Ultrasound- assisted extraction propolis and its kinetic study

IOP Conf. Series: Materials Science and Engineering **736** (2020) 022089 doi:10.1088/1757-899X/736/2/022089

Ultrasound- assisted extraction propolis and its kinetic study

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Abstract. In current times, there has been a growing attention in the consumption of functional food such as propolis due to the abundant of available bioactive compounds such as polyphenols. There are several health properties of propolis reported on having a huge spectrum of biological properties including anti- inflammatory, antioxidant, anticancer and antimicrobial. Maceration is known for its longer extraction time issue. Ultrasonic- assisted extraction is seen as an alternative to solve this problem. This study focused on indicating the factors influencing solid liquid ultrasonic- assisted extraction of total polyphenols content (TPC) from propolis using ethanol as solvent. The effects of three main factors; temperature (25°C, 35°C, 45°C, 55°C, 65°C), reaction time (5, 10, 15, 20, 25 minutes) and concentration of ethanol (50:50, 60:40, 70:30, 80:20, 90:10) (v/v) were studied to achieve maximum total phenolic content. The optimum conditions for ultrasonic- assisted extraction of phenolic content from propolis are with 70% ethanol, at 65°C for 25 mins. From the experimental data of kinetics study, the ultrasonic extraction of phenolic compounds from propolis is the second- order kinetic model and the extraction constant (k_2) was 2.8998 g mg^{-1} min^{-1}.

1. Introduction

Natural products such as propolis, have been attributed as valuable sources of substances used for discovery and development of new therapeutic agents. These days, products made from bee (honey, propolis, royal jelly, bee pollen or bees wax) are building reputation due to the medicinal beneficial values of the bioactive compounds [1]. Propolis is composed of resin- based substance collected by bees from exudates and bud of the plants and mixed with was and bee enzymes [2]. Propolis has a various diversified chemical composition and they are unique to their geographical regions. So far, there are more than 300 compounds that have been recognised to exist in propolis [3]. There is a broad spectrum of biological effects of propolis which include anticancer and antioxidant to antiviral and anti-inflammatory properties [4]. Phytochemicals; phenolic compounds particularly are acknowledged to be valuable to human health because they have the potential to reduce the risk of degenerative diseases by reducing oxidative stress and preventing the oxidation of macromolecular [1]. Mohdaly et al., (2015) stated that phenolic compounds have recently drawn awareness among scientists for having diverse properties, such as antimicrobial, anti- diabetic, anti- hyperlipidemia or anti- inflammatory effects but antoxidant has been the main activity reported for these compounds. Phenolics can be obtained from

propolis extracted from ethanol, methanol [5], ethanol- water mixture [6- 10] and methanol- water mixture [11]. Among all the extracts, propolis extract obtained from ethanol was mostly favored because they have a plentiful of phenolic acids and flavonoids [2].

The most common extraction method is maceration. Maceration method is generally is similar to producing alcohol tincture; the raw material is immersed in the solvent (normally alcohol) and the extract will be obtained after certain period of time. Most researchers obtained propolis extract from maceration due to its simplicity and no special requirement of advanced technology equipment or devices. However, maceration is time- consuming, demanding extraction duration from 2 to 10 days [12]. Ultrasonic-assisted extraction is seen as an alternative to solve this problem. The mechanical effect offered by ultrasound enhances the solvent diffusion into the sample matrix, increasing the contact surface area between the solid sample and solvent [13]. The major advantages of this method are the method can be reused due to a various sample sizes, the sudden decline in tie required to execute a greatly efficient extractions of polar organic compounds [14].

