Available online at www.sciencedirect.com

ScienceDirect

Materials Today: Proceedings 00 (2018) 0000–0000

materialstoday: PROCEEDINGS

www.materialstoday.com/proceedings

3rd GCET_2017

Ultrasound Assisted Methods for Enhanced Extraction of Phenolic Acids from *Quercus Infectoria* Galls

N. Sukor[a], R. Jusoh[a]*, S. A. Rahim[a], N. Kamarudin[a]

[a]*Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia.*

Abstract

Quercus Infectoria galls or known as Manjakani, is one of the potential herbs that have multiple therapeutic properties and are widely used in folklore medicine. Phenolic acids, including gallic acid and tannic acid, have been extracted from the galls by using different ultrasound extraction system (probe-type and bath-type) and the results were compared with the conventional extraction system. The effect of sonication time, solvent types and solvent concentration on the extraction yield of the phenolic acids has been investigated. The maximum extraction yield obtained for gallic acid and tannic acid are 2155.77 mg/kg and 15236.83 mg/kg, respectively in 8 h extraction time, operating temperature of 70°C, solid to solvent ratio of 1:10, ultrasonic power of 11 W and ultrasound frequency of 20 kHz with 0.1 M hexadecyltrimethylammonium bromide as the solvent. The obtained yield was significantly higher as compared to the conventional extraction system where only about 794.57 mg/kg yield was achieved. Peleg's model was used to describe the kinetics of probe-type ultrasound extraction system and the model showed a good agreement with the experimental results. Therefore, it can be concluded that probe sonication system significantly induces the extraction efficiency to increase the bioactive constituents yield.

Selection and/or Peer-review under responsibility of The 3rd International Conference on Green Chemical Engineering and Technology (3rd GCET): Materials Science, 07-08 November 2017.

Keywords: Phenolic acids; *Quercus Infectoria*; Hexadecyltrimetylammonium bromide; Conventional extraction; Ultrasonic-assietd extraction.

* Corresponding author. Tel.: +60-95492815
E-mail address: rohayu@ump.edu.my

Selection and/or Peer-review under responsibility of The 3rd International Conference on Green Chemical Engineering and Technology (3rd GCET): Materials Science, 07-08 November 2017.

Introduction

Gall of *Quercus Infectoria* has been used for centuries in folklore medicines in several Asian countries and widely known for its therapeutic benefits. It is also known with names like Majuphal, gall tree, gall oak, masikai, magic nut and aleppo oak. This herb belongs to the Fagaceae family that can be widely found in Greece, Asia Minor and Iran [1]. The gall arising in the branches of the tree are the one that is widely known for its therapeutic benefits and uses also contributes in the folklore medicines. In Asian, the galls of *Quercus Infectoria* have been used as traditional medicine for centuries in treating the inflammatory disease while in Malaysia, the gall of the *Quercus Infectoria* has been used as herbal drink for women to restore the elasticity of the uterine wall after their childbirth [2]. Apart from that, this herb also shows promising results in cosmeticeutical field reported by Nur Syukriah in 2014 [3]. In her report, she stated that the galls possess high potential in skin whitening aside other benefits. Besides, it can also be used as mouth antiseptic and can control the inflammation of tonsils, while the direct application of it onto the skin cures swelling or inflammation [2]. This herb also has potential in osteoblast function and bone metabolism [4].

As mentioned in previous report, the *Quercus Infectoria* galls contains phenolic acids such as gallic acid, tannic acid and ellagic acid that has been proven to have multiple biological effects such as high antioxidant activity and the ability to become antimicrobial, antibacterial and antifungal agent [5]. The antioxidants main function is to delay the oxidation processes of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and the presence of gallic acid as one of the important phytochemical is believed can contributed to it [6]. Moreover, it has also been reported that the phenolic acids has higher antioxidant activity compared to vitamin C and E towards the reactive oxygen species [7].

High power ultrasound can be applied using two types of devices, ultrasonic bath or probe-type ultrasound equipment. Both systems are based on a transducer as a source of ultrasound power. The ultrasonic bath is the most commonly known type of ultrasonic device usually consists of a stainless steel tank with one or more ultrasonic transducers. It is usually operate at a frequency of around 40 kHz and can be equipped with temperature control. They are readily cheap, available and large numbers of samples can be simultaneously treated. However, compared with probe systems, the low reproducibility and low power of ultrasound delivered directly to the sample are major drawbacks. Indeed, the delivered intensity is highly attenuated by the water contained in the bath and the glassware used for the experiment. High power ultrasonic probes are generally preferred for extraction applications. The probe system is more powerful due to an ultrasonic intensity delivered through a smaller surface (only the tip of the probe), when comparing to the ultrasonic bath. They are generally operated at around 20 kHz and use transducer bonded to probe which is immersed into the reactor resulting in a direct delivery of ultrasound in the extraction media with minimal ultrasonic energy loss [8].

This work focused on examining the effect of extraction methods, type of solvent and its concentration on the phenolic acids content from *Quercus Infectoria* galls. The kinetic analysis using Peleg's model for various effects was also studied.

2. Material and Methods

2.1. Plant material

The plant material, *Quercus Infectoria* (Manjakani) galls were purchased locally from an Indian herb store in Bandar Kuantan, Pahang. It was first washed, cleaned and air dried at room temperature. Then, the gall was crushed using pestle and mortar until it become fine powder before it can be used prior to the extraction process.

2.2. Reagents and standards

Deionized water and hexadecyltrimethylammonium bromide, CTAB reagent (Sigma-Aldrich, Germany) were used as the extraction solvent. Deionized water was obtained from FKKSA Lab, UMP. Gallic acid (Merck, Germany) and Tannic acid (Merck, Germany) were used for the quatification of phenolic acids and as calibration standard. Acetonitrile (Merck, Germany) and orthophosphoric acid, 85% (Fisher Science, UK) were used as mobile phase for HPLC analysis. All reagents and standards were purchased from Nano Life Quest Sdn Bhd.

