Original Article

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# Validated TLC-densitometric method for determination of oxyresveratrol contents in ma-haad (*Artocarpus lakoocha*) heartwood extracts

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## Abstract

*Atrocapus lakoocha* Roxb. or Ma-haad, is a tree in Moraceae family. The main stilbenoid in the heartwood of this plant, oxyresveratrol is an active substance that promotes strong tyrosinase inhibitory and antioxidative effects. In this study, thin layer chromatographic (TLC) – densitometric method was developed and validated to quantitative analyze of oxyresveratrol contents in aqueous and ethanolic extracts from *A. lakoocha* heartwood. All validated parameters including linearity, precision and accuracy were in acceptable ranges. The validated TLC-densitometric method was applied for quantitative analysis of oxyresveratrol content in *A. lakoocha* heartwood extracts. Ethanolic extract contained oxyresveratrol in a higher amount than aqueous extract (7.66 and 6.80 %w/w dry weight, respectively). The developed TLC densitometric method was simple accurate and precise, which could be used for quantitative analysis of oxyresveratrol content in *A. lakoocha* heartwood extracts.

**Keyword:** *Artocarpus lakoocha*, mahaad, oxyresveratrol, TLC-densitometry, validated method

## 1. INTRODUCTION

*Artocarpus lakoocha* which is called in Thai as Ma-haad, is a medium to large deciduous tree with a spreading crown. The bark is grey and the slash is deep red with milky latex[1]. This plant is native in the regions of South and South-East Asia including Bangladesh, Bhutan, Cambodia, India, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam[1]. In Thai traditional medicine, the heartwood of this plant has been used as anthelmintic agent for the treatment of tapeworm and ascaris while the roots and stem bark have been used as antipyretic agents[2]. Water extract from heartwood of *A. lakoocha*, Puag-haad is a yellow solid which has also been used for anthelmintic effect[3]. The extract from the heartwood of this plant was reported to exhibit *in vitro* tyrosinase inhibitory effect with the inhibition of melanin production and whitening effect in clinical trial[3]. The active component in Ma-haad heartwood is 2,3,4, 5,-tetrahydroxystibene or oxyresveratrol. This compound was reported to promote strong tyrosinase-inhibitory and skin-whitening effects and antibrowning activity for fruit juice. It could inhibit tyrosinase 20 times stronger than resveratrol[4,5]. Moreover, oxyresveratrol also exhibited antiviral, anti-herpes simplex virus, anti-inflammatory, antioxidant and neuropreotective effects[6-11]. From various pharmacological effects and cosmetic advantages, the development of pharmaceutical and cosmetic products from heartwood of *A. lakoocha* is now popular.

Quality control of oxyresveratrol contents in raw material, heartwood extract and finished product could be developed. Some analytical method including gravimetric, high-performance liquid chromatographic (HPLC), capillary zone electrophoretic and thin layer chromatographic (TLC)-densitometric techniques were developed for quantitative analysis of oxyresveratrol contents in Puag-haad and *A. lakoocha*[12-15]. However, there is no report for validated analytical method that compares oxyresveratrol

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content in Ma-haad heartwood extracts prepared by different methods of extraction.

## 2. MATERIALS AND METHODS

### *2.1 Preparation of Artocarpus lakoocha heartwood samples*

The heartwoods of mahaad (*A. lakoocha*) were purchased from traditional drugstore in Sampantawong district, Bangkok in June 2013. Plant samples were identified by Assistant Prof. Dr. Pongtip Sithisarn, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. Plant samples were cleaned, chopped into small pieces and dried in hot air oven (60 $^{o}$C, 30 min). After that, plant samples were powdered using electronic mill (60 mesh sieve). *A. lakoocha* powder was kept at -20 $^{o}$C until used.

### *2.2 Preparation of Artocarpus lakoocha heartwood extracts*

For decoction, *A. lakoocha* powder was boiled with distilled water (1: 10 w/v) for 1 h and then filtered using Whatman filter paper No. 1. The extraction method was repeated for 2 times. The filtrates were combined and dried by evaporation on a water bath to yield dried *A. lakoocha* heartwood aqueous decoction extract.

For maceration, *A. lakoocha* powder was macerated with 70% ethanol (1: 10 w/v) for 6 h with continuous shacking and left overnight (12 h) then filtered using Whatman filter paper No. 1. The extraction method was repeated for 2 times. The filtrates were combined and dried by evaporation on a water bath to yield dried A. lakoocha heartwood ethanolic maceration extract.

### *2.3 Preparation of Standard Oxyresveratrol Solution*

Standard stock solution of oxyresveratrol (1 mg/ml) was accurately prepared by dissolving oxyresveratrol (10 mg) in methanol (10 ml). A working standard solution with a concentration of 200 mg/ml was then prepared from the dilution of 2 ml stock solution into the volume to 10 ml. From this solution, 4 additional standard solutions (0.08, 0.04, 0.02 and 0.01 mg/ml) were prepared by serial dilution. The amounts of 200, 400, 600, 800 and 1000 ng/band were the used for preparation of oxyresveratrol standard curve for TLC-densitometric analysis of oxyresveratrol content in Ma-haad extracts.

