PHARMACOGNOSTIC STUDY OF *ARTOCARPUS LAKOOCHA* HEARTWOOD

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ABSTRACT: A pharmacognostic study of *Artocarpus lakoocha* heartwood was performed on 13 samples collected from five different geographical areas of Thailand. Evaluation of the crude drug was conducted according to the World Health Organization (WHO) guidelines for herbal standardization. Microscopic examination of the powdered drug revealed the presence of parenchyma and fiber cells of the medullary ray, as well as bordered pored tracheids and vessels. The contents of foreign matter, acid-soluble ash, total ash, moisture and oxyresveratrol were determined to be 0.04, 2.06, 2.51, 9.57 and 1.44 %, respectively, whereas the ethanol-soluble extractive, water-soluble extractive and loss on drying values were found to be 7.93, 5.27 and 9.79 %. In addition, a thin-layer chromatographic system for rapid detection of oxyresveratrol was described, and a method for quantitative analysis of oxyresveratrol content in the crude drug using capillary zone electrophoretic technique was developed.

Keywords: *Artocarpus lakoocha*, pharmacognostic specification

INTRODUCTION: For centuries, plants and plant products have been used for treating various illnesses. Today, several medicinal plants and their products are still in use, being employed as home remedies, over-the-counter drugs as well as raw materials for the pharmaceutical industry, and they represent a substantial proportion of the global drug market[1].

Artocarpus lakoocha Roxb. (Moraceae), also known as *Artocarpus lacucha* Roxb., is a tropical tree widely distributed in the regions of South and South-east Asia , including Nepal, India, Srilanka, Myanmar, Southern China, Vietnam, Thailand, Malaysia and Indonesia[2]. In Thailand, the plant is called 'Ma-haad', and a dried aqueous extract prepared from the heartwood of this plant and known as 'Puag-haad' has been traditionally used as an anthelmintic[3].

Previous chemical investigations of the heartwood of this plant have shown that oxyresveratrol (2,4,3',5'-tetrahydroxystilbene) is the major constituent[4,5] and the compound is responsible for the anthelmintic activity[5]. Recently potent skin depigmenting effect[6] and moderate anti-herpetic activity[7] have been reported for oxyresveratrol, suggesting the potential of the compound as a skin whitening agent or an anti-herpetic drug. Because of its high content of oxyresveratrol, *A. lakoocha* heartwood appears to be an excellent natural source of the compound. However, it can be envisaged that the pharmacognostic properties of the crud drug have to be fully determined before its international commercial opportunities can be realized. A previous comprehensive study on the pharmacognostic and chemical properties of *A. lakoocha* described the morphology and microscopic characteristics, and the oxyresveratrol content of the stems, branches and roots of the plant collected from the wild in five provinces of Thailand[5]. The present report provides additional and more update information on the pharmacognostic properties of *A. lakoocha* heartwood, based on the samples currently available in drugstores in Thailand

MATERIALS: Samples of *A. lakoocha* heartwood were purchased from 13 different Thai traditional drugstores. Three of them were located in Bangkok. The remaining drugstores were situated in 10 different provinces and distributed in five geographical areas of Thailand as

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follows: (1) Central: Nakorn Pathom and Kamphaeng Phet; (2) Northern: Pichit and Nakorn Sawan; (3) Eastern: Chon Buri; (4) Northeastern: Nakorn Ratchasima and Ubon Ratchathani; (5) Southern: Songkhla, Trang and Nakorn Si Thammarat.

METHODS: Macroscopic and microscopic properties, and constant numbers due to quality of *A. lakoocha heartwood* were examined by standard methods of the World Health Organization (WHO)[1].

Macroscopic and microscopic examination

Each sample of *A. lakoocha* heartwood was identified by visual examination of the physical properties such as texture, size, colour and other visual inspections. For microscopic examination, the powdered sample (ground and sifted through a 250 micron sieve) was inspected under a microscope equipped with a micrometer.

Determination of foreign matter

The sample (50.0 g) was spread in a thin layer, and the pieces of foreign matter were sorted out by visual inspection. The powder of the foreign matter was sifted through a 250 micron sieve. All portions of the foreign matter were pooled and weighed.

Determination of total ash

The ground sample (3.0 g, accurately weighed) was placed in a previously ignited and tared crucible. The sample was spread in an even layer and ignited by gradually increasing the heat to 500-600°C until white ash was obtained. The ash was then cooled in a desiccator and weighed without delay.

Determination of acid-insoluble ash

To the crucible containing the total ash was added 25.0 ml of hydrochloric acid (70 g/l). The crucible was then covered with a watch-glass, and the mixture was boiled gently for 5 minutes. The watch-glass was rinsed with hot water (5 ml), and this liquid was added into the crucible. The insoluble matter was collected on ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator and weighed without delay.

