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Ultrasonic extraction method for alizarin from roots of *Morinda citrifolia*

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ABSTRACT

An ultrasonic method for the extraction of alizarin from roots of *Morinda citrifolia* was investigated. The influence of four extraction variables on extraction efficiencies of alizarin was evaluated and compared with maceration. The optimal extraction conditions found were 0.1 g dried plant sample with soaked for 3 h in 15 mL of 60% aqueous ethanol and then extraction for 15 min under ultrasonic irradiation in ultrasonic bath. Under the optimum conditions, the extraction yield of alizarin was 14.80 ± 0.02 mg/g with 95 - 102 % recovery. The reproducibility of the extraction method were in the range of 2.1 -2.5% for intra-day and inter-day extraction.

KEYWORDS

Ultrasonic extraction;
Ultrasound;
Alizarin;
Morinda citrifolia.

INTRODUCTION

Alizarin (1,2-dihydroxyanthraquinone) is a red dye which has been used for dyeing textile to produce yellow or red color[1] and normally used as complexing agent for some metal ions[2,3]. Its pigment code is Pigment Red 83 and its color index number, CI, is 75,330[4]. The natural sources of alizarin were found from the roots of madder plants (*Rubia tinctorum*)[4] and the roots of Noni plants (*Morinda citrifolia*)[5]. The alizarin component became the first natural dye to be synthetically reproduced in 1860 by the German chemists: Carl Graebe and Carl Liebermann, and the English dye chemist: William Perkin[4]. Although, the synthetic dyes is still the most useful for dyeing textile, the development of the alternative for the preparation of the natural dyes is increased by the world-wide demand for fibers and colorants of natural origin.

Morinda citrifolia (Noni), a plant originated in tropical Asia or Polynesia, has been used as food and medicine for over 2000 years. All parts of this plant, which include fruits, flowers, leaves, bark, stem and roots contain about 160 phytochemical compounds and the major micronutrients are phenolic compounds, organic acids and alkaloids[5]. The Noni plant roots contain several of some hydroxyanthraquinones such as Morindone, Damnacanthal, Morenone, Alizarin etc.[5]. The principle dyes occurring in these dried roots are alizarin.

Many research projects have been used some extraction techniques such as solvent extraction[4], ultrasound-assisted extraction (UAE)[1], microwave-assisted extaction (MAE)[6,7], pressurized hot water extraction (PHWE)[8] and micelle-mediated pressurized hot water extraction (MMPHWE)[9] for extraction of some anthraquinones from the roots of *Morinda citrifolia* and the roots of *Rubia tinctorum*. Among these, the use of ultrasound trends to be the suitable extraction technique for bioactive constituents from plant materials[10,11]. The ultrasound enhances the extraction efficiency by increasing the yield and by shortening the time for extraction[1]. Also, the ultrasonic-assisted extraction is not difficult to perform on a laboratory scale using a simple cleaning bath by direct or indirect extraction[11].

In the present work, a simple, fast, precise and reliable extraction method for alizarin in roots of *Morinda citrifolia* was developed using ultrasound in ultrasonic bath. The extraction apparatus on ultrasonic bath was performed. The various effects on the release of alizarin from the roots of *Morinda citrifolia* such as types of solvent, compositions of solvent, extraction time and solvent volume/sample weight ratio were investigated and optimized for high recovery and high precision.

EXPERIMENTAL

Plant materials

The fresh roots of *Morinda citrifolia* were collected in Chiang Mai Province, Thailand. The samples were chopped to small pieces and then dried in an oven at 50 °C for 24 h. The dried root samples were ground to powder and kept in a dry place until use.

Maceration

Maceration was performed with 0.1 g of dried root samples and 10 mL of solvent in glass vial. The mixture was left at ambient temperature for desirable time. The extract was then filtered with a filter paper (Whatman No.1). The concentration of anthraquinones as alizarin was measured by spectrophotometer.