There is lack of sufficient studies on the kinetic studies of ultrasonic- assisted extraction of propolis. The kinetic study of the extraction is carried out after one factor at time (OFAT) process achieved [15]. With the assistance of ultrasound, ultrasonic- assisted extraction of propolis is considered as a second- order mechanism solid liquid extraction. Second- order mechanism model simply means that there are two simultaneous phases in the extraction [16]. The rate of dissolution of phenolic compound is determined by:

$$\frac{dCs}{dt} = k(C_s - C_t)^2 \tag{1}$$

k is represented as second order extraction rate constant (g mg^{-1} min^{-1}), C_s is the concentration of total phenolic content saturation (mg g^{-1}) and C_t is the concentration of total phenolic content at any time (mg GAE g^{-1} propolis). In order to figure out the kinetic parameters, the above equation is integrated under the boundary conditions, $C_t = 0$ to C_t and t = 0 to t, and written in a linearized form:

$$\mathrm{Ct} = \frac{\mathrm{C_{s^2}} \,.\, \mathrm{k.\,t}}{1 + \mathrm{Cs.\,k.\,t}} \tag{2}$$

$$\frac{t}{C_t} = \frac{1}{k.C_{s^2}} + \frac{t}{C_s} = \frac{1}{h} + \frac{t}{C_s} \tag{3}$$

Where h refers to the initial extraction rate (mg g^{-1} min^{-1}) when t and C_t approach 0. Lazar et al., (2016) suggested that the total phenolic content concentration at any time can be expressed as (after rearrangement):

$$C_t = \frac{t}{\left(\frac{1}{h}\right) + \left(\frac{t}{Cs}\right)} \tag{4}$$

By plotting a graph of t/ C_t versus t, the extraction capacity (C_s), the second order extraction constant (k) and the initial extraction rate (h) can be determined from experimental works. The objectives of the present study were to investigate the factors contributing to solid liquid extraction of phenolic

compounds of propolis via ultrasonic- assisted extraction and to study the kinetics and extraction mechanism based on the second- order extraction model.

2. Materials and Method

2.1 Material

Crude propolis was obtained from a local farmer in Kuantan, Malaysia. The propolis was grinded and sieved through a 68μm mesh. The sieved propolis powder was stored in an airtight glass bottle and kept at 4°C until use. All chemicals and solvents used were of analytical grade.

2.2 Methodology

2.2.1 Effects of Extraction Solvent Types, Ethanol Concentration, Extraction Time and ExtractionTemperature

2.2.1.1 Selection of extraction solvent

0.5 g of crude propolis was weighed and dissolved in 5 ml of 70% (v/v) ethanol, 70% (v/v) methanol and 70% (v/v) acetone in individual test tube. They were kept for sonication at ambient temperature. The crude propolis sample weights were maintained for all ethanol volumes.

2.2.1.2 Ethanol Concentration

0.5g of crude propolis was macerated with 50% (v/v), 60% (v/v), 70% (v/v),80% (v/v) and 90% (v/v) ethanol, and sonicated for 20 minutes at ambient temperature.

2.2.1.3 Extraction Time

Crude propolis (0.5 g) was macerated in 5 ml of 70% (v/v) ethanol, and then left for sonication at different extraction times starting from 5 to 25 minutes at ambient temperature (25°C)

2.2.1.4 Temperature

Half a gram of crude propolis were soaked with 70% (v/v) ethanol, and exposed to sonication for 20 minutes with the extraction temperatures at 25°C, 35°C, 45°C, 55°C and 65°C.

The extracts obtained were used for the determination of the total phenolic content for all experiments.

2.2.2 Ultrasonic- assisted extraction method

The extraction was executed with a sonicator (Q500, QSonica, USA) with probe size (0.5 inches, CL-334). The sonication generator working frequency was maintained constant at 20 kHZ. Samples of crude propolis powder were placed into test tubes (15 ml) and the volumes were made up to 5 ml with the extracting solvent and the test tubes were arranged in a beaker water bath. They were sonicated for different times at the required temperatures. By applying pulses mode ON for 10 seconds, the temperature was controlled. Once the extraction was done, the test tubes were removed from the bath and centrifuged by using (5810 R type, Eppendorf, Germany) at 4000 rpm for 10 minutes. Whatman No. 1 filter paper was used to filter through the propolis extracts and the solution was retrieved and stored in an amber bottle, and used for the quantification of TPC.