Determination of ethanol-soluble extractive

The ground sample (5.0 g) was macerated with absolute ethanol (100.0 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extract was filtered rapidly to avoid loss of ethanol. The filtrate (20.0 ml) was evaporated to dryness in a tared small beaker and then dried with heat to constant weight.

Determination of water-soluble extractive

The ground sample (5.0 g) was macerated with distilled water (100.0 ml) in a closed conical flask in shaking bath for 6 hours and allowed to stand for 18 hours. The extract was filtered, and the filtrate (20.0 ml) was evaporated to dryness in a tared small beaker and dried at 105°C to constant weight.

Determination of loss on drying

The ground sample (5.0 g) was accurately weighed in a tared small beaker and then dried at 105°C to constant weight.

Determination of water content

The ground sample (50.0 g) in water-saturated toluene (200.0 ml) was subjected to azeotropic distillation. As soon as the water was completely distilled, the inside of the condenser tube was rinsed with toluene, and the distillation was continued for 5 more minutes. The heat was then removed, and the receiving tube was allowed to cool to room temperature. The water and toluene layers were allowed to separate, and then the volume of water was read off.

Determination of volatile oil content

Volatile oil distillation was performed on the ground sample (100.0 g) in water (600.0 ml) using a clevenger apparatus. When the distillation was complete, the heat was removed, and the receiving tube was allowed to cool to room temperature. The volatile oil and water layers were allowed to separate, and then the volume of volatile oil was read off.

Thin-layer chromatographic identification

The ground sample (1 g) was macerated with methanol (20 ml) of for 12 hours. The extract was filtered and evaporated to dryness. The residue was dissolved in

methanol (0.5 ml), and 10 μl of this solution was applied on to a thin-layer plastic plate coated with siliga gel GF254 (Polygram® SIL G/UV254, 0.25 mm thickness, 20 cm x 20 cm). The TLC plate was then placed in a chamber with chloroform and methanol (9:1) as mobile phase. After development, the plate was removed, and allowed to dry in air and examined under ultraviolet light (254 nm and 366 nm). Then, the plate was sprayed with vanillin-sulfuric acid reagent and heated in an oven at 120 °C for 10 minutes.

Quantitative analysis of oxyresveratrol by capillary zone electrophoresis

Sample was ground and sifted through a 250 micron sieve. The ground sample (2 g) was extracted with methanol (250 ml) in a soxhlet extractor for 5 hours. The extract was collected, and its volume was adjusted to 250 ml in volumetric flask. The obtained extract was diluted with a running buffer (1:4 v/v). Standard solutions of oxyresveratrol (25, 50, 75 and 100 mg/l in running buffer) were prepared from a solution of oxyresveratrol (1.0 mg/ml in methanol) by serial dilution. Benzoic acid (1.0 mg/ml in distilled water) was used as internal standard. Solutions of analytes were filtered through a 0.45 μm syringe filter before injection.

Capillary zone electrophoresis conditions were as follows: ***Instrument:*** P/ACE System 5010 Beckman; ***Buffer solution:*** Borate buffer 25 mM, pH 9.24 prepared from Sodium tetraborate; ***Capillary column:*** Uncoated fused silica capillary 57 cm length (50 cm to detector) x 50 μm i.d., thermostated 25°C, voltage 30 kV; ***Detector:*** UV detector at 280 nm; ***Sample, Standard and Internal Standard injection:*** 2 seconds from each vial.

Validation of analytical method

Accuracy and precision were determined by standard addition method. The methanolic extract was spiked with standard oxyresveratrol (20.0 and 50.0 mg/l). The spiked sample was analyzed as described above in duplicate. Precision of 4 days spiked assay was measured as the coefficient of variation (CV).

RESULTS AND DISCUSSION: The heartwood of *Artocarpus lakoocha* has brown color (Figure 1). It is hard and termite resistant with a weight of about 640 kg/cu.m[8]. Figure 2 shows a branch of *A. lakoocha* with leaves alternate and fruits in axillary position. The powdered sample of *A. lakoocha* heartwood under microscope (Figure 3) displayed large amounts of lignified fiber and medullary ray cells, typical of wood tissues. Bordered-pored tracheids and vessels were also abundant. The microscopic characteristics observed in this study were in agreement with those earlier reported by Sambhandharaksa et al[5]. Table 1 shows the other pharmacognostic properties of *A. lakoocha* heartwood. The contents of foreign matter, acid-soluble ash, total ash, moisture and oxyresveratrol were determined to be 0.04, 2.06, 2.51, 9.57 and 1.44 %, respectively, whereas the ethanol-soluble extractive, water-soluble extractive and loss on drying values were found to be 7.93, 5.27 and 9.79 %. These constant numbers have been reported for the first time in this study, except for the water and oxyresveratrol contents.[5]

