Preliminary Study on the Effects of Processing Parameters on Tannin Extracted from *Quercus infectoria* Galls

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Abstract
Preliminary phase is greatly essential to assess the possibilities, outcomes and patterns of the experimental data before proceeding to further stage. Processing parameters are the main crucial factors affecting the whole extraction system and they are extraction time, temperature, solvent to raw material ratio and many others. These factors play a significant role especially in the extraction of desired active compounds from its sources. Hence, the effects of processing parameters (solvent to raw material ration, extraction temperature and duration) on tannin extraction from *Quercus infectoria* galls are studied. Series of experiments were designed using One Factor at a Time (OFAT) involving three different parameters to be analysed. Extraction temperature was set at the range between 50°C to 100°C, extraction duration (60 min to 210 min) and solvent to raw material ratio (15:1 to 30:1) was analysed accordingly using aqueous extraction method. Active compound found abundantly in *Q. infectoria* galls; tannin was quantified using High Performance Liquid Chromatography (HPLC) while yield of extract and antioxidant activity present in the extract was examined thoroughly. Overall, this study mainly present the preliminary work involved in the extraction of tannin from *Q. infectoria* galls. Conclusively, processing parameters functions at its best condition at the temperature of 70°C, 120 min of extraction duration and solvent ratio of 1:21.

Keywords: *Quercus infectoria*, Tannin, Aqueous extraction, Antioxidant, Preliminary study

INTRODUCTION

Quercus infectoria Olivier (Family: Fagaceae) is a small tree being widely distributed in Greece, Asia and the Middle East. This tree comprises its own galls that emerge on its shoot as a result from the attack of gall wasp, *Cypnis gallae-tincotoriae* (Samuelson, 1992). The galls are commonly known as Manjakani ni Malaysia and is common in traditional medicines practices since decades ago. Locally, the galls are used with combination of other herbs as a drinking remedy by women after childbirth. This to ensure the uterine wall elasticity is restored. On the other hand, in India this medicinal plant is widely known as Majuphal which has been used extensively as dental powder and also in the treatment of toothache and gingivitis. Besides that, scientific literature has stated that gargling Q. infectoria galls extract with hot water can subsequently reduce inflammation of tonsils while direct usage of boiled galls on skin can effectively cures any swelling or inflammation (Chopra *et al.,* 1956). The constituents of *Q.infectoria* galls comprise a large amount of tannin (50-70%), traces amount of gallic, syringic and ellagic acid (Ikram and Nowshad, 1977; Dar and Ikram, 1979; Hwang *et al.,* 2000). The main constituent of this galls are tannins which posses various beneficiary in terms of biological and chemical functions can actually being studied thoroughly for further scientific applications. *Q.infectoria* galls able to exhibit high potency in antioxidant (especially in scavenging free racidals) and anti-inflammatory properties as well as pharmacologically proven to be astringent, antiparkinsonian, antidiabetic and antitremorine (Dar *et al.,* 1974; Dar and Ikram, 1979; Hwang *et al.,* 2000; Kaur *et al.,* 2004; 2008). Hence, this particular medicinal gall has been studied extensively in terms of its active component identification, extraction processes and its relative affecting factors, biological and chemical functions for pharmaceuticals, medicinal and herbal technology industry.

Extraction can be defined as the separation of active components of plant or animal tissues from other inactive or inert components by using several solvents selectively chosen in accordance to standard extraction procedures. Standardization of extraction procedures contributes to a defined and final quality of the herbal product (Handa, 2008). The extraction of these standardized herbal extracts with its specific amount of bioactive

compounds is highly important. Hence, varying processing conditions will give a great impact on every extraction processes. In this preliminary study, the effects of *Q. infectoria* galls active constituent; tannin, antioxidant ability and its overall yield will be evaluated based on the processing conditions selected. This is to ensure that despite from different processing parameters applied on the extraction process, the quality and quantity of active constituents extracted can still be well preserved and is able to exhibit its biological and chemical functions greatly. Previous literatures have only focused on the extraction process and the identification of active components found in *Q. infectoria* galls. There is no scientific studies have been performed in assessing the best conditions for processing parameters to extract out the active components at its best form and quantity. Hence, a preliminary study on these processing parameters is crucial to determine the relative effects of different range used in the selected processing parameters against the extraction of tannin from *Q. infectoria* galls. Different range of processing parameters can possibly display different extraction conditions and how this scenario can actually affects the quality of extracted compounds. Overall, this study aims to determine the significance of processing parameters range selected towards its response variables during the extraction of tannin from *Q. infectoria* galls.