2.2.3 Determination of Total Phenolic Content (TPC)

The TPC of propolis extracts was quantified by applying Folin- Ciocalteu method [18] method. Briefly, 0.1 ml of propolis extract was combined with 0.2 ml Folin- Ciocalteu reagent (diluted 1/10) and 1 ml of distilled water. After a brief shake and left to stand for 5 minutes, 1 ml of 6% (w/v) sodium bicarbonate was added and the mixture was shaken again. The mixture was incubated in the dark for 30 minutes at ambient temperature. After the incubation was done, the solutions were measured for their absorbances at 760 nm using UV- Vis spectrophotometer (UV- 1800 type, Shimadzu, Japan). The total phenolic

content of extracts was calculated from the gallic acid calibration curve (Y = 1.885x + 0.0735, R^2 = 0.9354) and presented as milligram gallic acid equivalent (mg GAE/ g propolis). Data are presented as means ± SD for at least three replications.

3. Results and Discussion

3.1 Effects of Types of Solvent

Figure 1 shows the relationship between different types of solvent with the same concentration, 70% (v/v) and the amount of total phenolic content extracted from each solvent. It was discovered that 70% acetone (v/v) yields the greatest amount of total phenolic content which is 0.071 mg GAE/ g propolis extract, whereas 70% methanol (v/v) has the lowest. Even though 70% acetone (v/v) has the highest amount of total phenolic content extracted, 70% ethanol (v/v) was chosen in the current work. Ethanol was selected as the solvent of the extraction in this study because they are safer and less toxic as compared to acetone, methanol and other organic solvent [19]. Besides, the amount of total phenolic content obtained between these two solvents; ethanol (70% v/v) and methanol (70% v/v) are not significantly different as the value of $p>0.05$.

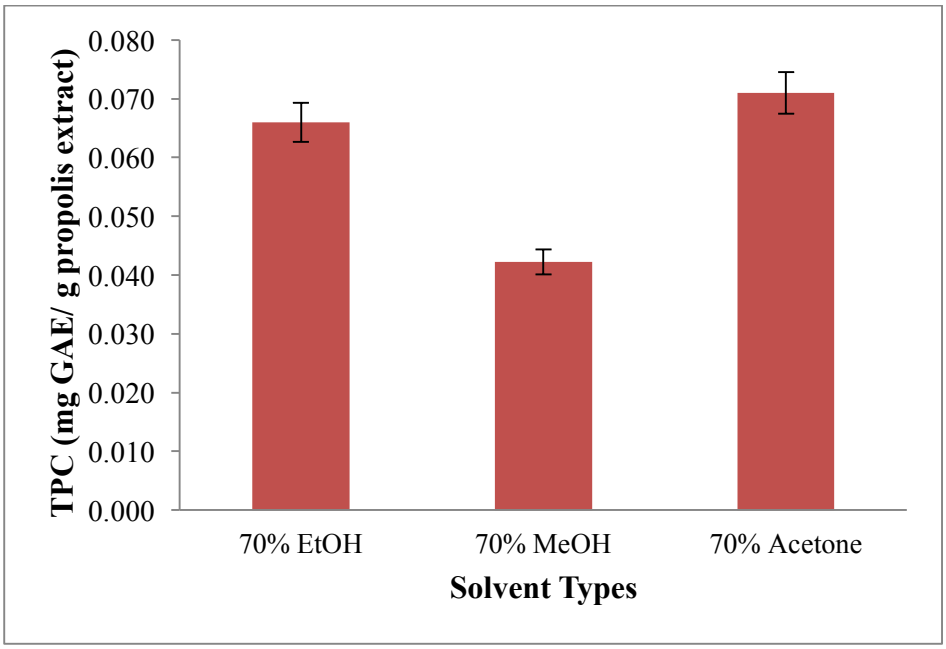

Figure 1. Effects of different types of solvent on total phenolic content extracted from propolis