Ultrasound extraction method

An ultrasonic bath was used as an ultrasonic source for the ultrasound-assisted extraction experiments. The Tru-Sweep ultrasonic benchtop cleaner Model 575HT (Crest Ultrasonics, USA) was basically a rectangular container (29.2 cm x 15.2 cm x 15.2 cm) with 38.5 – 40.5 kHz transducers at the bottom. The sonic power rating was 135 W. The extraction of anthraquinones was performed by adding 0.1 g of dried root samples into 15 mL of solvent in ≈ 20 mL of glass vial. The dried samples were soaked for 3 h and the glass vial was then partially immersed into the ultrasonic bath, which contains ca. 2 L of water. To fix the position of the extraction bottle, the stainless steel rack with iron wire was used as shown in Figure 1. The bottom of the glass vials were contacted with the bottom of the bath. The solvent surface in the flask was kept under the level of the water in ultrasonic bath. After extraction was completed at desirable time, the extract was then filtered with a filter paper (Whatman No.1). The concentration of anthraquinones as alizarin was measured by spectrophotometer.

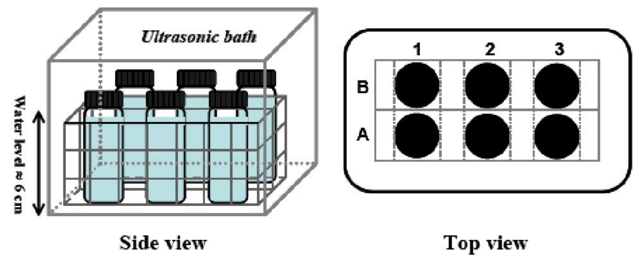

Figure 1 : Extraction apparatus on ultrasonic bath

Conventional extraction method

Extraction was carried out by magnetic stirrer of one gram of the roots of *Morinda citrifolia* in 150 mL of 60% ethanol in a clean glass beaker. The beaker was tightly covered with aluminum foil to prevent the evaporation of water from the beaker. The absorbance values were monitored till there was no appreciable change, i.e. 24 h.

UV-visible spectrophotometric analysis

The quantitative analysis of the anthraquinones extracts as alizarin from the roots of *Morinda citrifolia* in various solvents was directly analysed using UV-visible spectrophotometer Lamda 25 (Perkin Elmer, USA). A calibration curve of alizarin (1,2-dihydroxy-anthraquinone) solution in various solvents was constructed in the range of $2.5 \times 10^{-5} – 2.0 \times 10^{-4}$ M and measuring the absorbance at 436 nm.

RESULTS AND DISCUSSION

Effect of type of solvents

Generally, the choice of an extracting solvent was the first important step towards parameter optimization, which has a strong impact on the yield of extraction. In the present study, several polar solvents such as acetone, ethanol and water were selected as extracting solvents, due to their polarity relatively to alizarin which are slightly polar compounds. The results in Figure 2 reveals that the extraction efficiency could generally be enhanced, by application of ultrasound, however the degree of enhancement differed depending on the type of solvent. For both cases, ethanol gave the highest extraction yield, followed by water and acetone. However, these results are difference from the previous study[1] which acetone trend to providing the highest extraction yield. For subsequent investigation, ethanol and water were selected as extracting solvent due to their extraction efficiency.

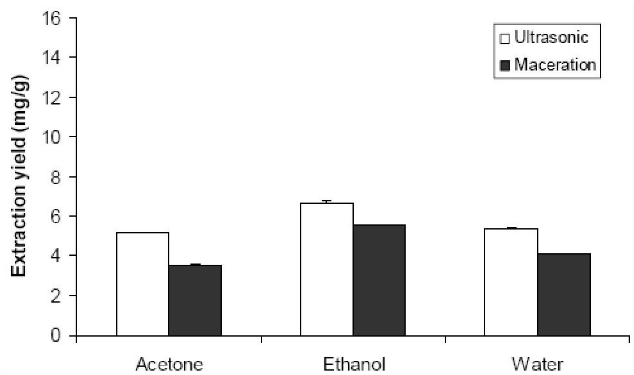

Figure 2 : Effect of solvent type on the extraction yield of alizarin. Ratio of solvent to sample: 10 mL/0.1g; extraction time 10 min

