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Influences of Anthraquinone Extraction Techniques from *Morinda* sp. on Extraction Efficiency

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ABSTRACT

The comparison of anthraquinone levels in root, stem, bark and leaf of *Morinda* sp. were investigated by HPLC with Alltima™ C8 column using 32%(v/v) acetonitrile and 0.5% (v/v) acetic acid as mobile phase at 40°C for 20 min. The roots contained the highest specific concentration of anthraquinone at 103.2 ± 4.8 mg/g dried mass, followed by stem (45.3 ± 6.3 mg/g), bark (6.90 ± 0.3 mg/g) and leaf (1.20 ± 0.6 mg/g). Four extraction techniques were employed to extract anthraquinone from the roots of *Morinda* sp and the anthraquinone content was analyzed using spectrophotometer. Soxhlet apparatus was utilized in the first technique with 50% (v/v) methanol as an extraction solvent. It was found that 14.6 ± 1.0 mg/g dried root powder was extracted with the expenses of 3.97 ± 0.17 Baht/mg anthraquinone. This was compared to the second extraction method at room temperature with 80%(v/v) acetone and 50%(v/v) ethanol that led to the highest concentration of extracted anthraquinone at 38.9 ± 1.6 and 27.0 ± 6.9 mg/g with the accompanied cost of 2.94 ± 0.12 and 1.42 ± 0.36 Baht/mg, respectively. The third extraction procedure was carried out in the pressurised steamer at 1 bar and 100°C for 5 min with 80% (v/v) ethanol. The highest level of extracted anthraquinone was 95.3 ± 0.6 mg/g at the cost of 4.28 ± 0.03 Baht/mg. The last method of extraction was performed in the closed-circuit solid-liquid extraction unit at room temperature. Although the extraction cost was relatively small (1.50 ± 0.08 Baht/mg), the specific concentration obtained was not high (only 2.27 ± 0.11 mg/g).

Key words: anthraquinone extraction, extraction cost, *Morinda* sp.

INTRODUCTION

Anthraquinone (Figure 1a) plays an important role as a biocatalyst in the pulp paper production industry. Ibrahim and Osman (1994) discussed the potential of using anthraquinone as laxative and growth inhibitor of microbes such as fungi. Furthermore, anthraquinone can also be used as substrate in the production of various dyes and pigments such as alizarin. This chemical is commonly found in plants such as Noni (*Morinda* sp.) and Cassod (*Cassia* sp.) (Kaewdok and Tubsombat, 2002). Noni plant or Indian Mulberry tree is commonly available in the northern part of Thailand.

Shotipruk *et al*. (2004) examined the anthraquinone extraction from *M. citrifolia* with hot water at high pressure within the temperature range of 110-220°C and flow rate speed of 2-6 ml/min. The best extraction condition was obtained

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at 220°C, 4 ml/min and 7 MPa with maximum anthraquinone level of 43 mg/g dried root. Aobchey *et al*. (2002) reported anthraquinone extraction from the root of *M. augustifolia* (Figure 1b and 1c) and biomass obtained from root cell culture. The extraction solvents were chloroform and methanol at atmospheric pressure. The highest level of anthraquinone obtained was 15 mg/g dried root which was compared to 8.9 mg/g of root cell culture.

The aim of this research was to determine anthraquinone content in root, stem, bark and leaf of *Morinda* sp. in order to select the part of plant with the highest anthraquinone level for further investigation on the extraction technique. The effects of four extraction techniques, which included (1) soxhlet extraction (2) simple solvent extraction at room temperature, (3) pressurized steamer extraction and (4) the extraction in closed-circuit solid-liquid extraction unit, on the extraction cost and extraction efficiency were examined so that the cost effective method of anthraquinone extraction from *Morinda* sp. root for large scale production could be established.

