CHAPTER IV

RESULTS AND DISCUSSION

4.1 Ultrasound assisted extraction

Effect of ultrasonication and its duration on extraction

Figure 4.1 shows the effect of ultrasonication and its durations (15, 30, 45, 60, and 90 minutes) on the release of anthraquinones. The experiments were carried out at 25 °C and at the power setting of 3 (≈15.7 W). The control experiments were performed using maceration without ultrasonic exposure at the same temperature. Compared with maceration, ultrasound assisted extraction was found to enhance the extraction yield due to the cavitational effects, which cause the intensification of mass transfer and thus closed interaction between the solvent and the plant tissues. The collapse of cavitation bubbles near tissue surfaces produces microjets, causing tissue disruption and a good penetration of the solvent into the tissue matrix *(Fairbank, 2001; Mason, 1992; Povey, 1998; Lorimera&Paniwnyka, 1996*). As illustrated in the figure, the percent recovery increases with increasing duration of ultrasonic treatment as the contact time between liquid and solid plant materials is increased. During the first 15 minutes, the rate of anthraquinones extraction was high, after which it decreased considerably. One reason for this was due to the large difference between the initial anthraquinones concentration in the extraction solvent and its solubility. Another reason for the initially high rate could be that anthraquinones located in the outer region of particle was more readily accessible than that in an inner part in which the plant tissues are more intact. The extraction from the outer part is attributed to external mass transfer, which in this case is convective since fluid motion was provided as a result of ultrasonic cavitation. At later time, anthraquinones from the inner part of the root particles must diffuse through the pores of the root materials, resulting in much slower extraction rate.

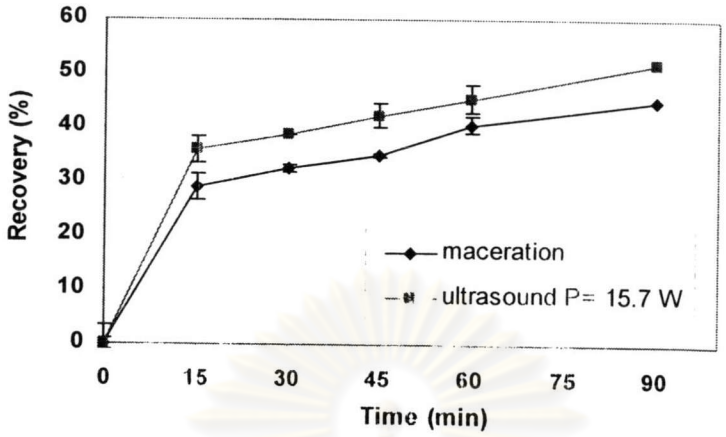

Figure 4.1 Effect of ultrasonication and its duration on extraction at 25 °C.

Effect of extraction temperature

The effect of ultrasonic extraction temperature on the release of anthraquinones is shown in Figure 4.2. The comparison shown in this figure was made for extraction in ethanol at a power setting of 3 at 25 °C, 45 °C, and 60 °C. Again, the product was rapidly released in the first 15 minutes, after which the release rate decreased. The percent recovery was found to increase with increasing temperature due to the increased solubility of anthraquinones in ethanol at higher temperature. In addition, at higher temperature, the liquid viscosity and density decrease, and thus resulting in increased mass transfer. Furthermore, as a result of decreased solvent viscosity, cavitation within the fluid as a result of ultrasonic treatment occurs more easily as the cohesive force, and thus the tensile strength of the liquid is reduced. This explanation is also supported by the study of *Albu et al.*, (*2001*) in which ultrasonic assisted extraction was investigated for extraction of carnosic acid from rosemary.

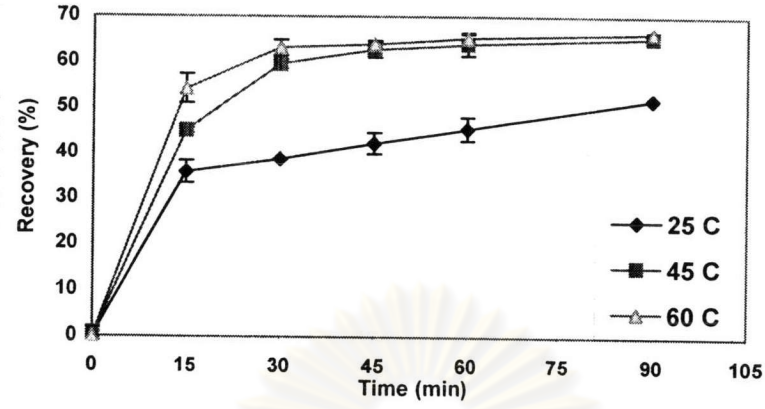

Figure 4.2 Effect of extraction temperature for ultrasonic extraction in ethanol at a power 15.7 W.