MATERIALS AND METHODS

Materials

Quercus infectoria galls were purchased from a local herbal shop at Pasir Larkin, Johor Bahru. They materials were sent to Institute of Bioproduct Development, Universiti Tekonologi Malaysia, Skudai, Johor for further treatment. The galls were sent for a pre-treatment process where they were cleansed thoroughly with tap water and dried at 40^{o}C using drying oven (Memmert, Germany) until excess water has completely drained out. The powdered galls were stored in a sealed plastic container and stored in a dry cool place until further usage.

Chemicals and reagents

All the chemicals used in this experiment were in analytical and also HPLC (High Performance Liquid Chromatography) grade. Tannic acid, ortho-phosphoric acid (85%), acetonitrile (41.05 g/mol), L-ascorbic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (394.32 g/mol) were purchased from Sigma- Aldrich Malaysia Sdn Bhd.

Extraction of tannin from *Q. infectoria* galls

An amount of *Q.infectoria* galls were weighed accordingly and subjected to decoction extraction method using water as solvent. Using hot plate stirrer, the process of extraction was carried out using several selected temperatures, solvent ratio and duration of extraction as illustrated in Table 1 below. Once extraction process has completed, the filtrate was sent to rotary evaporator to remove excess water and therefore sent to drying process in a drying oven for 48 hours at 40^{o}C.

Table 1: Processing parameters in OFAT preliminary study

Parameter	Conditions
Group A	
Extraction Temperature	50^{o}C, 60^{o}C, 70^{o}C, 80^{o}C, 90^{o}C, 100^{o}C
Extraction Time	180 min
Solvent to Raw Material Ratio	30:1
Group B	
Extraction Time	60 min, 90 min, 120 min, 150 min, 180 min, 210 min
Solvent to Raw Material Ratio	30:1
Extraction Temperature	Selected from Group A
Group C	
Solvent to Raw Material Ratio	15:1, 18:1, 21:1, 24:1, 27:1, 30:1
Extraction Temperature	Selected from best results in Group A
Extraction Time	Selected from Group B

Preliminary study of processing parameters range

The preliminary study was mainly assessed on the effect of processing parameters towards the extraction of tannin from *Q. infectoria* galls. The range was set accordingly using OFAT preliminary study. As such, the experiment was carried out one at a time to thoroughly assed the significance of using such range in the extraction process or any further experiments. In common cases, OFAT is usually being used in order to analyze abundance of data and to predict the significance of most data before proceeding to other experimental designs. The parameters with its own conditions involved in this OFAT study were classified into three different groups (A, B, C) with their respective response variables; were further illustrated in Table 1. All experiments were carried out in triplicate and all data represented in this study are assessed statistically using Analysis of Variance (ANOVA) test.

Yield of extract

Yield of *Q. infectoria* aqueous extract was calculated based on the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Dry mass of crude extract (g)}}{\text{Mass of sample used for extraction (g)}} \times 100$$

Quantification of Tannic Acid

Tannin from *Q. infectoria* extract was determined through High Performance Liquid Chromatography (HPLC) using the method described by Asghari *et al.* (2011) with slight modifications. 1mg/ml of *Q. infectoria* extract was mixed thoroughly with distilled water as solvent and was filtered using 0.45 µm nylon filter membrane. The mixture was then injected to the HPLC system. The chemical marker used for the quantification of tannin was tannic acid. The mixture was injected with column C18 with particle size of 5 micron. 1 % of ortho-phosphoric acid was used as solvent A while 100% acetonitrile as solvent B. With isocratic pump mode, the separation of tannic acid was conducted on the basis of 95% solvent A and 5% solvent B with flow rate of 1ml/min at 280 nm. Every injection was set until they achieve 20 µl. Data was collected and sample peaks were identified by comparing with standard peaks of tannic acid solution obtained from the assay. The amount of tannic acid was calculated using appropriate calibration curves.

Scavenging of free radicals

This assay was conducted according to the method of Miliauskas *et al.* (2004) with slight modifications. In order to conduct scavenging assay, 1mL of methanolic solution of gall extract was incubated with 0.5mL of 0.05mM DPPH solution for 30 min. Absorbance was read there-after at 517nm measured using UV-vis spectrophotomer. A decrease in absorbance reading indicated that antioxidant activity is high and DPPH radicals are greatly scavenged. The capability to scavenge DPPH radicals was calculated using the formula below:

$$\text{DPPH quenched (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Statistical Analysis

All values were expressed as mean ±S.E. Statistical analyses were performed using Analysis of Variance (ANOVA) test. Correlations were established using Pearsons correlation coefficient (r) in bivariate linear correlations ($p < 0.05$). These statistics were calculated using Microsoft office Excel 2007 and SPSS version 21.0 (IBM corporation, New York, U.S.A).The values of P lower than 0.05 were considered as significant (P is probability).