2.3. Extraction procedures

2.3.1. Conventional Extraction

In this study, sample *Quercus Infectoria* galls were used in powder form. The schematic diagram of the conventional extraction set-up can be seen as depicted in Figure 1(a). Powdered *Quercus Infectoria* galls were immersed in the extraction solvent and the mixture was heated on a hotplate with continuous stirring for 8 hours. The extraction temperature was set at 70°C, the sample-to-solvent ratio at 1:10 (sample: water) with volume of infusion was set at 100 ml. The mixture was covered with aluminium foil throughout the extraction to minimize the evaporation in order to maintain the sample-to-solvent ratio.

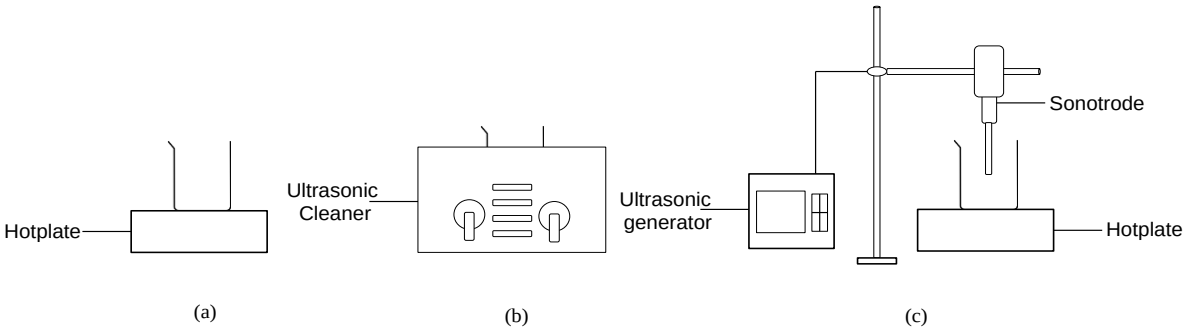

Figure 1 Schematic diagrams (a) Conventional extraction (b) Ultrasonic cleaner (c) Ultrasonic probe

2.3.1. Ultrasonic Assisted Extraction (Bath-type)

The extraction of phenolic acid from *Quercus Infectoria* galls were conducted using 2.75L Fisherbrand Scientific ultrasonic cleaner with electronic timer, quick degassing and heating. Figure 1(b) shows the schematic diagram of the ultrasonic cleaner extraction set-up. Powdered *Quercus Infectoria* galls were immersed in the extraction solvent and the mixture was heated and sonicated [9] for 8 hours with the volume of infusion at 100 ml. Sample-to-solvent ratio 1:10 with water as solvent were fixed in this study. This method used the same procedure as the conventional method whereby the mixture was covered with aluminium foil throughout the extraction to minimize the evaporation in order to maintain the sample-to-solvent ratio. Each sample was filtered with nylon syringe filter (pore size of 0.22 μm). Extracted product was left to cool at room temperature and then was kept at 4 ˚C prior to analysis for determination of active compounds by using High Performance Liquid Chromatography (HPLC).

2.3.3. Ultrasonic Assisted Extraction (Probe-type)

Ultrasonic processor Q700 (700 watts, 20kHz) from QSonica, Newtown, U.S.A with a replaceable flat tip ultrasonic probe (sonotrode) was used to conduct an extraction process of gallic acid and tannic acid from *Quercus Infectoria* galls. It was made of titanium alloy that had a tip diameter of 12.7 mm and 127 mm length. The ultrasonic probe was immersed in the extraction medium and the energy is transmitted via the sonotrode directly into the sample [6]. In probe-type ultrasonic extraction process, the ultrasound power level could be varied by adjusting the amplitude setting of the sonotrode while the cumulative average ultrasound dose could be varied by adjusting the duty cycle. The sonication intensity was calculated using the following equation:

$$I = \frac{P}{A}$$

Where A (cm^2) was the area of the sonotrode tip. The A value was 1.27 cm^2. The conventional extraction has no sonication process. The amplitude was set at position 1 to correspond to a power input P of 11W, and 8.66 W/cm^2 sonication intensity, I using 40% duty cycles (A duty cycle of 40%, was obtained by sonicating for 4s followed by a rest period of 6s). Each sample was filtered with nylon syringe filter (pore size of 0.22 μm). Extracted product was left to cool at room temperature and then was kept at 4^oC prior to analysis for determination of active compounds by using High Performance Liquid Chromatography (HPLC).

2.4. Kinetic model

Mathematical modelling can help in the design, optimization and control of the processes under investigation, as well as providing useful information for scale up of the equipment. The empiric and relatively simple Peleg's model can provide a proper fitting to extraction processes [10], besides describing the kinetics of sorption processes [11]. Previous researchers has used Peleg's model to explain the extraction of natural compounds such as ursolic acid from *Ocimum sanctum* [12], esveratrol and viniferin from grape cane [13] and curcumin from *Curcuma amada* [14]. In the present study, Peleg's model has been used to estimate extraction rate constant, initial extraction rate and equilibrium concentration. The influence of process conditions on the kinetic parameters has also been analysed. The model equation describing the kinetics of extraction of materials can be given as follows:

$$C_t = C_o + \frac{t}{K_1 + K_2 t} \quad (1)$$

where C_t is the concentration of gallic acid at time t (mg GA/g sample), K_1 is Peleg's rate constant (min.g/mg) and K_2 is Peleg's capacity constant (g/mg). C_o is the initial concentration of gallic acid which is zero, as fresh solvent is used. The extraction typically occurs in two stages with the first order trend in the initial stage and zero order in the later. The modified Peleg's equation which represents the concentration of target solute (gallic acid) in extraction solvent against time can be written as follows:

$$C_t = \frac{t}{K_1 + K_2 t} \quad (2)$$

The graph between $1/C_t$ vs. $1/t$ can be plotted to calculate K_1 (Peleg's rate constant) and K_2 (Peleg's capacity constant) values from the slope and intercept respectively. C_t was also subsequently calculated using Eq. (2) at different times for checking the fitting of the model.

2.5. High performance liquid chromatography (HPLC) analysis of phenolic acids

The measurements of separation and determination of phenolic acids; as gallic acid from the *Quercus Infectoria* galls were performed using an High Performance Liquid Chromatography (HPLC) system Agilent Series 1100 equipped with diode array detection (DAD) and a column Phenomenex Prodigy 5µ (250 X 4.60 mm). The wavelength for detection of gallic acid was set at 270 nm. Separation was achieved by flow rate of 1 ml/min with 3.0% Phosphoric acid (90%) / Acetonitrile (10%), in an isocratic programme. The injection volume was 10 µl. Each sample and standard was filtered with nylon syringe filter (pore size of 0.22 µm). For standard preparation, the mobile phase of phosphoric acid and acetonitrile were prepared, degassed in an ultrasonic bath and injected through the chromatographic column.