### *2.4 Instruments and Chromatographic Conditions*

Ten microliters of each sample solution was spotted in form of bands of a length of 5.0 mm, on a precoated silica gel aluminum plate 60F-254 (20 x 10 cm) using a Camag Linomat 5 syringe. A constant application rate of 100 nl/s was employed while a space between each band was 10.0 mm. The slit dimension was kept at 4.00 x 0.30 mm while 10 mm/s scanning speed was employed. The mobile phase consisting of hexane : ethyl acetate : chloroform : methanol : formic acid (3 : 3 : 2: 1: 1) was used. Linear ascending development was carried out in 20 x 10 cm twin trough glass chamber saturated with the mobile phase. The length of each chromatogram run was 8 cm. After developing, the TLC plate was dried using an air dryer. Densitometric scanning was performed on Camag TLC scanner 3 in the reflectance-absorbance mode at 366 nm, operated by CATS software (V 1.2.6, Camag, Switzerland). The source of radiation utilized was a deuterium lamp. Video densitometry of the TLC-chromatogram was carried out with the support of Camag Reprostar 3 with cabinet cover and mounted digital camera. The oxyresveratrol content was analyzed from the TLC chromatographic band at the $R_f$ value of 0.49.

### *2.5 Method Validation*

The TLC-densitometric analytical method was validated in terms of linearity, accuracy, and precision.

#### *Linearity*

Linearity was determined over the range of 200 – 1,000 ng/spot. Five concentrations of standard oxyresveratrol solutions (20,000, 40,000, 60,000, 80,000 and 100,000 ng/ml) were applied on to the TLC plate to give bands

containing various amounts of oxyresveratrol (200, 400, 600, 800 and 1,000 ng/spot). A plot of average area under curve (AUC) versus concentration (ng/spot) was obtained. Linearity was expressed as the correlation coefficient ($r^2$).

### *Accuracy*

Determination of accuracy was done using standard addition method. Standard oxyresveratrol (200 mg/ml) 0, 1, 2 and 3 ml were separately added with 0.5 ml of *A. lakoocha* heartwood extracts (4 mg/ml) then the volume of each solution was adjusted into 5 ml. Oxyresveratrol content was then analyzed using TLC-densitometry with the analytical as mentioned above and oxyresveratrol standard curve. The percentage of recovery was then calculated.

### *Precision*

Precision was evaluated in terms of intra-day and inter-day precision. Three concentrations of standard oxyresveratrol solution (200, 400 and 600 mg/ml) were added to *A. lakoocha* heartwood extracts. The percentage of relative standard deviation (%RSD) of oxyresveratrol content determined in the same day and three different days were calculated for intra-day precision and inter-day precision, respectively.

### *2.6 Quantitative Analysis of Oxyresveratrol in Ma-haad Extracts*

Fifty milligram of each Ma-haad extract was dissolved in 10 ml methanol. The obtained solution was then analyzed for oxyresveratrol content using validated TLC-dentisomteric method.

### *2.7 Statistical Analysis*

All data are reported as means ± standard deviation of triplicates. Least significant difference was used to compare means ($p < 0.05$). All analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., USA).

## 3. RESULTS AND DISCUSSION

### *3.1 Preparation of Artocarpus lakoocha heartwood extracts*

Maceration with 70% ethanol gave Ma-haad extracts with the higher yield (39.45 ± 0.92 %w/w) than the yield of the extracts obtained from decoction method (23.95 ± 1.34 %w/w). The extracts from both methods appeared as dark brown sticky extracts with specific odor. Ma-haad heartwood raw material and the chemical structure of oxyresveratrol are shown in Figure 1.

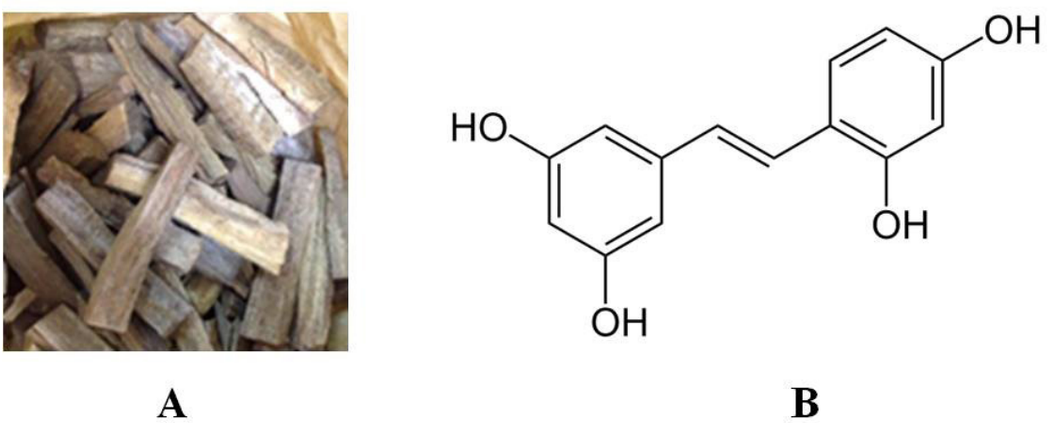


**Figure 1.** Physical characteristic of Ma-haad (*Artocarpus lakoocha*) heartwood (A) and chemical structure of oxyresveratrol (B).