Effect of type of solvents

The effect of the type of solvents on ultrasound assisted anthraquinones extraction was determined for four commonly used solvents: acetone, ethanol, methanol, and acetonitrile. The ultrasound assisted experiments were conducted at 25 °C and at the power about 15.7 W for 45 minutes and the results were compared with maceration at the same temperature as shown in Figure 4.3.

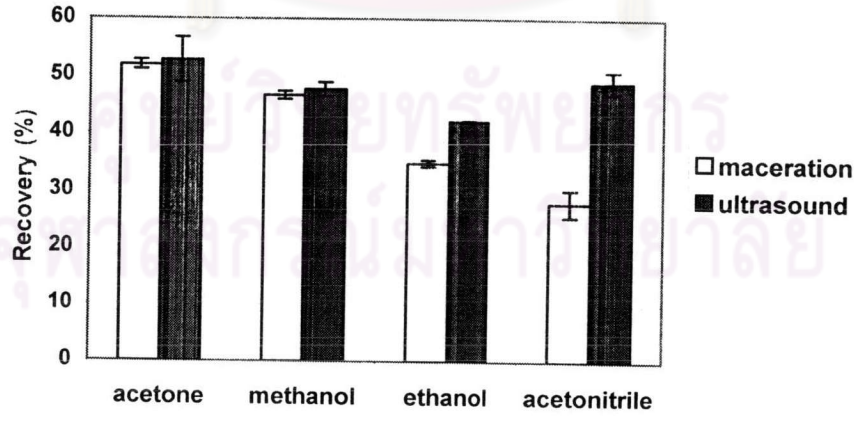

Figure 4.3 Effect of type of solvents on maceration and ultrasound extraction at ambient temperature

As seen in the figure, for the case of maceration, acetone gave the highest percent recovery, followed by methanol, ethanol and acetonitrile, respectively. The different extraction efficiencies of these solvents may be attributed to their differing polarities and viscosities. Some properties of the solvents used in this study is listed in Table 4.1. Note that the polarity indices of acetone, methanol and ethanol are similar. Acetone has the lowest viscosity and thus the solvent could most easily diffuse into the root matrix thus resulting in the highest extraction efficiency after the 45 minutes. Surprisingly however, acetonitrile on the other hand has the lowest viscosity compared to the other three solvents, but gave the lowest extraction efficiency. This could be due to its higher polarity that results in lower solubility than the other solvents.

When ultrasound was applied, acetone yields the highest anthraquinones in the extract, followed by acetonitrile, methanol, and ethanol. The results in Figure 4.3 reveals that the extraction efficiency could generally be enhanced, by application of ultrasound, however the degree of enhancement differs depending on the type of solvent. As seen here, there was almost no increase in the extraction efficiency as a result of ultrasound for extraction in acetone and methanol. However, for ethanol and acetonitrile, the increase in the efficiency of extraction was significant. This is due to the influence of solvent properties on the occurrence and the intensity of cavitation. As summarized in Chapter 2, solvent properties impacting the behavior of ultrasonic cavitation includes vapor pressure, viscosities, and surface tension. Of these properties, medium vapor pressure is most conducive to ultrasound activity. Ultrasonication in low vapor pressure liquid produces few cavitational bubbles as a result of higher cavitation threshold; however the bubbles implode with relatively greater force, which enhances plant tissue disruption during extraction. High vapor pressure liquid on the other hand is not very effective - more bubbles are created, but they collapse with less intensity due to a smaller internal/external pressure differential. As for the liquid viscosity, acoustic cavitation occurs more easily in liquid with low viscosity as the ultrasonic intensity applied could more easily exceeds the molecular forces of the fluids. Furthermore, liquid with lower viscosity has lower density and higher diffusivity, and can more easily able to diffuse into the pores of the plant materials (*Li et al., 2004*). Surface tensions of liquid also influence cavitational effects. In liquid with small surface tension, the energy required in

order to produce cavitation bubbles, thus cavitation occurs more readily. These values at ambient temperature for the solvents tested in this study are summarized in Table 4.1.