2.6. Scanning Electron Microscopy (SEM)

SEM imaging was performed using Scanning Electron Microscope Quanta 450 on the *Quercus Infectoria* galls sample particles to visualize and characterize the surface. 5nm coating of Pt was applied to the surface of the samples before proceeding with imaging.

3. Results and discussion

3.1. The Chromatographic Separation and Detection of phenolic acids

A 99% gallic acid and tannic acid standards were used during sample analysis as a positive control. The same method of extraction and analysis carried out for the sample was used to produce a standard curve, which was used to identify and quantify the phenolic acids; gallic acid and tannic acid in the samples. This procedure was performed by prepared the standard solution for analysis ranging from 10ppm to 60ppm concentration of gallic acid and tannic acid at 270nm and 280nm, respectively. The peak obtained was due to the various concentrations of phenolic acids used to construct the standard curve.

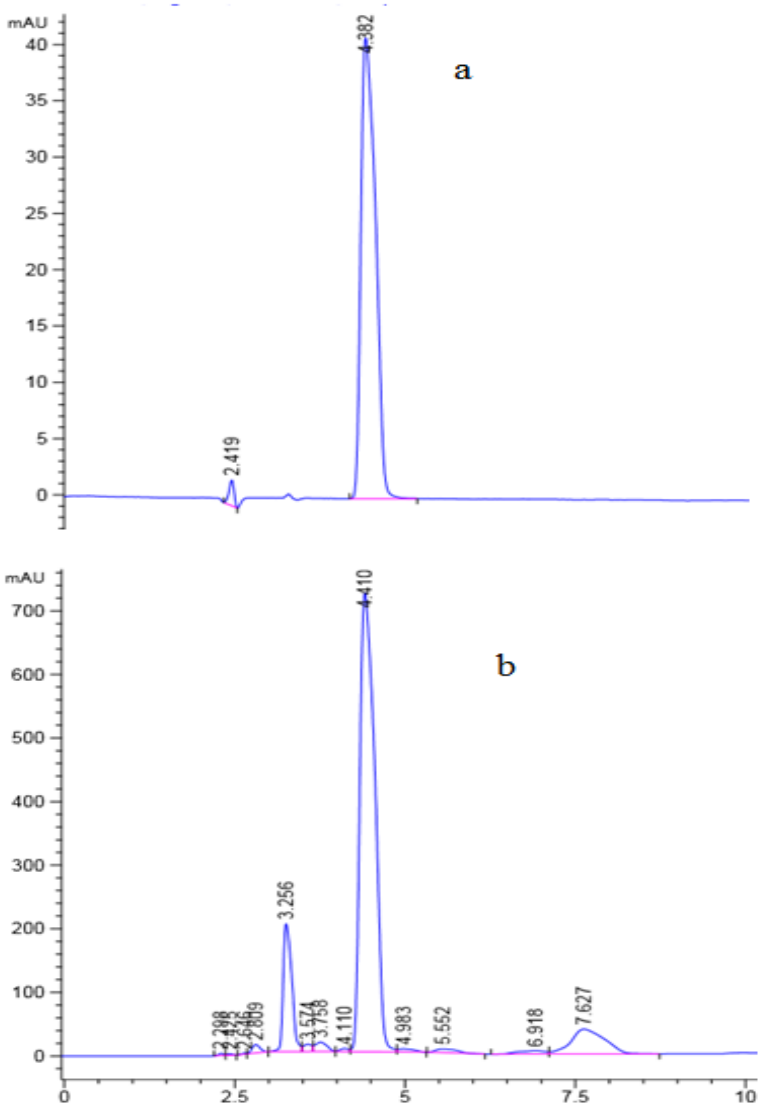

Figure 2 Chromatographic analysis of phenolic acid; as gallic acid (a) standard solution, (b) extraction sample from *Quercus Infectoria* galls

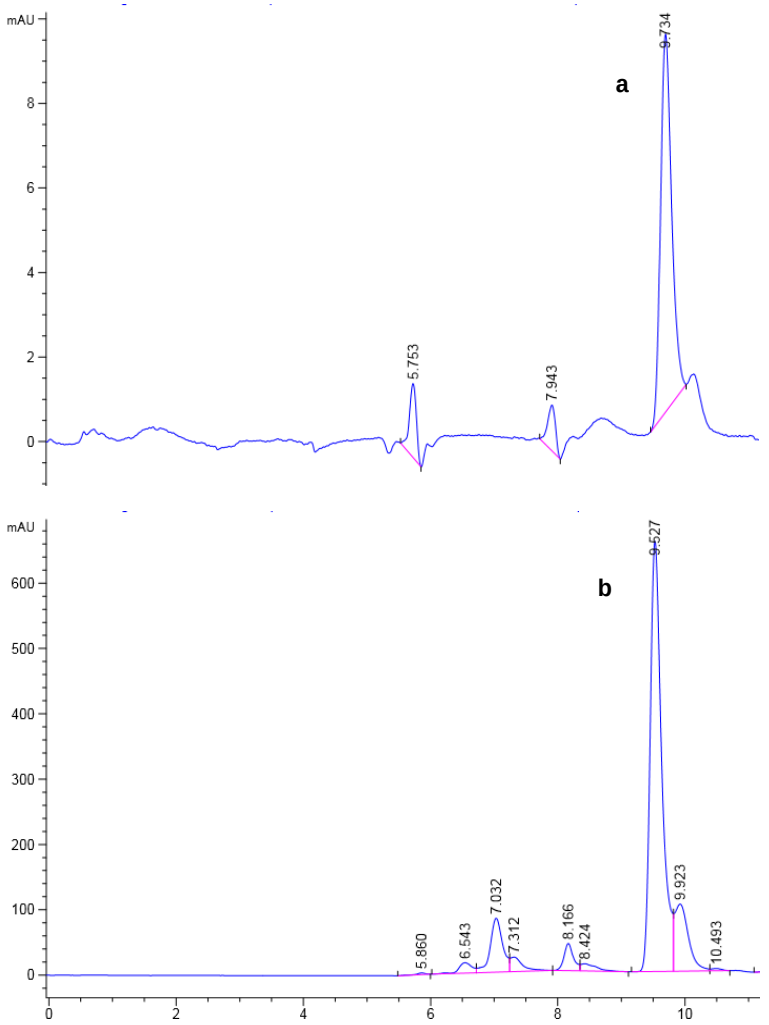

Figure 3 Chromatographic analysis of phenolic acid; as tannic acid (a) standard solution, (b) extraction sample from *Quercus Infectoria* galls