3.2 Effects of Ethanol Concentration

Figure 2 illustrates the influence of different concentrations of the ethanol on total phenolic content of propolis extract. It was found that when the ethanol concentrations elevated from 50% to 80% (v/v), the amount of TPC increased from 0.038 to 0.110 mg GAE/ g propolis. The highest total phenolic content was recorded at 80% ethanol (v/v). Similar finding was reported for extraction of rambutan (*Nephelium lappaceum*) peel using 80% ethanol (v/v), resulted the highest total phenolic content [20]. From the economical point of view, 70% ethanol (v/v) was selected to study the effects of extraction time and temperature on total phenolic content in this study. When ethanol concentration increased to 90%, the

total phenolic content measured declined to 0.103 mg GAE/ g extract. There is a possibility that if the higher ethanol concentration was used to extract propolis, a number of lipids constituents were also extracted and this might restrict the phenolic extraction from propolis as reported by [21] which studied on total phenolic content extracted from wheat bran. It is noticed that ethanol has a biphasic effect on the extraction of phenolic compounds.

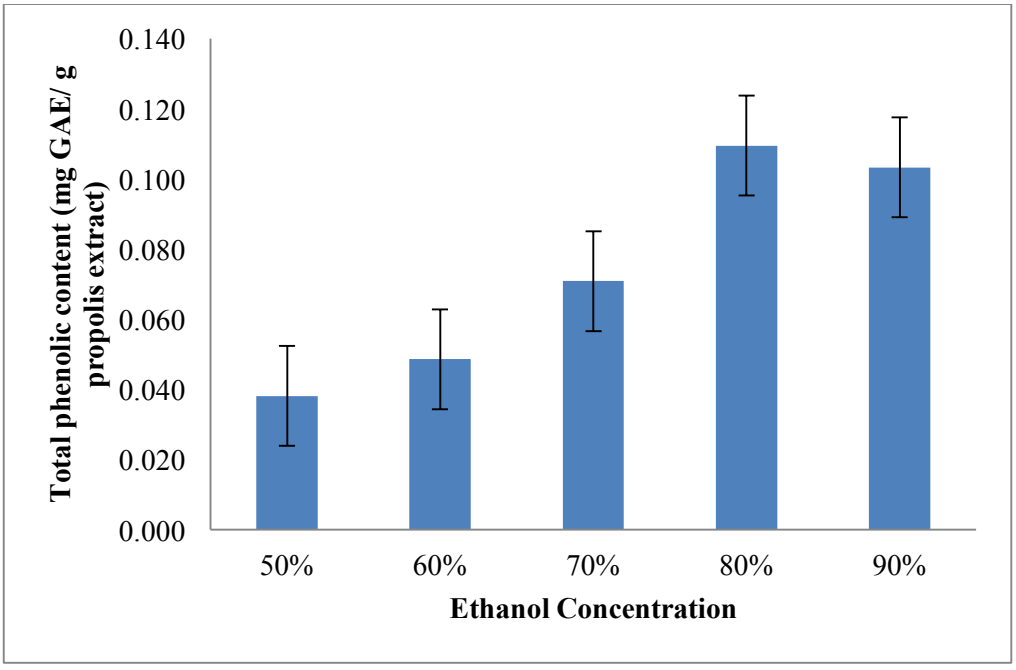

Figure 2. Effects of different ethanol concentration on total phenolic content from propolis

3.3. Effects of Extraction Time

Figure 3 shows the influence of different extraction times and total phenolic content extracted from propolis at 25°C. As the extraction times increased from 5 mins to 20 mins, the concentration of propolis measured was increased from 0.058 mg GAE/ g to 0.065 mg GAE/ g. The highest amount concentration was shown at 25 mins, which was 0.0079 mg GAE/ mg propolis. With constant temperature throughout the experiment, the total phenolic content increased as the extraction time raised due to the longer duration of the propolis sample being in contact with the extraction solvent. Similar finding was reported by [22], which studied on orange peels' polyphenols that were extracted by the sonicated extraction. As stated by Chew et al., (2011), prolonged extraction would increase the possibility of the occurrence of oxidation of phenolic compounds and thus reducing the quantity of extracted phenolic compounds.