MATERIALS AND METHODS

Materials and chemicals

All of the chemicals used in the experiment with the exception of 95% (v/v) industrial grade ethanol (OV chemical, Chiang Mai, Thailand) were analytical reagent grade: absolute ethanol (Merck, Darmstadt, Germany, Product No. 1.00983.2500), acetone (Lab-Scan, Dublin, Ireland, Cat. No. A3501), ethyl acetate (Lab-Scan, Dublin, Ireland, Cat. No. A3511), methanol (Merck, Darmstadt, Germany, Product No. 106009) and anthraquinone (Fluka, New York, USA, Cat. No. 2015490). The reasons of using 95% industrial grade (86 Baht/kg) instead of an analytical grade absolute ethanol (452 Baht/kg) were to lower the extraction cost and to provide experimental data that reflected the practical large scale extraction situation where industrial grade ethanol would be the more likely choice of extraction solvent.

Sample preparation

The roots of *Morinda* sp. with minimum age of 3 yrs were collected from households within the Muang and Saraphee District of Chiang Mai Province and Muang District of Lampoon Province during July - August. The samples were kept frozen at -20°C before applying the appropriate size reduction methods for each part of plant. The harder portion, namely, root, stem and bark were chopped while the leaf was cut with knife into smaller pieces. This was followed by drying step in the tray dryer (Armfield, Ringwood, UK, Model UOP 8) at 65°C and air flow rate of 1

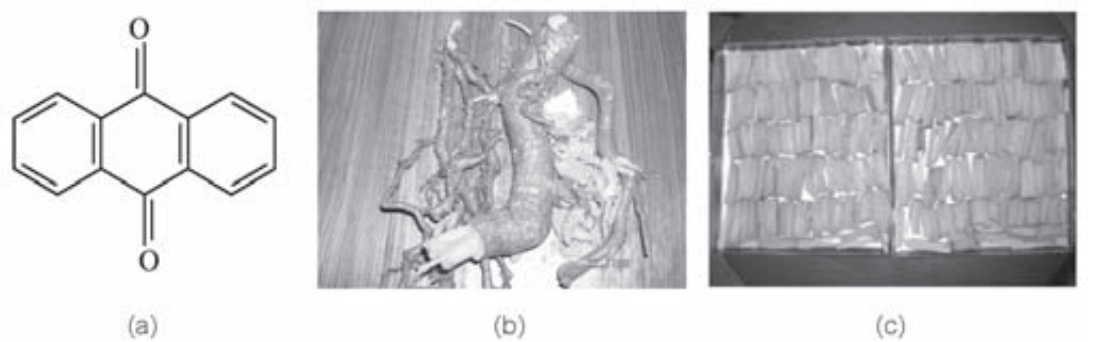

Figure 1 Structure of anthraquinone (a) (Kritsanapan and Nualkeaw, 2003) and roots of *Morinda* sp. before (b) and after (c) pretreatment for drying process.

m/s until the equilibrium moisture content was reached. All of dried samples were fed into the hammer mill with a 250 μm sieve tray.

Anthraquinone extraction with soxhlet apparatus

The original extraction method for *C. siamea* Britt. proposed by Kritsanapan and Nualkaew (2003) using soxhlet extraction unit (Siripanya Trading, Lampang, Thailand) was improved and modified for anthraquinone extraction from *Morinda* sp. root. Each extraction was repeated twice. The extraction was carried out with 150 ml of 50%(v/v) ethanol or 50%(v/v) methanol using four rounds of reflux followed by filtration with Whatman No. 4 filter paper.

Anthraquinone extraction with other methods

Three replicates were used for each extraction method by maintaining the ratio of root powder per volume of solvent at 0.17 g per 100 ml.

Extraction with different type of solvents at room temperature

Three types of solvents were used including ethanol, acetone and ethyl acetate. Each solvent was mixed with distilled water in the following %(v/v overall solution); 0, 5, 20, 35, 50, 65, 80, 95 and 100. The extraction of 0.05 g *Morinda* sp. root powder with 30 ml of each solvent was performed at room temperature for 6 h.