Figure 2(a) illustrated a good resolution of the gallic acid chromatographic peak obtained at retention time 4.4 ± 0.2 min. This is parallel with the previous studies using gallic acid where they also managed to obtain about the same retention time for the gallic acid at 4.9 ± 0.2 min [6] and 4.7 ± 0.2 min [5]. On the contrary, Figure 4(b) shows the peaks appeared from the extraction sample of *Quercus Infectoria* galls and the gallic acid can be clearly seen at peak 4.410 min which within the standard retention time. On the other hand, Figure 3(a) illustrated a chromatographic peak obtained at retention time 9.7 ± 0.2 min represented tannic acid. Retention time at 9.5 ± 0.2 min can be seen from Figure 3(b) showing the peak of tannic acid from the extraction sample of *Quercus Infectoria* galls. The calibration curve was obtained by plotting a straight line based on the concentration of phenolic acids at 6 differences concentration of standard solution. An excellent linearity $R^2 = 0.95$ and 0.99, ($R^2 \approx 1$) for both gallic acid and tannic acid, respectively were obtained with excellent regression factor.

3.2. Effect of Extraction Method and Sonication Time

Figure 4 and Figure 5 shows the comparison performance of different extraction methods towards the concentration of phenolic acids; gallic acid and tannic acid, respectively from *Quercus Infectoria*. Three type of extraction methods (conventional, ultrasonic bath-type and ultrasonic probe-type) were tested using the same parameters to observe the extraction yield. In attempt to obtain the maximum extraction efficiency of phenolic acids from the powder of *Quercus Infectoria* galls, the effect of sonication time ranging from 1 to 12 hours were tested. The samples were taken at every 2 hours until 12 hours. All three methods resulted to an increase yield every hour until 8th hours but eventually decreasing on 10th hours and onwards. Perhaps, this can be explained by the fact that the mass transfer is a time-dependent process [15]. Even though longer ultrasonic time will provide more thorough touching of the solvent with the powder of sample tissue, it can be degraded and oxidized under ultrasound, resulting in reduced yield at excessive extraction time [16]. On top of that, this phenomenon might be due to the active ingredient that was not dissolved when the solubility of dissolving-out substances became saturated [17] with the increase of extraction time, while the loss of phenolic acids were increased with the viscosity of extracts increased when extraction time increased. Therefore, based on the result, extraction time ought not to exceed 8 hours. Both gallic acid and tannic acid indicated about the same trends with ultrasonic probe extraction method resulted to the maximum yield compared to the other two methods. The highest extraction yield of gallic acid from Figure 4 after 8 hours were obtained by the following sequences: Ultrasonic probe-type > Ultrasonic bath-type > Conventional extraction, with 1287.816 mg GA/kg sample, 1103.441 mg GA/kg sample and 794.567 mg GA/kg sample respectively. On the other hand, tannic acid yield also resulted the same sequences of extraction method: Ultrasonic probe-type > Ultrasonic bath-type > Conventional extraction, with 10542.45 mg TA/kg sample, 9920.05 mg TA/kg sample and 9508.30 mg TA/kg sample respectively. It is widely known that the ultrasonic-based extraction is much better than the conventional extraction method due to its sonication effect towards the samples as reported before in the extraction of flavonoids [18], alkaloid [19], anthocyanins [20], natural pigments [21] and other bioactive compounds [22;23]. Based from the extraction yield resulted after 8 hours, the extraction using sonotrode was found to be almost 40% more efficient. The ultrasonic-assisted extraction (UAE) technique was able to shortened the extraction time and reduce organic solvent consumption [24]. The UAE performance is contributed by the factors of intensity, time, solvent, temperature, pulsation and matrix. Besides that, UAE involve mechanical vibrations which is sound waves with high frequency. Ultrasound can increase in the permeability of the cell wall, mechanical stressing and cavitation effect during the extraction process [6].

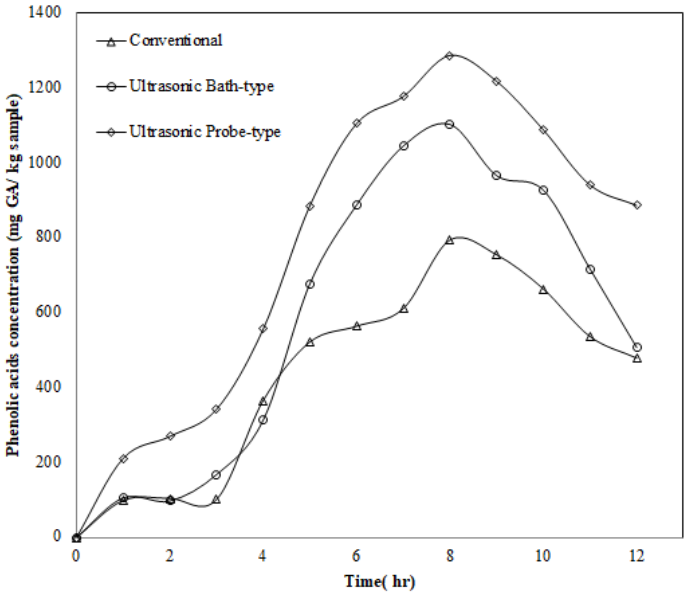

Figure 4 Comparison performances of extraction methods towards the gallic acids extraction amount from the *Quercus Infectoria* galls using water as solvent at temperature 70°C, with sample-to-solvent ratio, 1:10 for 8 hours