### *3.2 Optimized Mobile Phases for TLC*

Optimum TLC condition was obtained after running different mobile phases. The best result was obtained by use of a silica gel $GF_{254}$ plate with a mobile phase consisting of hexane : ethyl acetate : chloroform : methanol : formic acid (3 : 3 : 2: 1: 1) (v/v). TLC analysis resulted on good resolution of oxyresveratrol at $R_f$ 0.49 ± 0.05.

### *3.3 Method Validation*

The developed method was found to be linear, accurate and precise for the analysis of oxyresveratrol content of in Ma-haad heartwood extracts under the experimental conditions used. The validation parameters are shown in Table 1.

### *3.4 Quantitative Analysis of Oxyresveratrol in Ma-haad Extracts*

The validated TLC-densitometric method was then applied to determine the content of oxyresveratrol in Ma-haad extracts. Ma-haad 70% ethanolic maceration extract significantly contained higher oxyresveratrol content than the content in decoction extract (Table 2). The oxyresveratrol content in both extracts ranged from 6-8 %w/w. Thin layer chromatogram for analysis of oxyresveratrol in Ma-haad heartwood extracts is shown in Figure 2.

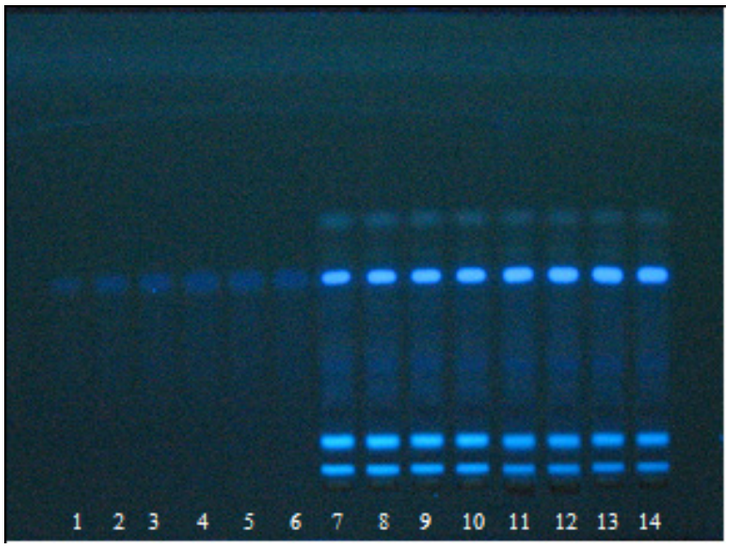


**Figure 2.** TLC chromatogram for analysis of oxyresveratrol in Ma-haad heartwood extracts; 1 = standard oxyresveratrol (200 ng), 2= standard oxyresveratrol (400 ng), 3 = standard oxyresveratrol (600 ng), 4 = standard oxyresveratrol (800 ng), 5 = standard oxyresveratrol (1000 ng), 6 = standard oxyresveratrol (1000 ng), 7-10 = Ma-haad heart wood decoction extracts, 11-14 = Ma-haad heart wood 70% ethanolic maceration extracts. Stationary phase : silica gel 60 F254, solvent system: hexane : ethyl acetate : chloroform : methanol : formic acid (3:3:2:1:1 v/v/v/v/v), detector UV 366 nm.

**Table 1.** Method validation data of the proposed TLC-densitometric method

| Validation parameter | Results |
|---|---|
| Linearity | y = 14.576x + 1432.787 ( $r^2$=0.9982 ) |
| Range | 200-1000 ng/band |
| Accuracy (% R) | 91.9-112.8 |
| Intraday precision (% RSD, n=6) | 5.4 -7.2 |
| Interday precision (%RSD, n=18) | 6.9 |

**Table 2.** Oxyresveratrol content in Ma-haad decoction and 70% ethanolic extracts

| Sample | Oxyresveratrol content % (w/w) |
|---|---|
| Ma-haad decoction extract | 6.80 ± 0.10a |
| Ma-haad 70% ethanolic maceration extract | 7.66 ± 0.11b |

The data was expressed as the mean ± SD of each result in 100 g Ma-haad heartwood. Different letters in the same column indicated significant differences between oxyresveratrol content in Ma-haad decoction and 70% ethanolic extracts ($p$<0.05)

## 4. CONCLUSION

Extraction by maceration with 70% ethanol promoted Ma-haad heartwood extract with high oxyresveratrol content. The developed TLC-densitometric method has acceptable validation parameters and could be applied for quantitative analysis of oxyresveratrol content in Ma-haad raw material and crude extract.

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