Extraction in pressurized steamer

The extraction was repeated as previous method with ethanol as a solvent. The sample was placed in the moisture can and the extraction was carried out in the stainless steel pressurized steamer (All American, Miami, USA, Model No.1925x). The pressure and temperature were controlled at 1 bar and 100°C respectively with extraction time of 5 min. The method of extraction was utilized in the comparison of anthraquinone content in root, stem, bark and leaf of *Morinda* sp.

Extraction in solid-liquid extraction unit

The 5 g root powder was extracted with 3 litres of distilled water in the closed circuit solid-liquid extraction demonstration unit (Armfield, Ringwood, UK, Model No. 12697) at room temperature with pump speed of 13.5 l/h. Sample was collected every 30 min for 5 h.

Analytical methods

The analysis of anthraquinone was performed either spectrophotometrically by taking measurement from a spectrophotometer (Perkin Elmers, Waltham, USA, Model No. Lambda 25) at 325 nm for comparison of extraction efficiency between each extraction method and chromatographically using HPLC (Shimadzu, Japan) for the determination of anthraquinone content in root, stem, bark and leaf of *Morinda* sp. with Alltima™ C8 column (Alltech, USA) at 40°C and a mobile phase flow rate of 2 ml/min whose composition was 32%(v/v) acetonitrile and 0.5%(v/v) acetic acid. The injection volume and run time were 5 μl and 25 min, respectively. Anthraquinone peak appeared between 15.0 - 15.8 min (Figure 2(a) and 2(b)) and was monitored at 263 nm. The dilution with corresponding solvent and appropriate dilution factor was applied accordingly. The quantification of anthraquinone in the sample was obtained from the standard curve of anthraquinone between 1.56 – 6.23 mg/ml. Both methods of analyses were comparable as evident from the relatively equivalent concentration of anthraquinone in root powder; 103.2 ± 4.8 mg/g for HPLC and 95.3 ± 0.6 mg/g for spectrophotometrical procedure. The determination of precision in terms of the coefficient of variation (CV) and accuracy in terms of relative error for each method were subsequently determined as described by Skoog