Table 4.1 Type of solvents properties (at 25 °C)

Type of solvents	Polarity index	Surface tension (mN/cm)	Vapor pressure (mmHg)	Viscosity (cP)
acetone	5.1	23.7	229.52	0.32
methanol	5.1	22.6	127.05	0.6
ethanol	5.2	23.7	59.02	1.2
acetonitrile	5.8	19.1	88.47	0.38
water	9	72.8	23.8	0.89

As seen in Table 4.1, acetone, methanol, and ethanol have similar surface tensions, but differing vapor pressures and viscosities. Although acetone and methanol have low viscosity that seem cavitation occur easily but it has high vapor pressure thus resulting acetone and methanol do not induced intensive cavitational collapses - more bubbles may be created, but they collapse with less intensity. Of all the solvents tested, the increase in the extraction efficiency of acetonitrile was found the greatest possibly because of its relatively low viscosity and surface tension which help increase the number cavitation bubbles, whose collapses were further intensified as a result of its relatively low vapor pressure. The effect of vapor pressure was also evident for the case of ethanol. Although ethanol has high viscosity, the intensity of cavitational collapses is violent due to its low vapor pressure.

Summarized in Table 4.2 is the percent increase in anthraquinones recovery as a result of ultrasonication in various solvents. It is clear that ultrasonication could enhance the extraction efficiency to the greatest extent in acetronitrile then followed by ethanol. However, acetonitrile is highly toxic solvent; we therefore select ethanol as extraction solvent for the further study.

Table 4.2 Percent increase in anthraquinones recovery by ultrasound assisted extraction

Type of solvent	Recovery (%) using maceration	Recovery (%) using ultrasound	Percent increase in recovery
Acetone	51.94 ± 0.043	52.78 ± 3.95	0.84
Methanol	46.68 ± 0.711	47.71 ± 1.23	1.03
Ethanol	34.81 ± 0.621	42.17 ± 0.107	7.36
Acetonitrile	27.87 ± 2.36	48.92 ± 1.93	21.05

Effect of ultrasonic power

The effect of ultrasonic power was determined for 45 minute extraction at 25 °C and the power of ultrasonic was measure by watt meter (energy check 3000, Germany). The results are shown in Figure 4.4 which illustrates that the product recovery was increased with increasing ultrasonic power. This is because as large amplitude of ultrasound wave travel through a mass medium, the bubbles collapse more violently.

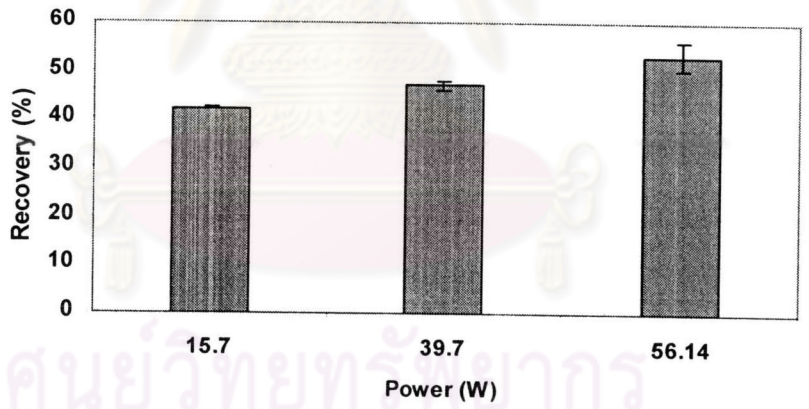

Figure 4.4 Effect of ultrasonic power at 25 °C for 45 minutes duration.

Effect of ethanol-water compositions

The effects of ethanol compositions on the anthraquinones recovery obtained after 45 minutes of maceration and ultrasound assisted extraction at ambient temperature are shown in figure 4.5.