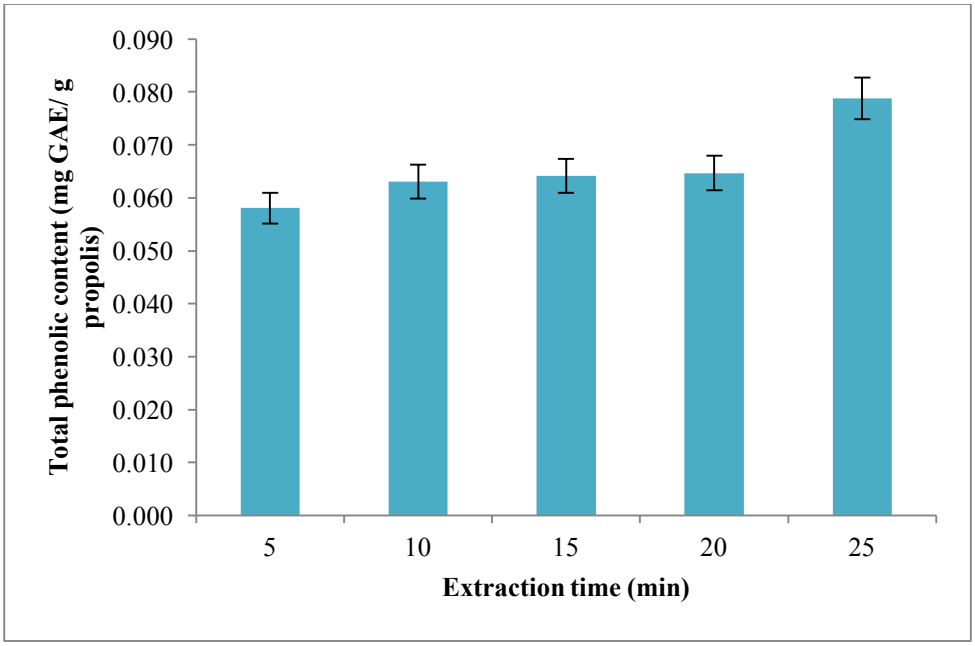

Figure 3. Effects of different extraction times on total phenolic content from propolis

3.4 Effects of Temperature

Figure 4 shows the effect of different extraction temperatures on TPC of propolis was measured at five different extraction durations (5, 10, 15, 20, 25 mins). As depicted by the figure, higher temperature would increase the amount of total phenolic content extracted. The highest temperature, 65°C yields the most total phenolic content, which is 0.093 mg GAE/ g propolis at 25 min. The lowest temperature 25°C recorded the lowest total phenolic content at 5 min which is 0.058 mg GAE/ g propolis..The temperature cause changes to physical properties; vapour pressure, surface tension, viscosity, of the solvent [23]. It also could affect the cavitation process in ultrasound- assisted extraction Higher temperature would lead to the cell wall breakage and thus increased the diffusivity of the phenolic compounds. Besides, it also caused the propolis to be less viscous and hence accelerating the extraction process. Increasing temperature favoured extraction by enhancing both the solubility and the diffusion coefficient from a solid matrix [24]. However, phenolics are heat- sensitive compounds which higher extraction temperature. By increasing the extraction temperature to 100°C, it is estimated that the amount of TPC would keep on decreasing as it way surpasses the boiling point of ethanol (78°C). The solvent will be evaporated to the air and the volume will be reduced hence, this would reduce the extraction efficiency.