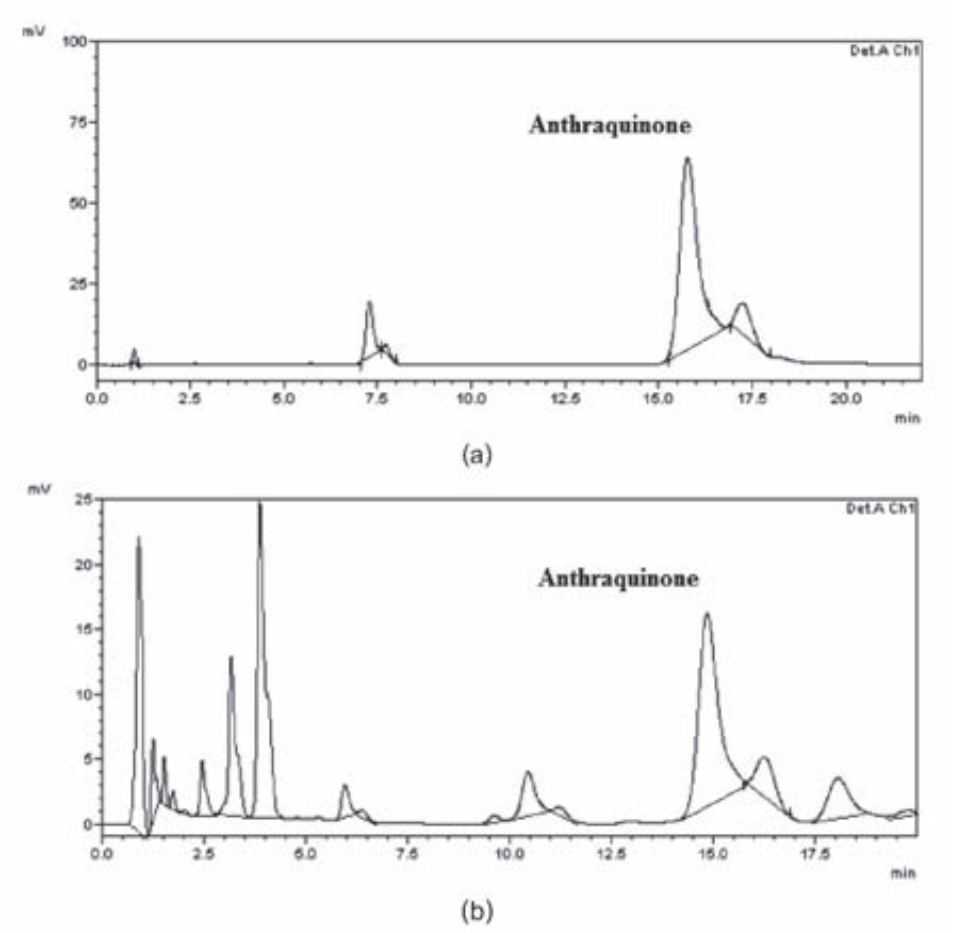

Figure 2 HPLC chromatogram of (a) anthraquinone standard and (b) sample from root powder.

et al. (1996). The corresponding CV and relative error for the analysis of anthraquinone based on HPLC were found to be 6.59% for root powder sample and -0.67% for anthraquinone standard, respectively. This was compared to the situation of spectrophotometrical analysis where both parameters were 0.89% for CV and 0.29% for relative error. The extraction cost was calculated from the initial cost involved in each extraction method such as water, electricity, natural gas, materials and chemicals. The statistical hypothesis testing on difference in the level of extracted anthraquinone and extraction cost were performed using experimental mean comparison technique (Skoog *et al*. 1996).

RESULTS AND DISCUSSION

Anthraquinone content in root, stem, bark and leaf of *Morinda* sp. by pressurized steam method

The roots contained the highest specific concentration of anthraquinone at 103.2 ± 4.8 mg/g dried mass (Figure 3, (0.172 ± 0.008 mg/ml)/(0.05 g/30 ml) = 103.2 ± 4.8 mg/g), followed by stem (45.3 ± 6.3 mg/g), bark (6.90 ± 0.3 mg/g) and leaf (1.20 ± 0.6 mg/g). This was compared to the maximum concentration of 1.2 mg/g anthraquinone glycoside and 2.0 mg/g total anthraquinone from *C. siamea* dried leaf (Kritsanapan and Nualkaew, 2003).

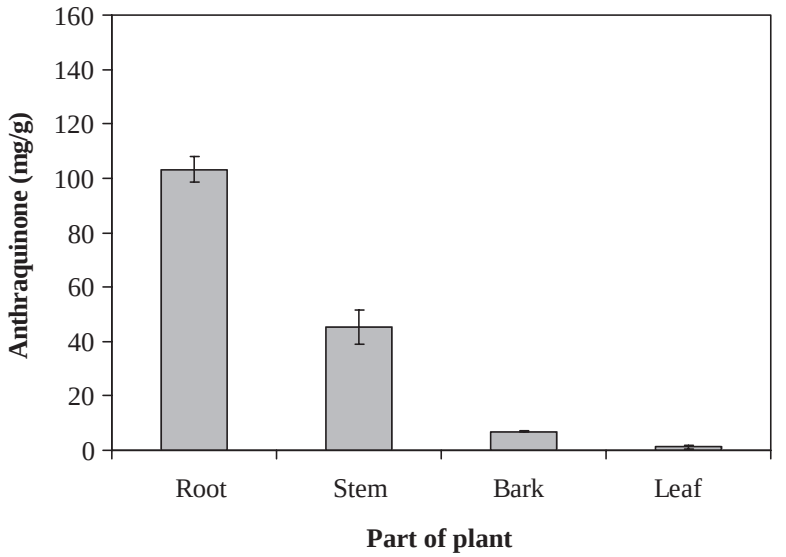

Figure 3 Anthraquinone content (HPLC method) in root, stem, bark and leaf of *Morinda* sp.