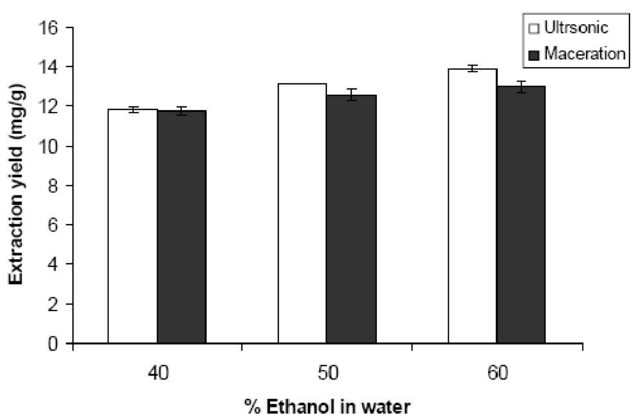

Figure 3 : Effect of ethanol-water compositions on the extraction yield of alizarin. Ratio of solvent to sample: 10 mL/0.1g; extraction time 10 min

Effect of ethanol-water compositions

In order to study the effect of ethanol-water on the extracting performance, different concentrations of ethanol were prepared as solvents to extract alizarin from the dried roots of *Morinda citrifolia*. The results revealed that the extraction yield was the highest when 60% ethanol was used as extracting solvents. Seen from Figure 3, when the concentration of ethanol was less than 60%, the yield increased with the increased concentration of ethanol. The results indicated that 60% ethanol was suitable for the extraction of alizarin from the plant. This was probably due to the relatively polarity and the increase in effective swelling of the plant by water, which can increase the surface area for solute-solvent contact[1].

When ultrasound was applied, extraction efficiency could generally be improved due to the effect of ultrasonic cavitation. The same trend was resulted as with maceration in which the extraction efficiency increased with the amount of water added until up to 60%. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture was also increased as the surface tension increased and the viscosity and the vapor pressure decreased[1].

Effect of extraction time

The influence of extraction time on the extraction yield of alizarin from the roots of *Morinda citrifolia* is shown in Figure 4. The results indicated that in both cases, the extraction yield is highly time-dependent. The rate of alizarin extraction was high, during the first 3 min, and then slightly increases. Compared with maceration, the ultrasound was found to enhance the extraction yield. The results in Figure 4 also show that the application of ultrasound increased the extraction rate only in the first 3 min. The rate thereafter was similar to that of maceration. This suggests that ultrasound was needed only at the beginning to initialize the fast extrac-

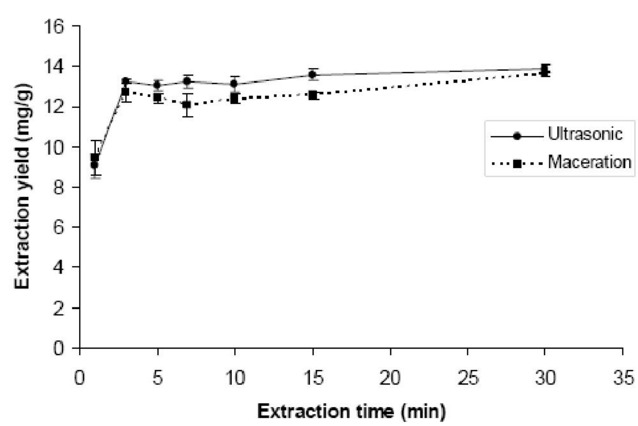

Figure 4 : Effect of ultrasonication time on the extraction yield of alizarin. Ratio of solvent to sample: 10 mL/0.1g; 60% ethanol

tion process. However, the utrasonication time at 15 min was selected from high extraction yield (13.56 ± 0.29 mg/g) within short extraction time.