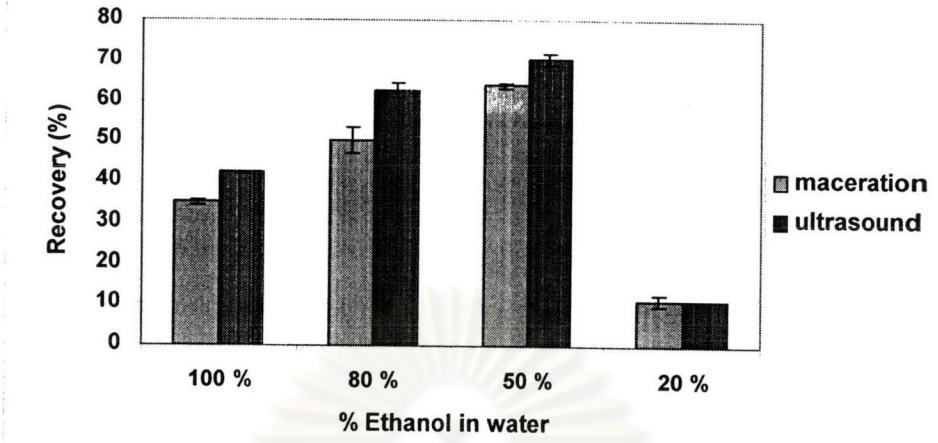

Figure 4.5 Effect of ethanol compositions on extraction efficiency of maceration and ultrasound extraction

This result demonstrates that the improvement of extraction efficiency with the addition of some amount of water was possible for both maceration and ultrasound assisted extraction. The percent product recovery increased with increasing percentage of water up to 50%. The increase in the extraction efficiency is attributed to the effective swelling of the plant, which helps increase the surface area for solute-solvent contact. Furthermore, the presence of water lowers the mixture viscosity, thus mass transfer is improved. At higher water concentration as in 20% ethanol however, the product recovery was the lowest as high content of water increases the mixture polarity to the degree that it no longer is favorable for extraction of anthraquinones.

When ultrasound (power 15.7 W, at 25 °C) was applied, extraction efficiency could be improved due to the effect of ultrasonic cavitation as mentioned previously. The same trend was resulted as with maceration in which the extraction efficiency increased with the amount of water added until up to 50 %. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture is also increased as the surface tension increases and the viscosity and the vapor pressure decrease. In addition to the cavitational effects, ultrasound has been shown to facilitate the hydration processes of dried materials which cause the plant materials to swell more readily. This rehydration is important to help regain the capacity of diffusion and osmosis that had been lost as a result of tissue desciccation that occurs during the drying process. Despite, the high

vapor pressure, surface tension, and low viscosity, the mixture of 20% ethanol has the polarity too high than it can be effective to dissolve the anthraquinones.

4.2 Microwave assisted extraction

In the second part of this study, microwave assisted anthraquinones extraction was investigated. For all experiments, 60% of power output (60% of 1200 W) was used. The ramping times for all extraction runs were 2 minutes and the sample to solvent ratios were fixed at 0.01 g/ml. The effects of various factors are investigated.

Effect of extraction times

The release profile of anthraquinones for various durations (5, 10, 15, 20, and 30 minutes total time, including ramping time and holding time) of microwave extraction is shown in Figure 4.6 for assisted extraction at 60 ° C. Similar to ultrasound assisted extraction, the yield of anthraquinones obtained using microwave assisted extraction increased with increasing times of extraction. The rate of anthraquinones release was the highest in the first 5 minutes, after which the release decreased considerably. When compared with ultrasonic extraction at the same temperature of 60 °C, the initial release rate for microwave extraction was higher. The percent recoveries of the product for both methods however approach the same value after about 18 minutes, and are much higher than that of maceration. The reason for this observation is that the heating rate in microwave equipment is more rapid than that in ultrasonic bath.