Extraction with soxhlet apparatus

The improved soxhlet extraction method with 50%(v/v) ethanol and 50%(v/v) methanol yielded 11.2 ± 2.8 and 14.6 ± 1.0 mg anthraquinone per g of dried root powder, respectively with 1 h extraction time. The extraction expenses per 1 mg of extracted anthraquinone were 4.98 ± 1.25 and 3.97 ± 0.17 Baht, respectively. Hemwimol *et al.* (2006) also performed a continuous extraction with soxhlet method with only 200 ml absolute ethanol from 2 g of dried root powder for the period of 4 h at 70°C with extraction efficiency of 97.7%. Further comparative analysis with extraction in the pressurized steamer at 1 bar and 100°C for 5 min with total anthraquinone level of 95.3 ± 0.6 mg/g dried root powder (Figure 6 at 80% ethanol, (0.159 ± 0.001 mg/ml)/(0.05 g/30 ml) = 95.3 ± 0.6 mg/g) indicated that soxhlet extraction methods had lower extraction efficiency than that of the extraction in pressurized steamer.

Extraction with different type of solvents

The roles of water in the enhancement of extraction efficiency were prominent in every case of anthraquinone extraction with various types of solvent. This might be described by a greater electrostatic force of water relative to other molecules (CYBERLAB 2007, LESA 2007). Therefore, the extraction with solvents other than water might also require a participating role of water at some extent to increase the extraction efficiency. Similar observation was also reported by Sakunpak *et al.* (2007) who investigated the roles of water in anthraquinone extraction from *Senna alata*. The concentration of water at 15% (w/w) helped enhancing anthraquinone extraction by 1.47 times when compared to the situation where the water was absence. Hemwimol *et al.* (2006) examined the optimal ratio of ethanol and water in anthraquinone extraction from *Morinda* sp. root by soaking 0.1 g of dried root powder in 10 ml of solvent. It was found that 50% ethanol was able to extract anthraquinone at 1.88 times greater than that of absolute ethanol. Such finding corresponded to the experimental results in Figure 4 (the highest level of 0.045 ± 0.009 mg/ml was obtained when 50% ethanol was used, comparing to 0.037 ± 0.013 mg/ml for absolute ethanol) and Figure 5 (0.065 ± 0.003 mg/ml for 80% acetone which was 2.6 times higher than

Ethanol

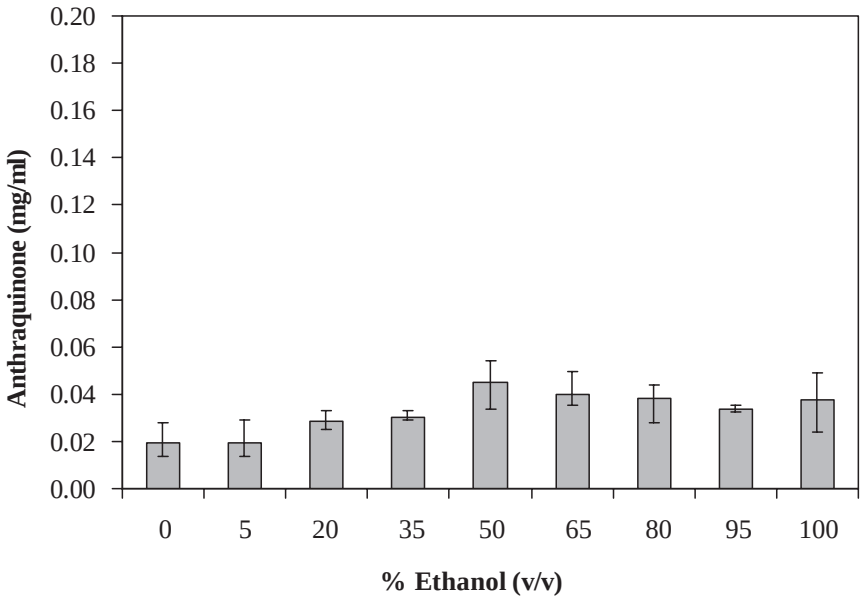

Figure 4 Anthraquinone extraction with a solvent consisted of ethanol and water at room temperature with various concentration level (%(v/v)). The analysis was carried out using spectrophotometer.