Table 1 The constant numbers due to quality of *A. lakoocha* heartwood

Specification	Mean ± SD*	Min - Max*
Foreign matter	0.04 ± 0.02	0.02 – 0.12
Acid-insoluble ash	2.06 ± 1.31	0.82 – 5.95
Total ash	2.51 ± 1.27	1.22 – 6.23
Ethanol-soluble extractive	7.93 ± 3.53	0.10 – 16.28
Water-soluble extractive	5.27 ± 2.38	0.91 – 9.90
Loss on drying	9.79 ± 0.67	8.60 – 11.24
Volatile oil content	–	–
Water content	9.57 ± 0.94	7.12 – 11.40
Oxyresveratrol	1.44 ± 0.66	0.44 – 2.35

*% dry weight

As for the thin-layer chromatographic identification, the methanolic extract showed the presence of oxyresveratrol as the major component, appearing as a dark spot at 254 nm and fluorescing at 365 nm with an Rf value of 0.27 on the TLC plate. The compound also developed a pink spot with vanillin-sulfuric acid reagent on the TLC plate (Figure 4).

For analysis of the oxyresveratrol content in the samples, a capillary zone electrophoretic method was developed. Standard solutions of oxyresveratrol with benzoic acid as internal standard were prepared and analyzed (Figure 5). The ratio of the area under peak of oxyresveratrol to that of benzoic acid was then calculated for each concentration of oxyresveratrol. A standard curve was obtained from the plot of the ratio against the concentration (Figure 6) and then used for determining the oxyresveratrol content of each sample (Table 1). This analytical method was validated for its accuracy and precision, and the results are shown in Table 2. In this study, it appears that the oxyresveratrol content in *A. lakoocha* heartwood varied from one sample to another, in the range of 0.44 – 2.35 % (Table 1). These values are, however, greatly different from those earlier reported by Sambhandharaksa et al[5], which were described as 11 – 13 %. A possible explanation for this discrepancy is that samples from different sources may contain different amounts of oxyresveratrol. It should be noted that in the study by Sambhandharaksa et al[5], the plant materials were collected from the wild, whereas the samples in this investigation were obtained from drugstores. This may suggest that for some reason, the quality of the commercial products is much lower than that of the samples collected from the wild, in terms of oxyresveratrol content. Neverthelsess, it should also be kept in mind that the analytical method used in this investigation is different from that in the previous report.[5] It is clear that a chemical study properly designed for comparing the oxyresveratrol content in the commercial and the wild samples is needed before any conclusion can be drawn.