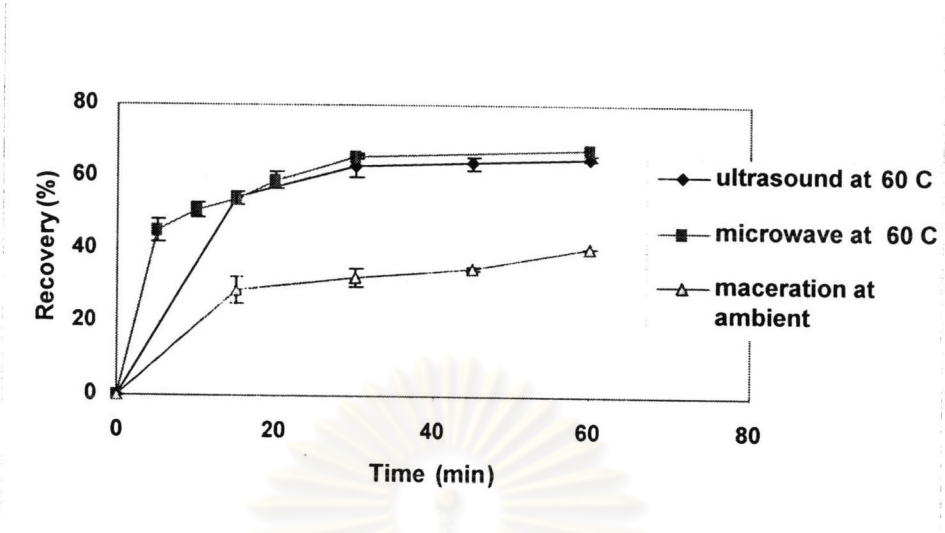

Figure 4.6 Effect of extraction times at power 15.7 W of ultrasound.

Effect of extraction temperatures

In a closed microwave vessel used in this study, the temperature of the solvent can be increased above the boiling point temperature. As a result, the solubility of anthraquinones in the solvent at elevated temperature can be greatly enhanced. The effect of extraction temperature is shown in Figure 4.7 which can be clearly seen that increasing the temperature from 60 to 120 ° C significantly increase the extraction efficiency due to the increased solute solubility and the increase in solvent diffusivity. Moreover, at high temperature, the viscosity of ethanol decreases, thus the efficiency of extraction increases.

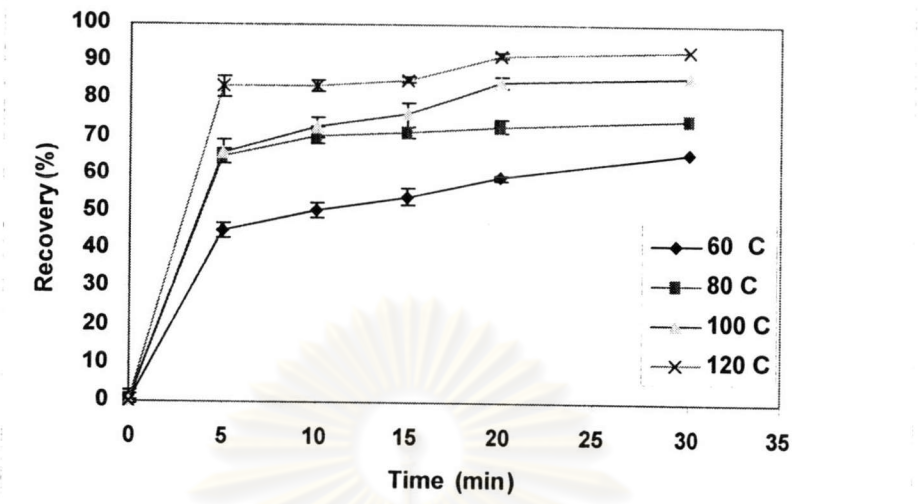

Figure 4.7 Effect of extraction temperature.

Effect of solvent type

The effect of the type of solvents on extraction efficiency of microwave extraction was determined at 60 ° C. The percent anthraquinones recovery after 15 minutes of microwave extraction in different solvents were compared. The results in Figure 4.8 demonstrate that methanol gave the highest extraction efficiency, followed by acetone, acetonitrile, and ethanol. As seen in this figure, the solvents behave differently in microwave assisted extraction and conventional extraction. Without microwave, the extractability of different solvents depends mainly on their polarity and viscosity as previously describe. Under the influence of microwave, the behavior of solvents is highly dependent not only on the solvent dielectric constant, but also its dissipation factor. The former is the ability of the solvent to absorb microwave energy, and the latter is the ability in which it dissipates the absorbed energy into heat. Thus, the higher the dielectric constant and dissipation factor, the higher heating rate could be resulted by the applied microwave energy.

