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Anthraquinone Extraction from *Morinda* sp. Root Powder Under Steam Pressurized Conditions

Taweyot Kunyotying and Noppol Leksawasdi*

ABSTRACT

Three types of containers and six strategies to control the evaporation of 80%v/v ethanol for extraction of anthraquinone from dried *Morinda* sp. root powder in a pressurized steamer at 20 psi for 30 min were investigated. These were the most extreme conditions possible in the system investigated. A tightly sealed 231 ml jam jar with a metal screw cap (diameter 5.4 cm, height 10.1 cm) was chosen as the most appropriate container with the ability to withhold 65.0 ± 5.6% of the original liquid volume after pressurization with saturated steam. The optimal pressure level and time for pressurized anthraquinone extraction in the jar containing 80%v/v ethanol was 5 psi for 5 min with the corresponding level of extracted anthraquinone being 30.9 ± 0.5 mg/g dried root powder, with a minimal extraction cost of merely 2.65 ± 0.04 baht/mg. Further experiments on the effect of varying the ethanol concentration revealed that 65%v/v ethanol achieved the highest extracted anthraquinone level of 38.4 ± 0.7 mg/g with a corresponding extraction cost of only 2.01 ± 0.04 baht/mg. The problem of reproducibility and the existence of outliers encountered previously with pressurized steam extraction were resolved and eliminated with the use of the 231 ml jam jar.

Key words: Morinda sp., anthraquinone, extraction in pressurized condition, spectrophotometry, saturated steam

INTRODUCTION

The roots of *Morinda citrifolia* L. have been a prevalent traditional source of red dyestuff for ritual textiles in South East Asia (Maxwell, 2003) and for use as a medicinal elixir in Polynesian cultures (McClatchey, 2002). Anthraquinones (9,10-anthracenedione, Figure 1(a)) can be identified in various parts of *M. citrifolia* (Wang *et al.*, 2002). Additional applications of anthraquinone include: as a biocatalyst in kraft wood pulp production process (Butterworth *et al.*, 2001), as a bird repellent (EPA, 1998; NIEHS, 2008) and as a laxative, and microbial growth inhibitor (Ibrahim and Osman, 1994).

Temiyaputra *et al.* (2008) reported the anthraquinone content in dried *Morinda* sp. root, stem, bark, and leaf of 103±4.8, 45.3±6.3, 6.9±0.3, and 1.2±0.6 mg/g, respectively. Anekpankul *et al.* (2007) extracted the highest amount of damnacanthal (Figure 1(b)) – an anticancer derivative of anthraquinones) from dried root of *M. citrifolia* at 170°C and 4 MPa. Similarly, Shotipruk *et al.* (2004) reported optimal water extraction conditions of 220°C, 4 ml/min and 7

Department Food Engineering, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand.
* Corresponding author, e-mail: noppol@hotmail.com, noppol@chiangmai.ac.th

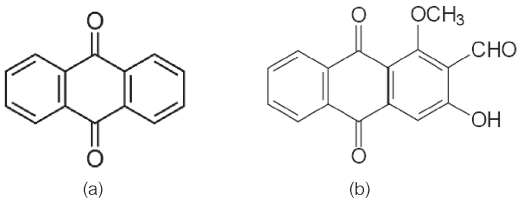

Figure 1 Chemical structure of (a) anthraquinone skeleton (NIEHS, 2008) and (b) one of its anti-cancer derivatives – damnacanthal (CosmoBio, 2008).

MPa to produce a maximum anthraquinone level of 43 mg/g dried root. A slightly lower content of 38.9±1.0 mg/g was reported for extraction at room temperature with 80%v/v acetone (Temiyaputra *et al.*, 2008). In addition, microwave (Hemwimol *et al.*, 2007) and ultrasound (Hemwimol *et al.*, 2006) could be used to extract anthraquinone. Extraction with 80%v/v ethanol in a pressurized steamer yielded up to 95.3±0.6 mg/g (Temiyaputra *et al.*, 2008).