RESULTS AND DISCUSSIONS

Table 2: Effects of extraction temperature on yield of extract, tannic acid content and antioxidant activity of *Q. infectoria* aqueous extract

Factor / Response	Temperature (°C)					
	50	60	70	80	90	100
Yield	52.477 ±0.000	65.850 ±0.001	82.543 ±0.000	80.643 ±0.001	78.940 ±0.000	73.840 ±0.004
Tannic Acid	1550.840± 0.039	1757.380 ±0.004	3155.243± 0.004	3010.823 ±0.012	2915.817 ±0.001	2011.440± 0.012
Antioxidant	71.560 ±0.000	74.993 ±0.001	91.150 ±0.000	90.84 ±0.000	88.673 ±0.004	81.820 ±0.001

Constant variable: Extraction time (180 min); Solvent ratio (1:30)

Table 3: Effects of extraction time on yield of extract, tannic acid content and antioxidant activity of *Q. infectoria* aqueous extract

Factor / Response	Time (min)					
	60	90	120	150	180	210
Yield	58.467 ±0.000	69.057 ±0.000	85.003 ±0.001	81.483 ±0.001	76.753 ±0.001	63.840 ±0.002
Tannic Acid	1350.373± 0.028	1658.62 ±0.014	3344.773 ±0.004	2996.823 ±0.018	2533.483 ±0.038	2196.167 ±0.014
Antioxidant	70.080 ±0.001	75.493 ±0.001	91.163 ±0.000	89.940 ±0.001	88.820 ±0.001	72.127 ±0.000

Constant variable: Extraction temperature 70°C (selected from Table 4.1 results); Solvent ratio (1:30)

Table 4: Effects of solvent to raw material ratio on yield of extract, tannic acid content and antioxidant activity of *Q. infectoria* aqueous extract

Factor / Response	Solvent Ratio (ml)					
	15	18	21	24	27	30
Yield	68.240 ±0.001	75.733 ±0.028	84.677 ±0.000	82.303 ±0.001	78.683 ±0.001	62.513 ±0.001
Tannic Acid	1358.997 ±0.000	1678.030 ±0.012	3175.810 ±0.002	2897.203± 0.004	2734.637 ±0.001	2193.210 ±0.001
Antioxidant	70.290 ±0.002	76.073 ±0.002	91.860 ±0.000	89.020 ±0.0012	78.217 ±0.004	72.123 ±0.001

Constant variable: Extraction temperature 70°C (selected from Table 4.1 results); Extraction time at 120 min (selected from Table 4.2 results)

Table 5: Best parameters condition based on OFAT preliminary study

Extraction Temperature (°C)	Duration of Extraction (min)	Solvent to Solid Ratio
70	120	21

Based on Table 2, 3, and 4 it can be seen that yield of extract, tannin content as well as antioxidant activity is greatly affected from the processing parameters which are extraction temperature, extraction time and solvent to raw material ratio. From the results itself, the trend of respective response variables are parallel with its processing parameters. When there is gradual increase of temperature, time and solvent ratio, there will be an increment of relative responses (yield, tannin content and scavenging activity). However, prolong heating; temperatures higher than 70°C as well as longer extraction time; more than 120 min will lead to a subsequent reduction of its yield of extract, tannin content and its scavenging activity.

The ability of *Q. infectoria* extract to scavenge free radicals is correlated with the high amount of tannin; active compound present in the extract itself. Since, free radicals attacks diverse classes of biomolecules including phenolic acids, esters and glycosides (Nimse and Pal, 2015), hence the basic principle lies in this concept is that tannin; phenolic compound present from *Q. infectoria* extract is strong enough to exhibit great antioxidant activity on its own compared to other phenolic compounds (Amarowicz *et al.,* 2004). Tannin has the ability to chelate metal ions and interfere in one of the reaction steps in Fenton reaction thereby retards oxidation process (Zhang *et al.,* 2004; Karamac *et al.,* 2006). Antioxidants are also crucial especially in halting oxidative damage which can implicate humans with several range of diseases such as cancers, cardiovascular diseases and also aging (Kehrer, 1993). However, they are many medicinal plants have found to be effective in combating these free radicals but in many scientific literatures, tannin has found to be the new natural antioxidants due to its effectiveness in oxidative damage defense mechanism (Amarowicz *et al.,* 2001; Pegg *et al.,* 2005; Amarowicz *et al.,* 2005). Rice-Evans and his colleagues also claimed that many naturally occurring phenolics with low molecular weight able to scavenge free radicals as effective as the antioxidant vitamins E and A. In fact, tannins have been tested clinically and were proven that both condensed and hydrolysable tannins are more effective natural antioxidants compared to small phenolics (Hagerman, 1988).