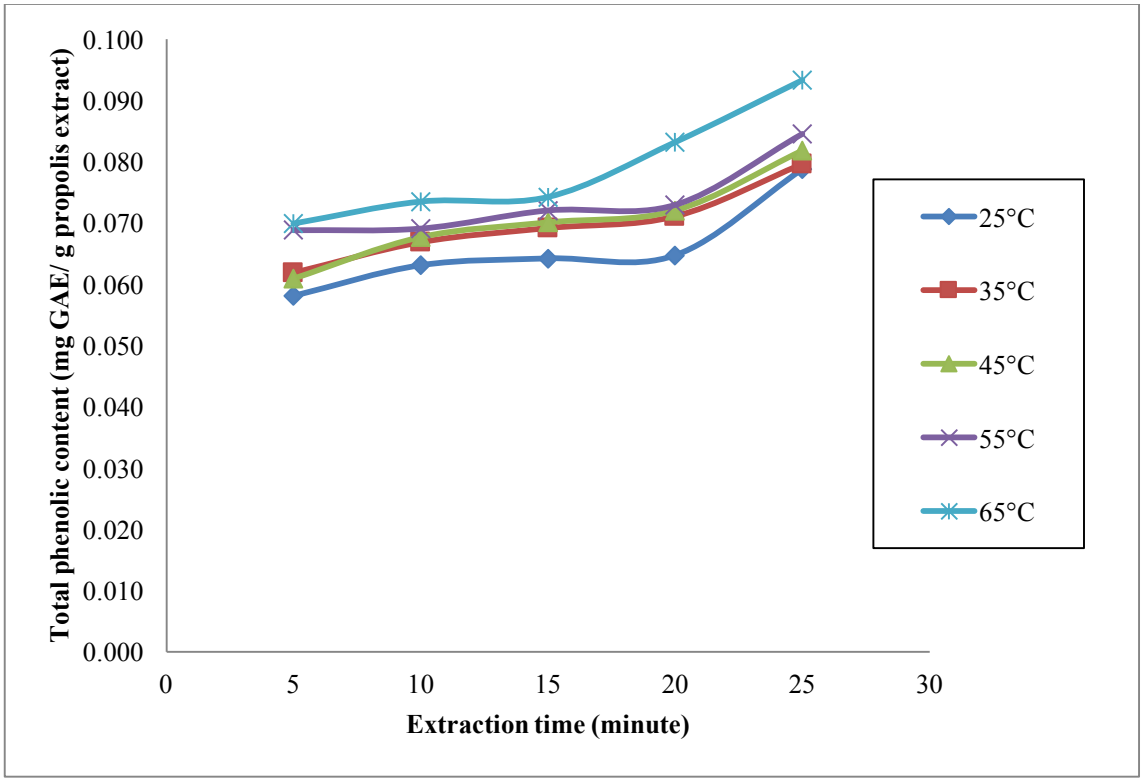

Figure 4. Effects of different extraction temperature on total phenolic content from propolis for specific times

3.5 Kinetic Study

Kinetic study was done to evaluate the relationship between the different extraction temperatures to extraction efficiency of TPC and also to identify the extraction model. There is an insufficient study on the kinetic study made on TPC. Nevertheless, there was a previous study done by [25] on the antioxidation capability by applying 2.2-diphenyl-1-picrylhydrazyl radical (DPPH) reagent and it was found that the second- order rate constant (k_2) for the oxidation of propolis extract was 0.17 $dm^3g^{-1}s^{-1}$.

In this work, a graph of t/ Ct versus extraction time (t) was plotted to determine the specific kinetic parameters such as initial extraction rate (h), extraction rate constant or concentration at saturation (C_s) and extraction rate constant (k) based on the linear mathematical regression equation. The extraction temperature of 65°C was selected in the kinetic study because it yielded the highest amount of total phenolic content. Figure 5 proves that the rate of extraction was higher at the early stage of the extraction and it got slower approaching 25 mins.