An anthraquinone extraction method with an air/steam mixture at 15 psi and 100°C for 5 min in a "moisture can" showed 40.1% errors and the presence of outliers (Temiyaputra *et al.*, 2008). The first objective of the current research was to determine whether different types of containers and restriction strategies for solvent evaporation played any role in maintaining the reproducibility of remaining volume of solvent after extraction in the presence of saturated steam. Secondly, the optimized pressure level and extraction time in the steamer were elucidated. The final objective was to examine the effect of various ethanol concentrations on the level of extracted anthraquinone from the extraction strategy developed with pressurized steam that could be used as a benchmark for future experiments.

MATERIALS AND METHODS

Materials and chemicals

Samples were prepared of 0, 5, 20, 35, 50, 65 and 80%v/v ethanol by diluting an appropriate volume of 95% (v/v) industrial grade ethanol (OV chemical, Chiang Mai, Thailand). Absolute ethanol (Merck, Darmstadt, Germany, Product No. 1.00983.2500) was used for 100%v/v ethanol, where specified. The preparation procedure for the *Morinda* sp. dried root powder was similar to that described by Temiyaputra *et al.* (2008).

Selection of an appropriate container for extraction in a pressurized steamer

The experiment was performed based on a 3 × 6 factorial design with five replicates. Three types of containers were selected: 1) 114 ml "moisture can" (diameter 6.1 cm, height 3.9 cm), 2) 163 ml jam jar with polypropylene screw cap (diameter 4.8 cm, height 9.0 cm), and 3) 231 ml jam jar with metal screw cap (diameter 5.4 cm, height 10.1 cm). The "moisture can" was the same container type previously used by Temiyaputra *et al.* (2008) and was included in the current experiment to provide a comparison with alternative jam jar containers. In an attempt to control the extent of 30±0.1 ml 80%v/v ethanol evaporation, six strategies were developed that were relatively simple and rather inexpensive: 1) control container with tightly sealed cap – "C", 2) addition of plastic covering before sealing with cap – "PC", 3) cap sealed with cellophane tape on the outside – "CT", 4) similar to (1) with an inner, small polypropylene bag with rubber band, in which the solvent was stored – "Ci", 5) similar to 2) with an inner bag – "PCi", and 6) similar to 3) with an inner bag – "CTi". The percentage of remaining liquid after extraction at 20 psi for 30 min in a pressurized steamer (All American, New York, USA, Model No.1952x) was recorded.

Determination of optimal pressure level and extraction time in the pressurized steamer

The extraction of 0.05 g *Morinda* sp. root powder with 30 ml of 80%v/v ethanol was

performed in a 231 ml jam jar with a metal screw cap, which was the most appropriate container as determined from the testing in the previous section. The root powder was poured into a side-threaded cotton cloth sachet (3.0 × 4.0 cm) before placing in the jam jar. The experiment was based on a 4 × 4 factorial design with 14 replicates. Four pressure levels of steam at 5, 10, 15 and 20 psi were investigated for four extraction periods of 5, 10, 15 and 20 min. The sample was analyzed for anthraquinone content.

Extraction with various ethanol concentrations in the pressurized steamer

This experiment was based on a complete random design (CRD) with 11 treatments using the same conditions as in the previous experiment involving the optimal extraction conditions of 5 psi for 5 min with the most appropriate container (231 ml jam jar). The 11 treatments involved 30 ml of different concentrations of ethanol; 0, 5, 20, 35, 50, 65, 80, 95 and 100%v/v. These were compared to two negative controls in which root powder samples were extracted by soaking in 80%v/v ethanol at 30°C for 5 and 25 min. Fourteen replicates were used in each experiment. Samples from each treatment were analyzed for anthraquinone content.

Analytical methods

The analysis of anthraquinone was performed spectrophotometrically at 325 nm as described by Temiyaputra *et al*. (2008). The extraction cost (baht per mg anthraquinone) was calculated from the initial cost involved, which included water, ethanol and consumed natural gas in each treatment. The calculation of extracted anthraquinone content was based on the concentration of the extracted anthraquinone (mg/ml) multiplied by the remaining extracted solvent after extraction (ml) and divided by the mass of root powder used (g). The statistical mean, standard error and hypothesis testing of experimental mean comparisons were based on the techniques described by Skoog *et al*. (1996).