Besides that, from the trend of data acquired it portrays a finding; prolong exposure to heat, longer extraction time and great difference in solvent to raw material ratio will greatly impact medicinal plants' yield of extract, active compound content as well as its scavenging ability. Therefore, the best range has been selected for each processing parameters examined and the results were tabulated in Table 5. Highest extract yield (85.003% ± 0.001), tannin content (3344.773 ppm ± 0.001) and most active scavenge activity (91.860% ± 0.002) was acquired at extraction temperature of 70°C, 120 min of extraction duration and solvent to raw material ratio of 1:21.

Figure 1 Effects of temperature, time and solvent ratio on *Q. infectoria* aqueous extract

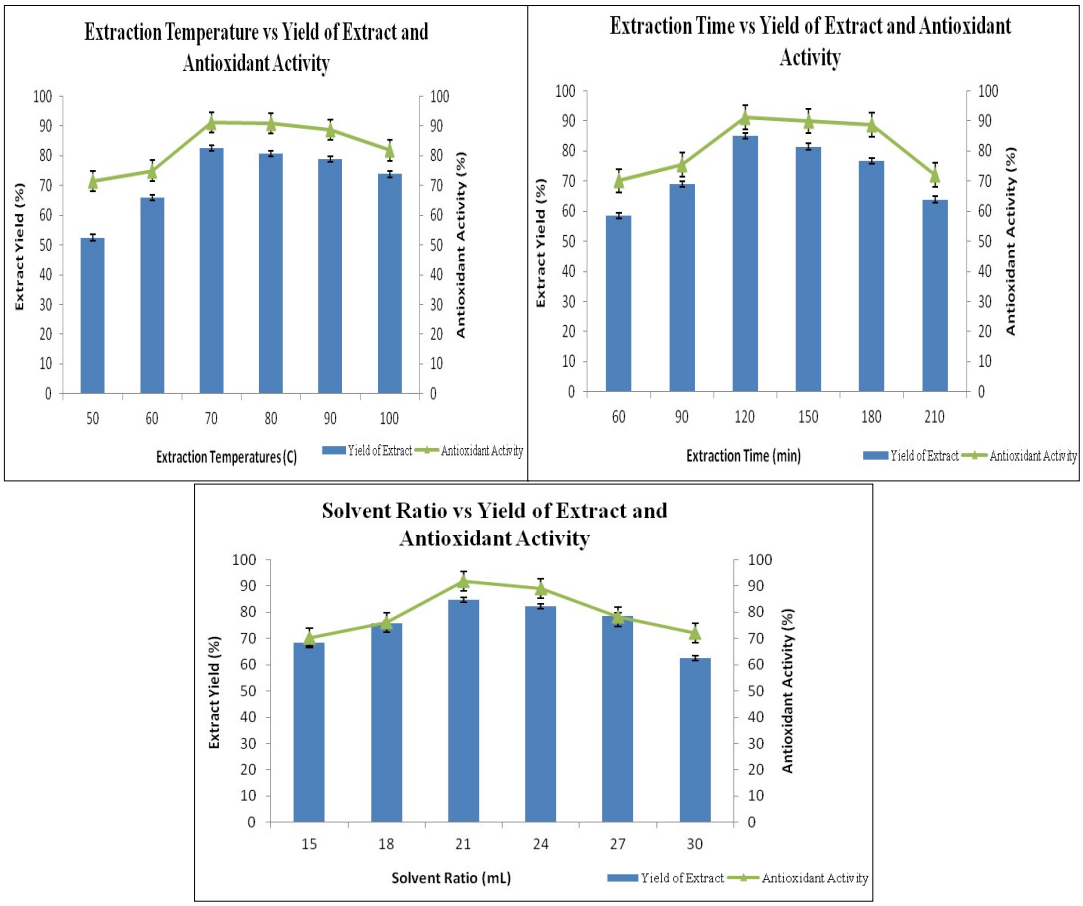
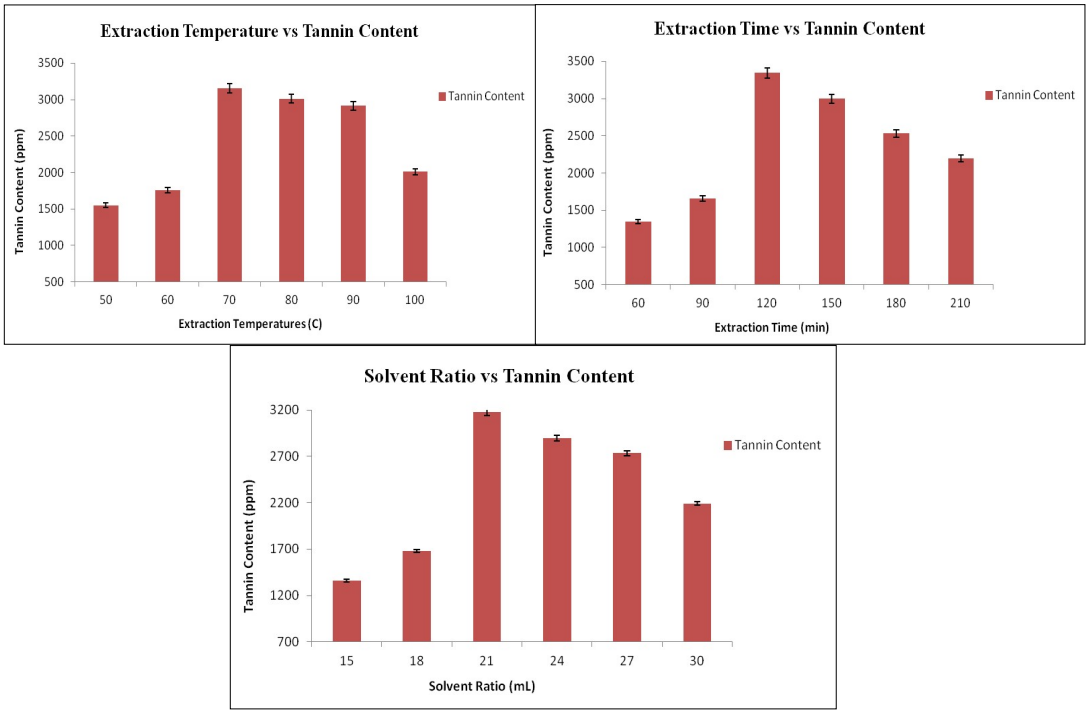