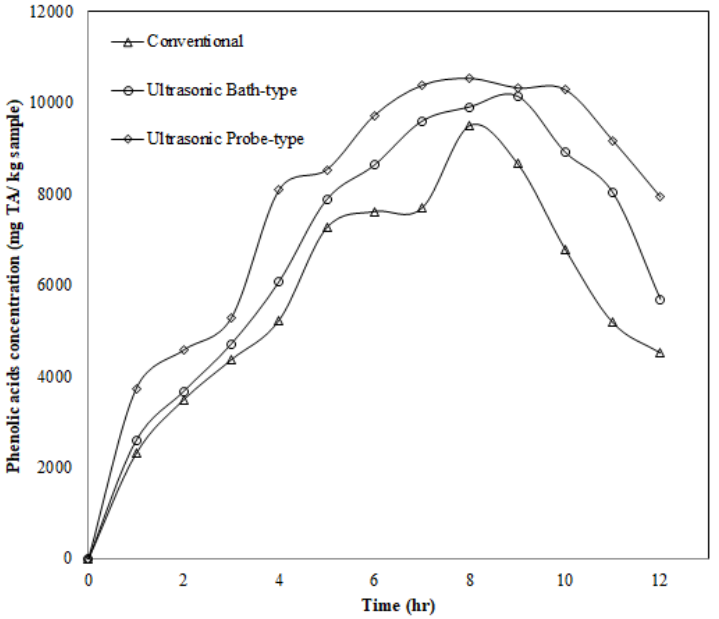

Figure 5 Comparison performances of extraction methods towards the tannic acids extraction amount from the *Quercus Infectoria* galls using water as solvent at temperature 70°C, with sample-to-solvent ratio, 1:10 for 8 hours

Comparing with the highest yield from conventional method, ultrasonic based extraction method need lesser time to obtained the same amount of yield. On the other hand, the efficiency of the ultrasonic probe-type was recorded to be about 15% much better than the ultrasonic bath-type. The ultrasonic bath-type method has disadvantage as it generally operates at a single frequency (20kHz or 40kHz) while the probe-type develop a power up to 100 times more than the provided ultrasonic cleaner [25]. On top of that, the ultrasonic irradiation of low power ultrasound only managed to irradiate through the walls where the sample is contained (indirectly), while the ultrasonic probe system is more powerful due to an ultrasonic intensity delivered through a smaller surface (only the tip of the probe) resulting in a direct delivery of ultrasound in the extraction media [8].

The calculated parameters of Peleg's model (constant K_1 and K_2), and the root mean squared deviation (RMSD) have been given in Table 1. High value K_1 on ultrasonic probe-type extraction method depicts that higher rate of phenolic acids concentration were produced compared to the other two methods. This demonstrates a good agreement of the experimental data with the calculated data with a small value of error from RMSD calculated.

Table 1 Values of Peleg's constant K_1 (min g/mg), Peleg's capacity constant K_2 (g/mg), and comparison of experimental and calculated GAeq (mg GAE/g) [Effect of extraction method] (sample-to-solvent ratio = 1:10, sonication time = 8hr, temperature = 70°C, solvent = water, duty cycle (probe) = 40%)

Extraction method	Experimental GAeq (mg GA/g)	K_1	K_2	Calculated GAeq (mg GA/ g)	RMSD (mg/g)
Conventional	0.79	0.62	1.07	0.87	0.05
Bath-type	1.10	0.71	0.35	2.28	0.68
Probe-type	1.29	0.87	0.48	1.71	0.24

3.3. Effect of Solvents

Figure 6 represented the extraction yield of phenolic acids from the *Quercus Infectoria* galls using water and with the additional surfactant, hexadecyltrimethylammonium bromide (CTAB), in comparing the efficiency of each solvent. It can be clearly seen that CTAB work best as surfactant to improve the extraction efficiency of the target analytes as the phenolic acids concentration was seen skyrocketed after 8 hours of extraction. Water as solvent resulted to a poorer phenolic acids concentration as much as 1287.82 mg/ kg for gallic acid and 11060.79 mg/kg for tannic acid. On the other hand, CTAB as extraction medium illustrated a very much higher increment of yield at 2155.772 mg/ kg and 15236.83 mg/ kg for gallic acid and tannic acid, respectively. This can be explained through the characteristic of the CTAB/cationic surfactant that possesses positive charge due to its quaternary ammonium cation/functional groups [26], whereas the phenolic acids; as gallic acid and tannic acid surface contains negative charges arising from carboxyl and hydroxyl groups. Adsorption of surfactant takes place through electrostatic interaction between positively charged groups of the cationic surfactant and the negatively charged phenolic acids compound. This polar interaction between both components somehow increase the effectiveness of the extraction as the CTAB has higher positive charge while water molecules have only a slight positive charge near its hydrogen atoms. In addition, the use of CTAB, a cationic detergent, facilitates the separation of polysaccharides during purification and can aid in removing phenolic acids [27].

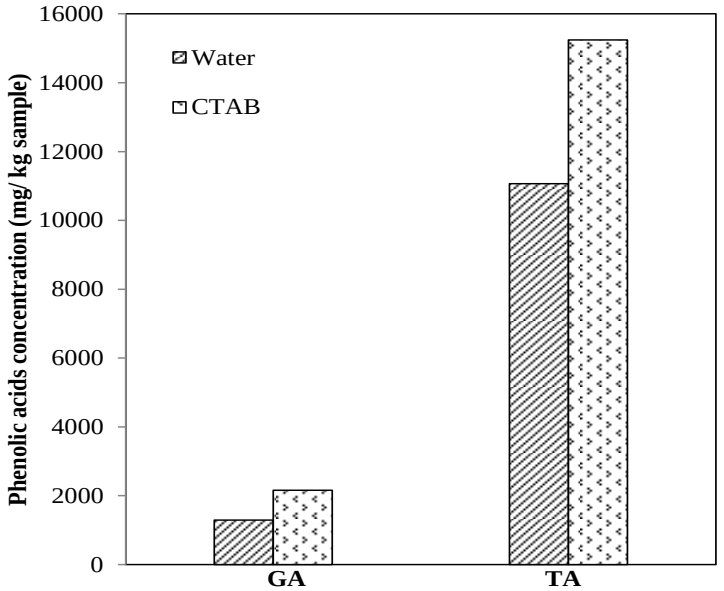

Figure 6 Extraction yield of phenolic acids from *Quercus Infectoria* galls using different solvents (water and hexadecyltrimethylammonium bromide, CTAB) by Ultrasound-assisted extraction (Ultrasonic probe-type) at temperature 70^{o}C, with sample-to-solvent ratio, 1:10 for 8 hours

The Peleg's model constants, K_1 and K_2, also the root mean squared deviation (RMSD) at different type of solvents have been given in Table 2. K_2 from Table 2 represented the capacity of the extraction using both water and CTAB as extraction medium to extract the target analytes; as gallic acid. CTAB with higher value of K_2 depicts that it is indeed a much better extraction medium compared to water.