Acetone

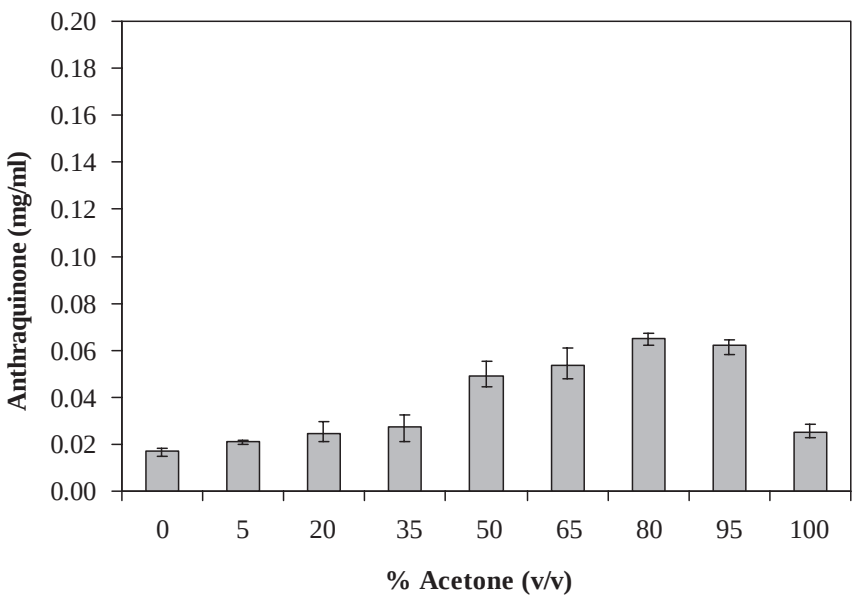

Figure 5 Anthraquinone extraction with a solvent consisted of acetone and water at room temperature with various concentration level (%(v/v)). The analysis was carried out using spectrophotometer.

Extraction in pressurized steamer

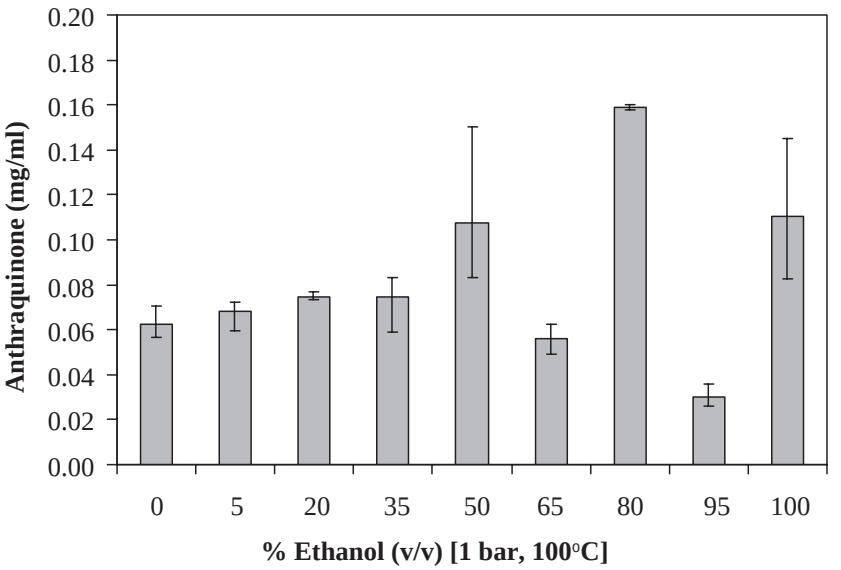

Figure 6 Anthraquinone extraction in a pressurized steamer with ethanol and water at various concentration levels (%(v/v)) at 1 bar, 100°C for 5 min. The analysis was carried out using spectrophotometer.