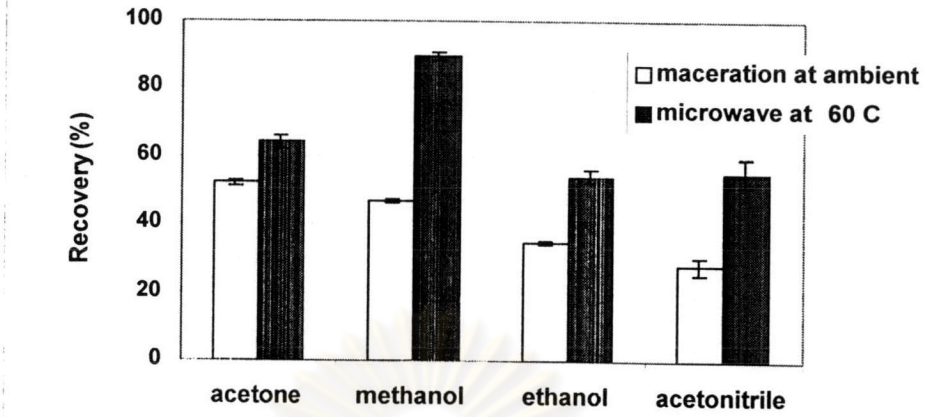

Figure 4.8 Effect of solvent types on efficiency of microwave extraction at 60 °C at 15 minutes.

The values for dielectric constants and dissipation factors are listed in Table 4.3. Methanol has relatively high dielectric constant and the highest dissipation factor, which means that it could absorb much of the microwave energy and transform it into heat better than the other solvents. Therefore, it is expected that the rate of microwave heating of methanol is the highest of all solvents tested in this study. This attributes to the highest extraction efficiency resulted. Although acetonitrile has higher dielectric constant than methanol, the dissipation factor of the solvent is low; the rate of heating of this solvent is therefore low. Next to methanol, acetone showed the highest extraction efficiency under the influence of microwave. Compared with ethanol, it has comparable dielectric constant but more than twice higher dissipation factor, thus acetone is expected to be more effective solvent than ethanol for microwave assisted extraction.

Table 4.3 Dielectric constant and dissipation factor of solvents

Type of solvents	Dielectric constant (F/m)	Dissipation factor
acetone	20.7	0.5555
methanol	32.7	0.6400
ethanol	24.3	0.2286
acetonitrile	37.5	0.062
water	80	0.15

Although methanol was shown to give the highest extraction efficiency, it is highly toxic and is not practical for use in food and pharmaceutical processing. Eventhough acetone showed the next highest efficiency, it is often more practical to start with the same solvent as is prescribed for the traditional extraction, which in this case is ethanol. In the next section, the effect of different composition of ethanolic solution in water would be investigated on the efficiency of microwave extraction.

Effect of ethanol-water compositions

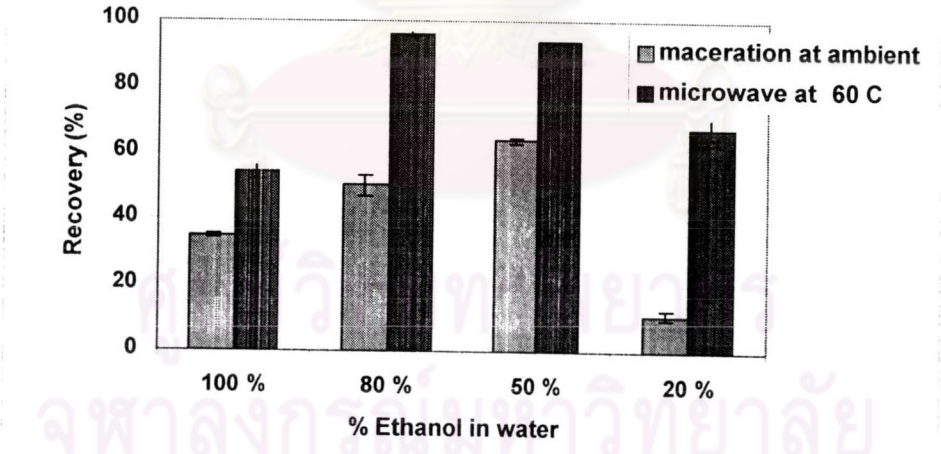

Figure 4.9 Effect of ethanol compositions on efficiency of microwave extraction at 60 °C at 15 minutes.