Table 2 Values of Peleg's constant K_1 (min g/mg), Peleg's capacity constant K_2 (g/mg), and comparison of experimental and calculated GAeq (mg GAE/g) [Effect of solvent] (sample-to-solvent ratio = 1:10, sonication time = 8hr, temperature = 70°C, extraction method = ultrasonic probe-type)

Solvent	Experimental GAeq (mg GA/g)	K_1	K_2	Calculated GAeq (mg GA/ g)	RMSD (mg/g)
Water	1.29	0.87	0.48	1.71	0.03
CTAB	2.16	0.63	0.63	1.41	0.53

3.4. Effect of Solvent's Concentration

As the addition of CTAB as surfactant indicated better extraction yield of the gallic acid, the effect of its concentration was then tested by varies the concentration ranging from 0.05M to 0.2M. In general, the extraction efficiency seems to also highly affect by the solvent's concentration. Table 3 compared the concentration of phenolic acids obtained with different type of solvent; water and CTAB at different concentration. The extraction of phenolic acid using probe-type extraction method with water as medium resulted to a much lower yield at 1287.82 mg/ kg and 10542.45 mg/ kg for gallic acid and tannic acid, respectively. At 0.10M CTAB concentration, it resulted to an obvious difference in yield after 8 hours compared to the others. After 8 hours, the yield of the phenolic acids; namely gallic acid clearly shoot up at the maximum amount of 2155.772 mg/ kg at 0.10M concentration of CTAB followed by concentrations 0.05M, 0.15M and 0.20M with yields 1706.159 mg/ kg, 1629.396 mg/ kg and 1380.765 mg / kg respectively. Tannic acid concentration also shows the same pattern as gallic acid with the highest yield at 15236.83 mg/ kg, followed by 14741.13 mg/kg, 14568.69 mg/kg and 14397.21 mg/kg for tannic acid as illustrated in Figure 7. It was observed that when the concentration increased from 0.05M to 0.1M, The extraction yield of the phenolic acids increased. This is due to the solubility and extraction capacity of the extraction solvent were enhanced with the increased of CTAB concentration [18]. However, when the concentration of the CTAB increased from 0.10M to 0.20M, the peak area of the phenolic acids gradually decreased. This circumstance attributed to the increase in viscosity of the solution media. As the concentration of CTAB increase, it eventually influenced the mass transfer of the target component that lead to a poor penetration into the plant tissue. Hence, resulted in an inability for the active compound to dissolve quickly and completely, therefore deescalated the extraction efficiency [28].

Table 3 Analytical data of phenolic acids obtained from ultrasound-assisted extraction (ultrasonic probe-type) by high performance liquid chromatography (HPLC) system Agilent series 1100 at different solvents' (CTAB) concentration after 8 hours

Extraction medium	Water	CTAB			
Concentration of phenolic compound (mg/ kg)		0.05M	0.10M	0.15M	0.20M
Gallic acid	1287.82	1706.16	2155.77	1380.77	1629.40
Tannic acid	10542.45	14741.13	15236.83	14568.69	14397.21

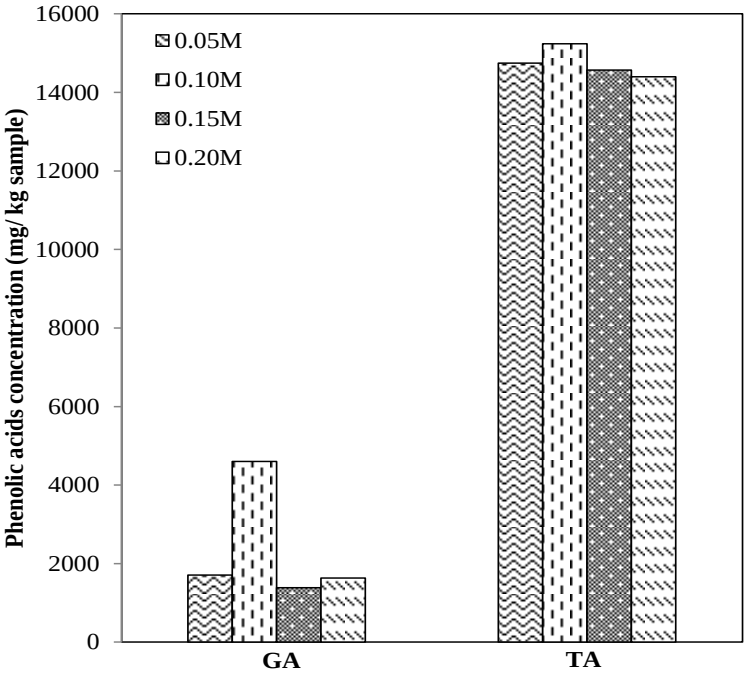

Figure 7 Extraction yield of phenolic acids from the *Quercus Infectoria* galls using different solvent's concentration (CTAB) at temperature 70°C, with sample-to-solvent ratio, 1:10 by Ultrasound-assisted extraction (Ultrasonic probe-type) for 8 hours

Table 4 depicts the Peleg's model constant and their comparison between the experimental data and the calculated one. There no much big different between the experimental data and the calculated data as computed in Table 4, showing that it is basically match each other.