RESULTS AND DISCUSSION

Selection of an appropriate container for extraction in the pressurized steamer

Anthraquinone extraction in a "moisture can" without an evaporation limiting strategy (C) and with a PC strategy showed the same volume loss of 80%v/v ethanol ($p > 0.05$) (Table 1). The addition of an inner bag to the "moisture can" mitigated volume loss as evidenced by the higher remaining solvent volume of 44.3±4.0% for Ci, 55.0±4.0% for PCi, and 47.7±4.7% for CTi. The application of both the 163 and 231 ml jam jars, however, resulted in less volume loss than for the "moisture can" as illustrated by the remaining volume of solvent in C of 45.3±2.3% and 65.0±5.7%, respectively. The PC strategy was less effective than C in retaining the solvent

Table 1 Percentage of the remaining volume of 80%v/v ethanol after the simulated pressurized extraction (20 psi for 30 min).

Strategies used	114 ml "moisture can"	163 ml jam jar	231 ml jam jar
C	0.00 ± 0.00^{a}	45.3 ± 2.3^{c}	65.0 ± 5.7^{df}
PC	0.07 ± 0.07^{a}	49.3 ± 2.0^{cd}	36.3 ± 3.7^{c}
CT	3.37 ± 0.07^{b}	22.4 ± 0.5^{e}	81.0 ± 7.7^{fg}
Ci	44.3 ± 4.0^{c}	72.0 ± 1.7^{f}	89.7 ± 1.7^{g}
PCi	55.0 ± 4.0^{d}	67.0 ± 7.3^{df}	60.7 ± 5.7^{df}
CTi	47.7 ± 4.7^{cd}	67.3 ± 3.0^{f}	85.7 ± 3.7^{g}

Note: Numbers with the same letter are not significantly different ($p > 0.05$).

volume in the 231 ml jam jar, with the percentage of remaining solvent volume from the PC strategy (36.3±3.7%) being significantly lower ($p \leq 0.05$) than for C (65.0±5.7%). The CT, Ci, and CTi strategies might be recommended for the 231 ml jam jar, in a situation where minimum volume loss of solvent was expected, as these procedures were able to preserve 81.0-89.7% of the original solvent volume. In the case of the 163 ml jam jar, all inner bag treatments (Ci, PCi, and CTi) provided results which were not significantly different ($p > 0.05$). The CTi strategy did not provide any significant suppression effect in the evaporation of solvent compared to the Ci strategy for all three containers. However, the 231 ml jam jar with the C strategy (65.0±5.7%) or the control without any modification to the cap were chosen as appropriate containers for the subsequent experiment instead of using the same jam jar with the CT strategy (81.0±7.7%) because: 1) it was considered acceptable to have the percentage of the remaining solvent volume at a level higher than 50% of the original volume; 2) the lower level of remaining solvent volume might help produce more concentrated anthraquinone extract; and 3) the preparation method with the jam jar involving attaching cellophane tape was considered too cumbersome and inconvenient.

Determination of optimal pressure level and extraction time in the pressurized steamer

The content of extracted anthraquinone and the corresponding extraction cost are shown in Tables 2 and 3, respectively, using the tightly sealed 231 ml jam jar with a metal screw cap for extraction in the pressurized steamer at pressure levels of 5, 10, 15 and 20 psi and extraction times of 5, 10, 15 and 20 min. The highest level of extracted anthraquinone was 31.0±0.8 mg/g under extraction conditions of 5 psi for 15 min. This was not significantly different ($p > 0.05$) from the extractions performed at 5 psi for 5 min (30.9±0.5 mg/g), 5 psi for 20 min (30.8±1.1 mg/g), 20 psi for 10 min (30.7±0.9 mg/g), and 20 psi for 20 min (30.4±0.9 mg/g). The optimal level of extracted anthraquinone was obtained at a pressure level of

Table 2 Content of extracted anthraquinone (mg/g dried root powder) at various pressure levels and extraction times in the pressurized steamer.