Figure 4.9 shows the effect of solvent compositions on efficiency of microwave extraction at 60° C. The results demonstrate that the use of microwave clearly enhanced the product yield in all compositions of solvents used. This is due to the increase in

extraction temperature. For microwave assisted extraction, 80% ethanol in water gave the highest percent recovery after15 minutes of extraction. Using both 80% and 50% ethanolic solution, 90% anthraquinones could be extracted within only 15 minutes. This is possibly due to the relative polarity of anthraquionones compound. Furthermore, some amount of water increases mixture dielectric constants, which helps absorb microwave energy, thus increasing the extraction efficiciency. The values for polarity indices and dielectric constant of ethanol mixtures at various compositions are shown in Table 4.4. The polarity indices of the mixtures increase with increasing water composition. The real polarity index of a mixture is very complicated to find but it may be determined approximately by the following linear equation

$$\Sigma P_{mix} = P_{ws} f_{ws} + P_{ss} f_{ss} \quad (4.1)$$

where P and f are the polarity index and volume fraction, respectively; and subscripts mix, ws, and ss represent values for the mixture, pure water, and pure solvent *(Perdersen et al., 1996; http://www.vydac.com/catalog/sections/Appendicies(204-229).pdf)*. In case of the dielectric constant of mixing solvents, it can be calculated from the following equation:

$$\varepsilon_r^{1/3} = \sum \left(\frac{V_i}{V}\right) \times \varepsilon_{ri}^{1/3} \quad (4.2)$$

where, ε_r is the dielectric constant of mixed solvent, *Vi* is the volume of *i* solvent, *V* is the total volume of mixed solvent, ε_{ri} is the dielectric constant of *i* solvent *(Hao et al., 2002)*.

In addition to the reason described above, the presence of water enhances swelling of plant material which increases the contact area between the plant tissues and the solvent, thus increasing mass transfer *(Palma et al., 2003; Li et al., 2005)*. However, if the amount of water was too high, the dielectric constant of the mixture increases but the dissipation factor decreases, the solvent mixture may be able to absorb high microwave energy but would not be able to dissipate the heat as effectively as solvent with lower percentage of water. It is, nevertheless, interesting to note here that, unlike maceration or ultrasonic extraction, microwave extraction with 20% ethanolic solution gave higher recovery than pure ethanol. A possible reason is that water could more easily penetrate into the plant material and reside within the cavity of plant matrix. When this water absorbs microwave and dissipate heat right next to the plant tissue, the heating

effect occurring within the plant matrix caused the product to more readily release. Furthermore, this heating effect within the plant tissue may enhance the structural changes of the plant matrix, thus further increasing the product mass transfer.

Table 4.4 Ethanol compositions properties

Ethanol compositions	Polarity index	Dielectric constant
100%	5.2	24.3
80%	5.96	32.126
50%	7.1	46.76
20%	8.24	65.27

4.3 Comparison of ultrasound and microwave with classical methods

The efficiency of extraction using ultrasound and microwave was compared with that of other classical methods. Table 4.5 summarizes the results.

Table 4.5 Comparison at maximum of percent recovery extraction times for each method.

Extraction methods	**Time**	**Temperature**	**Type of solvent**	**Recovery (%)**
Maceration	3 days	Ambient	Ethanol	63.33 ± 2.734
Soxhlet Extraction	4 hr.	Boiling point	Ethanol	97.74 ± 0.311
Ultrasound extraction	60 min	60 °C	Ethanol	66.43 ± 0.48
Microwave extraction	30 min	60 °C	Ethanol	65.88 ± 0.599
Ultrasound extraction	60 min	60 °C	Ethanol:water (50:50)	95.72 ± 0.532
Ultrasound extraction	60 min	60 °C	Ethanol:water (80:20)	95.07 ± 1.49
Microwave extraction	15 min	60 °C	Ethanol:water (80:20)	95.91 ± 0.716