Table 4 Values of Peleg's constant K_1 (min g/mg), Peleg's capacity constant K_2 (g/mg), and comparison of experimental and calculated GAeq (mg GAE/g) [Effect of solvent's concentration] (sample-to-solvent ratio = 1:10, sonication time = 8hr, temperature = 70°C, extraction method = ultrasonic probe-type, solvent = CTAB)

Solvent's concentration	Experimental GAeq (mg GA/g)	K_1	K_2	Calculated GAeq (mg GA/ g)	RMSD (mg/g)
0.05M	1.71	0.65	0.76	1.18	0.27
0.10M	4.60	0.50	0.52	1.73	1.44
0.15M	1.38	0.64	1.07	0.87	0.26
0.20M	1.63	0.33	1.07	0.90	0.37

3.5. Scanning Electron Microscopy (SEM) Analysis

In order to have a better interpretation of the phenolic acids extraction from *Quercus Infectoria* powder, SEM images from the surface of the untreated QI galls powder and ultrasonic probe-type treated QI galls at the desired operating condition of parameters were captured and compared as in Figure 8(a) and (b), respectively. The SEM images for the surface characteristics such as morphology relating, directing to the process of extraction on the powder samples were also analysed.

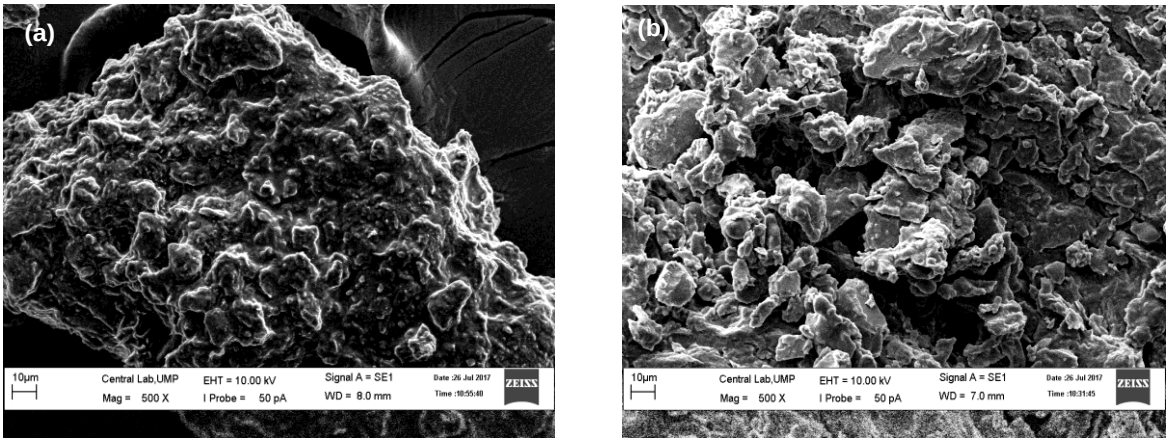

Figure 8 SEM Microscopic observation (magnitude 500x) of *Quercus Infectoria* surface at operating conditions T=70^{o}C, solvent= CTAB, solvent's concentration= 0.1 M, sample-to-solvent ratio=1:10 at 8 hours extraction time (a) control, (b) after UAE (US probe, 40% duty cycle)

Figure 8(a) illustrated the surface of *Quercus Infectoria* galls powder in 500x scale as control. Basically, the *Quercus Infectoria* galls looked alike ball-shaped with numerous protruding blunt horn-like lumps over the external surface. The surface is greyish-brown to brownish-black in colour.

Figure 8(b) represented the rough rugae-like surfaces governing the whole integument of the sample powder after extraction using ultrasonic probe-type. It can be clearly seen that the sonication power from the ultrasonic affected the hardness of the sample surface and make *Quercus Infectoria* galls powder looser than before. It can be proved that the gallic acid may easily be extracted from the severe ruptures on the surface of the samples by sonicated process using probe due the images attributed from both Figure 8(a) and (b).

Conclusion

This research was carried out to determine the performance of ultrasound-assisted extraction method in extraction of phenolic acids from *Quercus Infectoria* galls. The maximum phenolic acids yield extracted was at temperature 70^{o}C with sonication intensity 8.66 W/cm^{2} and 40% duty cycle, diluted in solvent with the addition of the CTAB surfactant at concentration 0.1 M with sample-to-solvent ratio 1:10 for 8 hours resulted to the maximum concentration of phenolic acids at 2155.772 mg/ kg and 15236.83 mg/kg for gallic acid and tannic acid, respectively. The efficiency of the ultrasound-assisted extraction (ultrasonic probe-type) procedure exceeds the extraction using ultrasonic cleaner and conventional extraction by improving the yield and shortens the extraction time. The kinetic analysis by using Peleg's kinetic model indicates that higher rate of phenolic acids concentration were produced by using ultrasonic probe-type extraction method, which demonstrates a good agreement between the experimental data with the fitted data.

Acknowledgement

The authors are grateful for the financial support from the Master Research Scheme (MRS), the Fundamental Research Grant Scheme from Ministry of Higher Education (Grant No. RDU160154) and Universiti Malaysia Pahang.

References

[1] Shrestha, S., Kaushik, V. S., Eshwarappa, R. S. B., Subaramaihha, S. R., Ramanna, L. M., Lakkappa, D. B. Pharmacognostic studies of insect gall *of Quercus infectoria* Olivier (Fagaceae*). Asian Pac J Trop Biomed*. 4 (2014) 35–39.

[2] Hasmida, M. N., Liza, M. S., Nur Syukriah, A. R., Harisun, Y., Mohd Azizi, C. Y., Fadzilah Adibah, A. M. Total Phenolic Content and Antioxidant Activity of *Quercus infectoria* Galls Using Supercritical CO2 Extraction Technique and Its Comparison with Soxhlet Extraction. *Pertanika J. Sci. & Technol*. 23 (2014) 287 – 295.

[3] Nur Syukriah, A. R., Liza, M. S., Harisun, Y., Fadzillah, A. A. M. Effect of solvent on antioxidant and antibacterial activities from *Quercus Infectoria* (Manjakani). International Food Research Journal. 21 (2014) 1067-1073.

[4] Hermizi, H., Hasmah, A., Ima Nirwana, S. The Potential Role of *Quercus infectoria* Gall Extract on Osteoblast Function and Bone Metabolism. *Open Journal of Endocrine and Metabolic Diseases*. 2 (2012) 82-88.

[5] Ab. Rahman, N. S., Md. Salleh L., Abd. Majid F. A., Harisun, Y. Quantification of Gallic Acid and Tannic Acid from *Quercus infectoria* (Manjakani) and their Effects on Antioxidant and Antibacterial Activities. *Pertanika J. Sci. & Technol.* 23 (2015) 351 – 362.