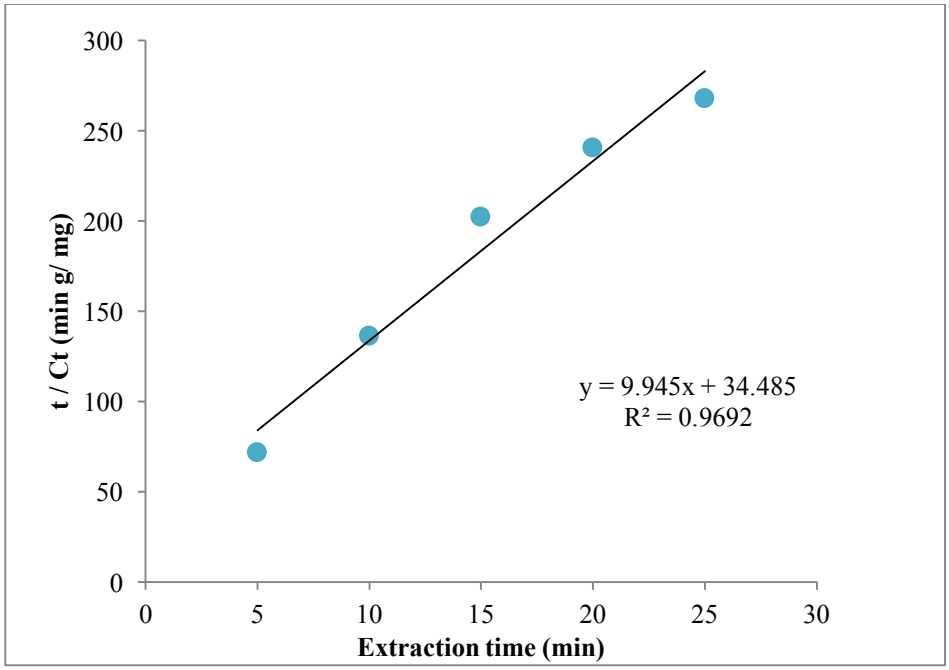

Figure 5. Second order extraction kinetics of total phenolic content extraction from propolis

At the beginning (first stage), the TPC measured was the lowest but as the duration of the extraction increased, the amount of TPC exponentially increased. From Figure 5, it is shown that for both 5 to 10 mins and 10 mins to 15 mins, the value of t/Ct increased to more than a half. Meanwhile, as the extraction time extended to 25 mins, the value was gradually decreased to 27.47 min g/ mg. It is proven that the rate of diffusion decreased as the extraction duration increased due to the high concentration of solute in the extraction solvent at the second phase [16]. Table 1 summarizes the kinetic parameters for the extraction of phenolic compounds from propolis with the assistance of sonication.

4. Conclusion

The mechanism of the extraction of phenolic compounds of propolis facilitated with ultrasound and macerated with ethanol can be divided into two parts; (1) washing and dissolution of phenolic compounds and (2) slow extraction or diffusion from the solid matrix to the liquid environment. The yield of total phenolic content and kinetic of solid- liquid extraction was affected by the type and concentrations of solvent, extraction time and extraction temperature. Therefore, it can be decided that the optimum conditions for ultrasonic- assisted extraction of phenolic content from propolis are with 80% ethanol, at 65°C for 25 mins. The experimental data of the kinetics yields the second order extraction constant (k_2) of 2.8998 g mg^{-1} min^{-1} and it is in agreement and well- suited with the second order extraction model.

Table 1. *Linearization of second order kinetic model of solid liquid extraction of propolis*

C_s (mg g^{-1})	k_2 (g mg^{-1} min^{-1})	h (mg g^{-1} min^{-1})	R^2
0.1	2.8998	0.0290	0.9692

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