Pressure level (psi)	Extraction time (min)			
	5	10	15	20
5	30.9 ± 0.5^{a}	30.2 ± 0.8^{bc}	31.0 ± 0.8^{a}	30.8 ± 1.1^{a}
10	26.9 ± 1.4^{d}	27.8 ± 1.7^{df}	25.9 ± 1.7^{de}	29.9 ± 1.0^{c}
15	25.0 ± 1.6^{e}	27.7 ± 1.4^{d}	26.4 ± 1.7^{d}	27.9 ± 2.1^{df}
20	28.7 ± 0.9^{f}	30.7 ± 0.9^{a}	30.1 ± 0.8^{bc}	30.4 ± 0.9^{ac}

Note: Numbers with the same letter are not significantly different ($p > 0.05$).

Table 3 Anthraquinone extraction cost (baht/mg) at various pressure levels and extraction times in the pressurized steamer.

Pressure level (psi)	Extraction time (min)			
	5	10	15	20
5	2.65 ± 0.04^{a}	2.77 ± 0.07^{b}	2.71 ± 0.06^{c}	2.77 ± 0.10^{b}
10	3.10 ± 0.16^{df}	3.08 ± 0.19^{df}	3.32 ± 0.23^{e}	2.95 ± 0.11^{ghi}
15	3.38 ± 0.22^{e}	3.15 ± 0.16^{d}	3.35 ± 0.22^{e}	3.27 ± 0.26^{e}
20	2.98 ± 0.09^{fg}	2.90 ± 0.09^{h}	3.01 ± 0.08^{fi}	3.10 ± 0.08^{d}

Note: Numbers with the same letter are not significantly different ($p > 0.05$).

5 psi and an extraction time of 5 min, as this treatment provided the most inexpensive extraction conditions with a relatively high content of extracted anthraquinone (30.9±0.5 mg/g). The lowest level of extracted anthraquinone of 25.0±1.6 mg/g was obtained at 15 psi for 5 min, which was not significantly different from the extraction at 10 psi for 15 min ($p > 0.05$). The extractions performed at 10 psi for 5 min, 10 psi for 10 min, 10 psi for 15 min, 15 psi for 10 min, 15 psi for 15 min and 15 psi for 20 min were not statistically different ($p > 0.05$). Therefore, the level of extracted anthraquinone was not influenced by an extraction duration of 5-15 min at 10 psi and 10-20 min at 15 psi.

Further extraction cost analysis (Table 3) indicated that the pressurized steam extraction performed at 5 psi for 5 min required the smallest amount of investment at 2.65±0.04 baht/mg of extracted anthraquinone. The most expensive strategies were 10 psi for 15 min (3.32±0.23 baht/mg), 15 psi for 5 min (3.38±0.22 baht/mg), 15 psi for 15 min (3.35±0.22 baht/mg), and 15 psi for 20 min (3.27±0.26 baht/mg). There was no significant difference ($p > 0.05$) between any of these strategies. The extraction performed at 20 psi for 20 min did not result in the highest investment because the level of extracted anthraquinone was reasonably high (Table 2). The extraction conditions at 10 psi for 5 min, 10 psi for 10 min and 15 psi for 10 min were not significantly different from 20 psi for 20 min ($p > 0.05$).

Effect of ethanol concentration in the pressurized steamer and anthraquinone extraction cost

The effect of varying ethanol concentration was investigated using tightly sealed 231 ml jam jars with metal screw caps at 5 psi for 5 min as indicated in Table 4. All eleven treatments were significantly different ($p \leq 0.05$). The highest level of extracted anthraquinone concentration of 38.4±0.7 mg/g was achieved with 65%v/v ethanol. Aobchey *et al*. (2002) reported 15 mg anthraquinone/g of dried root powder. Shotipruk *et al*. (2004) attained an extraction level of 43 mg/g of dried root powder. However, the extraction level was different from Temiyaputra *et al*. (2008) (95.3±0.6 mg/g and 103.2±4.8 mg/g using 80%v/v ethanol) who employed an air/steam mixture instead of saturated steam. Vermass and Kuun (1989) distinguished the drying effect on lumber

Table 4 Content of anthraquinone and expenses involved in extraction with pressurized steamer (PS) using various concentrations of ethanol/water mixture.