Effect of solvent volume/sample weight ratio

Under the above optimal conditions of extracting solvent (60% EtOH) and time of sonication (15 min), effects of solvent volume on the extracting yield of alizarin was evaluated. The results are displayed in Figure 5. The results indicated that an increase of extraction yield of alizarin could be observed with the increase of the solvent volume /sample weight ratio, especially when this ratio was increased from 5 to 10 mL. Normally, a larger solvent volume can dissolved target components more effectively leading to an enhancement of the extraction yield[12,13]. Hence, the solvent volume/ sample weight ratio of 15 mL was selected.

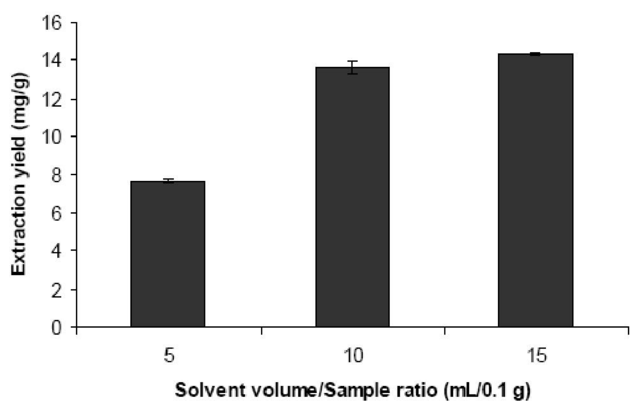

Figure 5 : Effect of solvent volume/sample weight ratio on the extraction yield of alizarin. 60% ethanol; ultrasonication time 15 min

Comparison between ultrasonic extraction and conventional method

The extraction yield between ultrasonic extraction and conventional method was compared as listed in TABLE 1. Conventional solvent extraction with magnetic stirrer for more than 24 h in 60% ethanol was able to give higher yield than ultrasonic extraction (1). For dried materials, the extraction yield can be improved by steeping them in solvent to facilitate swelling and hydration process in the pores of the cell wall which will improve the diffusion process and hence enhancing mass transfer[11]. Therefore, by soaking the dried roots of *Morinda citrifolia* in extracting solvent for 3 h before ultrasonic extraction for 15 min, the extraction yield increase up to 14.80 mg/g, which was comparable to the conventional method, but with shorter extraction time required.

TABLE 1 : Comparison between conventional method and ultrasonic extraction method

Methods	Extraction time	Extraction yield (mg/g)
Conventional	> 24 h	14.87 ± 0.45
Ultrasonic extraction (1)	15 min	14.30 ± 0.06
Ultrasonic extraction (2)	Soaked 3 h + 15 min	14.80 ± 0.02

Recovery

Under the above optimal conditions, the alizarin standards 0.5 and 1.0 mg were added to 0.1 g of the dried roots of *Morinda citrifolia*. The high percentage recoveries of alizarin 95-102 % were obtained as shown in TABLE 2.

TABLE 2 : The recovery of alizarin from dried roots of *Morinda citrifolia* (n=3)

Sample	Alizarin added (mg)	Recovery (%)
Dried roots of *Morinda citrifolia*	0.5	95.7 ± 2.1
Dried roots of *Morinda citrifolia*	1.0	101.8 ± 1.5

Reproducibility

The average extraction yield for 6 bottles which extract on the same experiment was 14.53 ± 0.27 mg/g with 1.9% RSD. To study the reproducibility of the ultrasound-assisted extraction method for alizarin from dried roots of *Morinda citrifolia*, the intra-day and the inter-day extraction with 3 times of experiments were performed. The extraction yields of alizarin showed a good reproducibility of experiments for intra-day and inter-day precision at 2.1 and 2.5%, respectively.

CONCLUSIONS

The laboratory scale for extraction of alizarin from the roots of *Morinda citrifolia* was developed using an ultrasonic bath. The proposed extraction method is simple and provides high extraction efficiency with good recovery and good precision for intra-day and inter-day extraction. The utility of crude alizarin extract solutions will be further investigated as colorant for small scale experiment.

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