0.025 ± 0.003 mg/ml for 100% acetone). Hemwimol *et al.* (2006) explained that a relatively high polarity index (p.i.) of water (p.i. = 9) in comparison to ethanol (p.i. = 5.2) might contribute to the enhancement of extraction efficiency. The swelling of *Morinda* sp. root cells also increased the surface area and facilitated the extraction efficiency by the solvents. In addition, the elevated mass transfer coefficient due to the decreased ethanol viscosity in the presence of water for up to 50% could all play parts in optimal extraction at such situation. The significant role of acetone in boosting the effect of anthraquinone extraction efficiency could be explained in term of higher mass transfer coefficient that allowed the better access of acetone to the anthraquinone reserve in each cell of *Morinda* sp. Furthermore, the similarity of carbonyl functional group (C=0) in acetone to that of anthraquinone might also play part in the improvement of anthraquinone extraction efficiency (Hemwimol *et al.*, 2006).

Compared to other methods, the pressurized steamer extraction procedure yielded the highest level of anthraquinone (0.159 ± 0.001 mg/ml in Figure 6 which was equivalent to 95.3 ± 0.6 mg/g dried root powder). The temperature increase accelerated diffusion process while the rise in pressure encouraged the movement of molecules or ions in the extraction medium (Jindawat, 2005) as evidence from the extraction of total soluble solid (TSS) from dried longan which found that boiling helped increase the extraction speed and resulted in higher concentration of extracted solute (Palakul and Fongduang, 2006). The concentration of extracted anthraquinone described in Figure 6, which was later converted to mg/g, was higher than those of Aobchey *et al.* (2002) by 6.35 times (15 mg/g of dried root powder) and Shotipruk *et al.* (2004) by 2.22 times (43 mg/g dried root powder). In addition, Shotipruk *et al.* (2004) suggested that the pressure level had no impact to the extraction

Table 1 Summary of expenses involved in anthraquinone extraction with four extraction techniques. The anthraquinone content (mg/g) was determined by spectrophotometer.

Extraction method	Solvent	Extraction conditions	Anthraquinone (mg per g of root powder)	Initial extraction cost of anthraquinone 1 mg (Baht)
Soxhlet	50 %(v/v) ethanol	Reflux 4 times	11.2 ± 2.8^{a}	4.98 ± 1.25^{I}
Soxhlet	50 %(v/v) methanol	Reflux 4 times	14.6 ± 1.0^{a}	3.97 ± 0.17^{I}
Solvent	50 %(v/v) ethanol	Room temperature	27.0 ± 6.9^{b}	1.42 ± 0.36^{II}
Solvent	80 %(v/v) acetone	Room temperature	38.9 ± 1.6^{c}	2.94 ± 0.12^{III}
Solvent	50 %(v/v) EA*	Room temperature	26.9 ± 5.1^{b}	$3.68 \pm 0.70^{I,III}$
PS#	80 %(v/v) ethanol	1 bar, 100°C	95.3 ± 0.6^{d}	4.28 ± 0.03^{I}
SLE!	Tap water	Room temperature	0.0038 ± 0.0002^{e}	1.50 ± 0.08^{II}

*ethyl acetate, #pressurized steamer, !solid-liquid extraction

The number with the same alphabet (a-e) and Roman numerical (I – III) indicate no significant difference at 95% CI

yield of anthraquinone within the temperature interval of 110-220°C. Such finding was in good agreement with the current study where the high level of extracted anthraquinone was still obtained even at atmospheric pressure. Hemwimol *et al.* (2006) also illustrated that up to 96% of available anthraquinone could be obtained when 80% ethanol was used in the extraction system utilizing ultrasound technology. However, it should be noted that the error associated with this extraction procedure was still relatively large as evidence from the presence of two outliers in Figure 6, in which the level of extracted anthraquinone (mg/ml) at 65 and 95% ethanol were much lower than those at 50, 80 and 100% ethanol. Further experimentation should be conducted to investigate whether extraction time beyond 5 min and extraction pressure (5 – 20 psi) play any part in stabilizing the extraction efficiency as well as the positioning or the number of extraction containers presence in the pressurized steamer that might influence or interfere the accessing pattern of steam to each container.