Extraction method	Solvent	Extraction conditions	Anthraquinone (mg/g root powder)	Extraction cost (baht/mg)
PS	Water	5 psi, 5 min	16.0 ± 0.3^{b}	3.48 ± 0.06^{VII}
PS	5%v/v ethanol	5 psi, 5 min	20.6 ± 0.2^{d}	2.78 ± 0.02^{V}
PS	20%v/v ethanol	5 psi, 5 min	24.6 ± 0.3^{f}	2.53 ± 0.02^{III}
PS	35%v/v ethanol	5 psi, 5 min	28.7 ± 0.2^{g}	2.35 ± 0.02^{II}
PS	50%v/v ethanol	5 psi, 5 min	36.4 ± 0.3^{j}	1.99 ± 0.02^{I}
PS	65%v/v ethanol	5 psi, 5 min	38.4 ± 0.7^{k}	2.01 ± 0.04^{I}
PS	80%v/v ethanol	5 psi, 5 min	30.9 ± 0.5^{h}	2.65 ± 0.04^{IV}
PS	95%v/v ethanol	5 psi, 5 min	31.7 ± 0.9^{i}	2.75 ± 0.08^{V}
PS	Absolute ethanol	5 psi, 5 min	22.5 ± 0.5^{e}	11.6 ± 0.3^{IX}
Solvent soaking	80%v/v ethanol	30°C, 5 min	12.0 ± 0.1^{a}	4.03 ± 0.03^{VIII}
Solvent soaking	80%v/v ethanol	30°C, 25 min	17.0 ± 0.1^{c}	2.83 ± 0.01^{VI}

Note: Numbers with the same letter or roman numeral are not significantly different ($p > 0.05$).

between the saturated steam and air/steam mixture. The appearance of wide cracks or splits in the wood and over-drying were less severe in the presence of steam only. Therefore, it might be possible that the application of an air/steam mixture instead of saturated steam had some influence over the level of extracted anthraquinone concentration. Further comparisons of the pressurized steamer method with two negative controls, in which the samples of *Morinda* sp. root powder were soaked in 80%v/v ethanol at 30°C for 5 and 25 min, revealed that the conventional solvent extraction procedure resulted in a 1.82-2.58 times lower level of anthraquinone (12.0±0.1 and 17.0±0.1 mg/g for an immersion time of 5 and 25 min, respectively) than its pressurized counterpart using 80%v/v ethanol (30.9±0.5 mg/g) . The cheapest extraction costs of 1.99±0.02 and 2.01±0.04 baht per mg of extracted anthraquinone were obtained with 50 and 65%v/v ethanol. Furthermore, there was no significant economic difference ($p > 0.05$) in using 5 or 95%v/v ethanol as extraction solvent in the pressurized steamer. The utilization of absolute ethanol should be avoided as the extraction cost was up to 11.6±0.3 baht/mg. The extraction costs of the conventional solvent (80%v/v ethanol) extraction procedure were also relatively high, with 4.03±0.03 and 2.83±0.01 baht/mg for an immersion period of 5 and 25 min, respectively.

CONCLUSION

The optimal pressure level and time for the extraction of anthraquinone from *Morinda* sp. roots in a pressurized steamer based on 80%v/v ethanol were 5 psi and 5 min using a 231 ml jam jar. The optimal, cost-effective ethanol concentration was 50-65%v/v producing an extracted anthraquinone content of 36.4-38.4 mg/g dried root at an extraction cost of 1.99-2.01 baht/mg anthraquinone.

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