Figure 2 Effects of temperature, time and solvent ratio on tannin content of *Q.infectoria* extract



On the other hand, Figure 1 illustrates the effect of extraction temperature, time and solvent ratio towards extraction process of *Q. infectoria* galls. Higher temperature gives a significant rise of extract yield, tannin content as well as antioxidant activity. However, extraction temperatures should not exceed the right limit to avoid thermal degradation of active compounds from happening. High temperatures can actually destroy protein complexes and its structures therefore damaging the active compound desired. Different compounds have different molecular weight and thermal capacity. Hence, preliminary study is absolutely crucial to screen for the right extraction temperatures and also other processing parameters before proceeding to the other phases of extraction. By regulating these factors prior the extraction process, such thermal degradation can be avoided and the right active compound can be successfully extracted from the selected medicinal plants. Additionally, Figure 2 the graph illustrates the effects of temperature, time and solvent ratio towards tannin content; the main active compound constitutes in *Q. infectoria* extract. From the results, it clearly depicts that gradual increase of time, temperature and solvent ratio leads to a subsequent increment of tannin contained in the extract. However, after prolonged extraction process leads to relative thermal degradation. Tannin content also reduces after more than 120 mins of extraction duration and solvent ratio higher than 1:21. This explains that every parameter convey their own limits to optimally produce the active compound. Conclusively, the range of parameters that have been considered to give significant impact towards these response variables are extraction time at 120 min, extraction temperature of 70^{o}C and solvent to raw material ratio of 1:21.

CONCLUSION

The results concluded processing parameters range gave significant effects towards its response variables whether enhancing or diminishing the total extraction process. High temperatures could lead to partial or full thermal degradation of the active compounds by disrupting the protein structures. Imbalance of solid to raw material ratio and long duration of extraction will negatively affect the extraction of phytochemicals and the quality and quantity of active compound extracted. Hence, the preliminary studies have clearly depicted the best appropriate range to be employed in further optimization study of extraction process or any other phytochemical related studies.

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