Comparison of anthraquinone extraction cost

Further analysis of the best extraction method could be done by finding the cost ratio between extracted anthraquinone (mg/g root powder) and the initial extraction cost of anthraquinone (Baht/mg) listed in Table 1. Even though, there was no statistical difference at 95% CI in the initial extraction cost between the soxhlet and pressurized steamer, the cost ratio from both methods differed by almost 10 times (2.25 for soxhlet method with 50% ethanol and 22.2 for pressurized steamer method).

CONCLUSION

In conclusion, anthraquinone extraction in the pressurized steamer with 80% ethanol provided the superior extraction in terms of extraction efficiency and cost ratio over that of a more expensive soxhlet and a less efficient solid-liquid extraction procedures.

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LITERATURE CITED

Aobchey, P., S. Sriyam, W. Praharnripoorab, S. Lhieochaiphant and S. Phutrakul. 2002. Production of red pigment from the root of *Morinda angustifolia* Roxb. var. scabridula Craib. by root cell culture. **CMU J.** 1: 66-78.

CYBERLAB. 2007. Water (online). Available: http://www.cyberlab.lh1.ku.ac.th/elearn/faculty/aid/id78/e-learning/water_menu2.htm. [February 8, 2007].

Hemwimol, S., P. Pavasant, and A. Shotipruk. 2006. Ultrasound-assisted extraction of anthraquinones from roots of Morinda *citrifolia*. **Ultrason. Sonochem.** 13: 543-548.

Ibrahim, D. and H. Osman. 1994. Antimicrobial of *Cassia alata* from Malaysia. **J. Ethnopharm.** 45: 151-156.

Jindawat, N. 2005. Diffusion (online). Available: http://www.e-learning.sg.or.th/act5/content2.html. [April 30, 2006].

Kaewdok, P. and S. Tubsombat. 2002. The anthraquinone extraction from the leaves of herbs in the Family Leguminosae. Research and Academic Services Institute, Rajbhat Mahasarakam (online). Available: http://research.rmu.ac.th/dataresearch/p1.php?ac=show&r=493. [March 30, 2007].

Kritsanapan, W. and S. Nualkeaw. 2003. Variation of anthraquinone content in *Senna siamea* leaves (online). Available: http://schoolbotany.haii.or.th/exhibition46/presentations/wandee/wandee.PPT. [July 28, 2005].

LESA. 2007. Water properties (online). Available: http://www.lesa.in.th/hydro/water_properties/water_properties.htm. [February 4, 2007].

Palakul, S. and S. Fongduang. 2006. Production of alcohol from dried longan, p.35 (online). Available: http://www.agro.cmu.ac.th/department/fe/student/student48/s48_9.html. [March 30, 2007].

Sakunpak, A., P. Panichayupakaranant and A. Sirikatitham. 2007. Preparation of *Senna alata* leaf extract and quantitative analysis of anthraquinone (online). Available: http://www.grad.psu.ac.th/grad_research/apply_file/full3920600305071.pdf. [March 30, 2007].

Shotipruk, A., J. Kiatsongserm, P. Pavasant, M. Goto and M. Sasaki. 2004. Pressurized hot water extraction of anthraquinones from the roots of *Morinda citrifolia*. **Biotechnol. Prog.** 20: 1872-1875.

Skoog, D.A., D.M. West and F.J. Holler. 1996. **Fundamentals of Analytical Chemistry**, 7th ed. p. 14-15, 33, 53 - 55.