlet extraction higher than SC-CO_2 extraction. Nevertheless, the quality of the extracts using SC-CO_2 extraction was better since less impurity were extracted due to high selectivity of the process. These can be explained by clear colour attained from the extraction process for the plant matrix.

Total Phenolic Content

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

Table 2: Total Phenolic Content of Q.infectoria

	Total Phenolic Content of plant
Soxhlet extraction	
(100% methanol)	95.86 ± 2.02
(70% methanol)	112.28 ± 3.03
(100% ethanol)	109.78 ± 6.57
(70% ethanol)	99.43 ± 2.02
(100% acetone)	107.64 ± 0.50
SC-CO_2 extraction	
Flow rate 2 ml/min	203.53 ± 10.56
Flow rate 3 ml/min	186.13 ± 7.45
Flow rate 4 ml/min	193.60 ± 9.88

For Soxhlet extraction method, better content of phenolic compound was found in 70% methanol extract (112.28 mg GAE/g sample) compared to the other solvents and it is well-matched with the previous result since it has highest percentage yield. Other than that, the phenolic content in the extracts when using 2 ml/min, 3 ml/min and 4 ml/min of CO_2 flow rate was found out to be 203.53, 186.13 and 193.60 mg GAE/g sample correspondingly. CO_2 flow rate of 2 ml/min shows high phenolic content suggested that higher solubility of the sample in the solvent. When the solubility is high, the amount of desired compound extracted is increased and the extraction of impurities along can be avoided.

Basically, the extracts from both extraction methods contain high amount of phenolic compound and it is useful for the prevention of oxidative activities of the plant's extract.

Antioxidant Activity of Q.infectoria

The antioxidant activity test was accomplished to investigate the ability of *Q.infectoria* to scavenge free radicals in vitro by the improved of scavenging activity percentage. The extracts shows that 70% methanol (94.35%) and water extract (94.55%) gives the highest DPPH scavenging activity and with slight difference by

100% methanol (93.38%). Still, other solvents also indicated high free radical scavenging varies between 92.60% (100% ethanol) and 92.83% (100% acetone). For the extraction using SC-CO_2, it shows that all of the extract using various CO_2 flow rate give significantly high activity (%). These results may explained by the fact that these extract rich with phenolic compounds which always play an important role in the antioxidant activity of the plant [21]. The table clearly shows that the highest antioxidant activity was attained by using 3 ml/min (96.965%) but the difference between 3 ml/min and 2 ml/min is too low (0.04%). The radical scavenging activity of the extracts is related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability.

Table 3:Antioxidant activity of Q.infectoria

	Antioxidant activity of plant (DPPH radical scavenging %)
Soxhlet extraction	
(100% methanol)	93.38 ± 0.18
(70% methanol)	94.35 ± 0.64
(100% ethanol)	92.60 ± 1.28
(70% ethanol)	92.86 ± 0.18
(100% aceton)	92.83 ± 1.61
SC-CO_2 extraction	
Flow rate 2 ml/min	96.93± 0.92
Flow rate 3 ml/min	96.96 ± 0.01
Flow rate 4 ml/min	95.84 ± 0.15

The contradictive result with the results found by extracting *Q.infectoria* was documented where methanolic extracts gives higher reduction activity followed by ethanol, aqueous and acetone in the extraction of wild edible plant *Digera muricata* (L.) mart [22]. The differences are due to the capability of the solvent to extract the bioactive compounds is differ for different biological plant. Until now, the antioxidant activity of *Q.infectoria* galls using different types of solvent has not been well documented but based on the results obtain, the types of solvents does not gives significant differences toward the scavenging of free radicals.

CONCLUSIONS

The methanolic, ethanolic, acetone and supercritical fluid extracts of *Quercus infectoria* galls extract contained total phenolic compounds and were capable of inhibiting, quenching free radicals to inhibit the free radical chain reaction, and acting as reducing agents. In addition, a linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compound could be major contributors to antioxidant activity.

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