As seen in Table 4.5, at the extraction temperature of 60 °C ultrasound and microwave with pure ethanol for 60 and 30 min respectively, gave comparable recovery (approximately 65%). This is also comparable to that resulted from maceration in pure ethanol at room temperature for 3 days. Clearly, by reducing the time required for extraction, ultrasound and microwave assisted extractions are promising methods that offer improved extraction efficiency. Soxhlet extraction for 4 hours in ethanol was able to give higher yields than ultrasound and microwave assisted extraction at 60 °C as the extraction was carried out at the temperature closed to the boiling point of ethanol, and the plant tissues were continuously extracted with the fresh condensed solvent for extended time period. While ultrasound and microwave assisted extractions on the other hand were conducted in a batch system. Nevertheless, using 80% ethanol as solvent in ultrasound and microwave extractions could increase the percent recovery up to approximately 96%, which is comparable to using soxhlet extraction in pure ethanol, but with shorter extraction time required. At the same temperature and solvent volume used, ultrasound and microwave extraction gave comparable percent recovery, with microwave extraction requires about half the extraction time of that of ultrasound assisted extraction.

4.4 Antioxidant activity

Antioxidant activity of the extracts obtained by various methods are tested and compared using 1,2-diphenyl-2-picrylhydrazyl (DPPH) radicals. The DPPH radicals at 517 nm give a strong absorption maximum but the absorbance is reduced after 2 hour incubation in darkness with the anthraquinones sample. For the purpose of comparing the antioxidant activity in various extracts, concentration of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index.
The IC_{50} values for various extracts are shown in Figure 4.10.

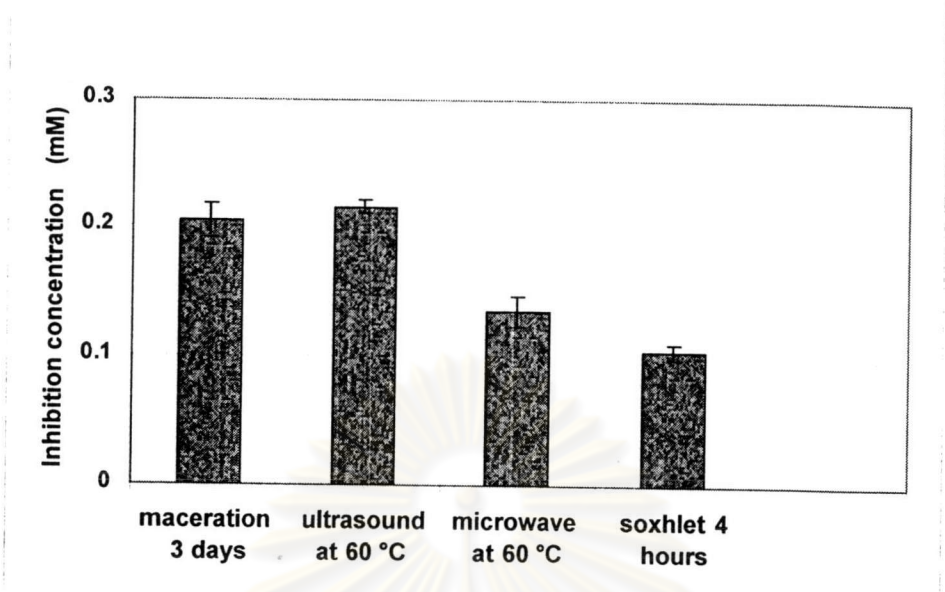

Figure 4.10 Antioxidant activities of anthraquinones in various methods at the maximum percent recovery.

The results show extracts obtained from microwave assisted extraction had higher antioxidant activities than that from ultrasound assisted extraction and maceration, but have comparable activity to that obtained by soxhlet extraction. Lower activity of the maceration extract was resulted from extended extraction that resulted in long exposure to unfavorable conditions such as light and oxygen. Although ultrasonic assisted extraction did not require long extraction time, it is commonly known that ultrasonic cavitation effect could induce free radicals formation within the liquid medium, thus oxidation and degradation of the anthraquinones could have occurred under this condition. When mixtures of ethanol and water were used as extraction medium, the antioxidant activity increased. This might be resulted from the fact that other water soluble compounds were extracted along with anthraquinones. Further study is needed to determine constituents of the extract.