[6] Md Salehan, N. A., Sulaiman, A. Z., Ajit, A. Effect of Temperature and Sonication on the Extraction of Gallic Acid from *Labisia Pumila* (Kacip Fatimah). *ARPN Journal of Engineering and Applied Sciences*. 11 (2016) 2192-2198.

[7] Chua, L. S., Lee, S. Y., Abdullah, N., & Sarmidi, M. R. Review on *Labisia pumila* (Kacip Fatimah): Bioactive phytochemicals and skin collagen synthesis promoting herb. Fitoterapia. 83 (2012). 1322-1335. doi: DOI 10.1016/j.fitote.2012.04.002.

[8] Chemat, F., Rombaut, N., Sicaire, A-G., Meullememiestre, A., Fabiano-Tixier, A-S., Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*. 34 (2017) 540-560.

[9] Annegowda, H. V., Bhat, R., Tze, L. M., Mansor, S. M. Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. Journal of Food Sci. Tech. 49 (2012) 510-514.

[10] Segovia, F. J., Corral-Perez, J. J., & Almajano, M. P. Avocado seed: Modelling extraction of bioactive compounds. Industrial Crops and Products. 85 (2016) 213-220.

[11] D'Alessandro, L. G., Dimitrov, K., Vauchel, P., Nikov, I. Kinetics of ultrasound assisted extraction of anthocyanins from *Aronia melanocarpa* (black chokeberry) wastes. Chemical Engineering Research and Design. 92 (2014) 1818-1826.

[12] Vetal, M.D. Lade, V.G., Rathod, V.K. Extraction of ursolic acid from *ocimum sanctum* by ultrasound: process intensification and kinetic studies. Chem. Eng. Process. 69 (2013) 24–30.

[13] Karacabey, E., Bayindirli, L., Artik, N., MAzza, G. Modelling Solid0Liquid Extrcation Kinetics of Trans-Resveratrol and Trans-ε-Viniferin from Grape Cane. *Journal of Food Process Engineering*. 36 (2013) 103-112.

[14] Shirsath, S. R., Sable, S. S., Gaikwad, S. G., Sonawane, S. H., Saini, D. R., Gogate, P. R. Intensification of extraction of curcumin from *Curcuma amada* using ultrasound assisted approach: Effect of different operating parameters. Ultrasonics – Sonochemistry. 38 (2017) 437-445.

[15] Xu, J. J., Li, Q., Cao, J., Warner, E., An, M., Tan, Z., Wang, S. H., Peng, L. Q., Liu, X. G. Extraction and enrichment of natural pigments from solid samples using ionic liquids and chitosan nanoparticles. *Journal of Chromatography A*. 1463 (2016) 32-41.

[16] Yang, Z., Tan, Z., Li, F., Li, X. An effective method for the extraction and purification of chlorogenic acid from ramie (Boehmeria nivea L.) leaves using acidic ionic liquids. Industrial Crops and Products. 89 (2016) 78–86.

[17] Wang, H. J., Pan, M. C., Chang, C. K., Chang, S. W., Hsieh, C. W. Optimization of Ultrasonic-Assisted Extraction of Cordycepin from Cordyceps militaris Using Orthogonal Experimental Design. Molecules. 19 (2014) 20808-20820. doi: 10.3390/molecules191220808

[18] Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Mayot, L., Ballesteros, R., Conidi, C., Cassano, A. Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (*Citrus paradise L.*) solid wastes. LWT – Food Science and Technology. 64 (2015) 1114-1122.

[19] Teng, H & Choi, Y. H. Optimization of ultrasonic-assisted extraction of bioactive alkaloid compounds from rhizoma coptidis (*Coptis chinensis* Franch.) using response surface methodology. Food Chemistry 142 (2014) 299–305.

[20] Rabie, K., Giovana, B. C., Amyl, G., Brooks, S. L. Marianne. Effect of Thawing Conditions on Polyphenol Content and Antioxidant Activity of Frozen Haskap Berries (*Lonicera caerulea L.*). Current Nutrition & Food Science. 11 (2015) 223-230.

[21] Maran, J. P., Priya, B., Nivetha, C. V. Optimization of ultrasound-assisted extraction of natural pigments from *Bougainvillea glabra* flowers. Industrial Crops and Products 63 (2015) 182–189.

[21]Majd, M. H., Rajaei, A., Bashi, D. S., Mortazavi, S. A., Bolourian, S. Optimization of ultrasonic-assisted extraction of phenolic compounds from bovine pennyroyal (*Phlomidoschema parviflorum*) leaves using response surface methodology. Industrial Crops and Products 57 (2014) 195–202.
[22]Maran, P., Manikandan, S., Nivetha, C. V., Dinesh, R. Ultrasound assisted extraction of bioactive compounds from *Nephelium lappaceum L*. fruit peel using central composite face centered response surface design. Arabian Journal of Chemistry 10 (2017) 1145–1157.
[23]Devgun, M., Nanda, A., & Ansari, S. H. Comparison of conventional and non-conventional methods of extraction of heartwood of *Pterocarpus marsupium Roxb*. Acta Pol, Pharma. Drug. Research. 69 (2012) 475-485.
[24]Torres, N. M., Talavera, T. A., Andrews, H. E., Contreras, A. S., Pacheco, N. Ultrasound Assisted Extraction for the Recovery of Phenolic Compounds from Vegetable Sources. Agronomy. 7 (2017) 1-19. doi: 10.3390/agronomy7030047.
[25]Jeevan, K. S. P., Garlapati, V. K., Dash, A., Scholz, P., Banerjee, R. Sustainable green solvents and techniques for lipid extraction from microalgae: A review. Algal Research. (2017) 138-147.
[26]Tamari, F., Hinkley, C. S., and Ramprashad, N. A Comparison of DNA Extractionn Methods using Petunia hybrid Tissues. Journal of Biomilecue Techniques. 24 (2013) 113-118.
[27]Ma, C. H., Wang, S. Y., Yang, L., Zu, Y. G., Yang, F. J., Zhao, C. J., Zhang, L., Zhang, Z. H. Ionic liquid-aqueous solution ultrasonic-assisted extraction of camptothecin and 10-hydroxycamptothecin from Camptotheca acuminata *samara*. Chem. Eng. Process. 57 (2012) 59-64.