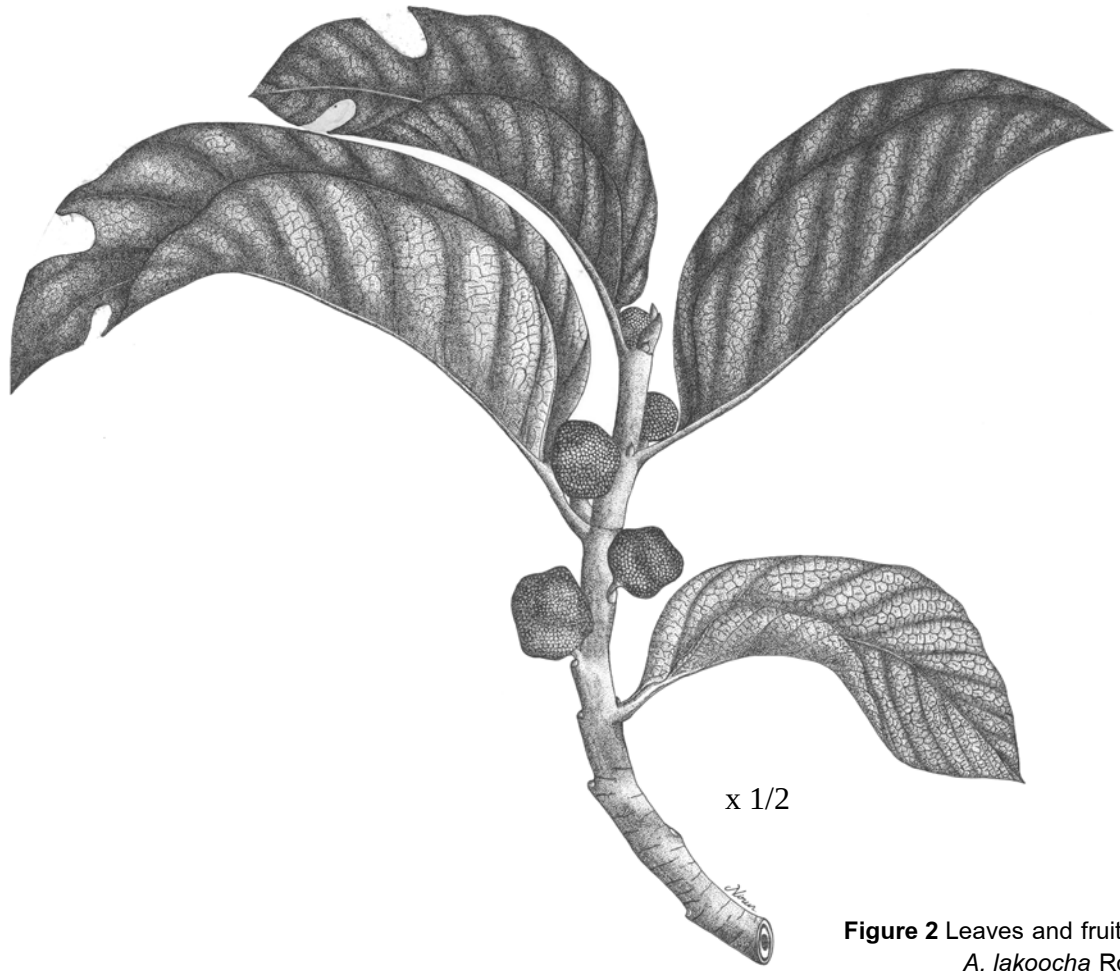

Figure 2 Leaves and fruits of *A. lakoocha* Roxb.

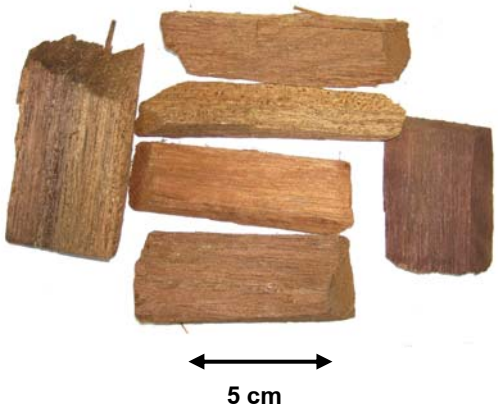

Figure 1 Heartwood of *Artocarpus lakoocha* Roxb.

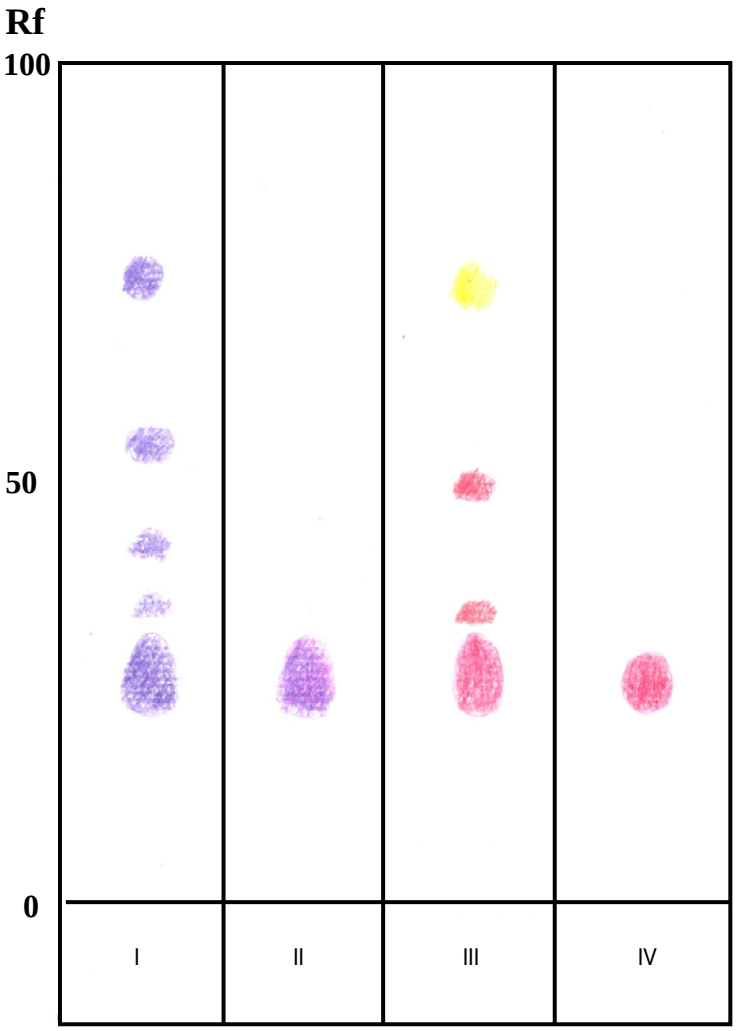

Figure 4 Thin-layer chromatogram of *A. lakoocha* heartwood extract
I = detection under UV light 254 nm
II = detection under UV light 366 nm
III = detection with vanillin-sulfuric acid
IV = standard oxyresveratrol, detection with vanillin-sulfuric acid

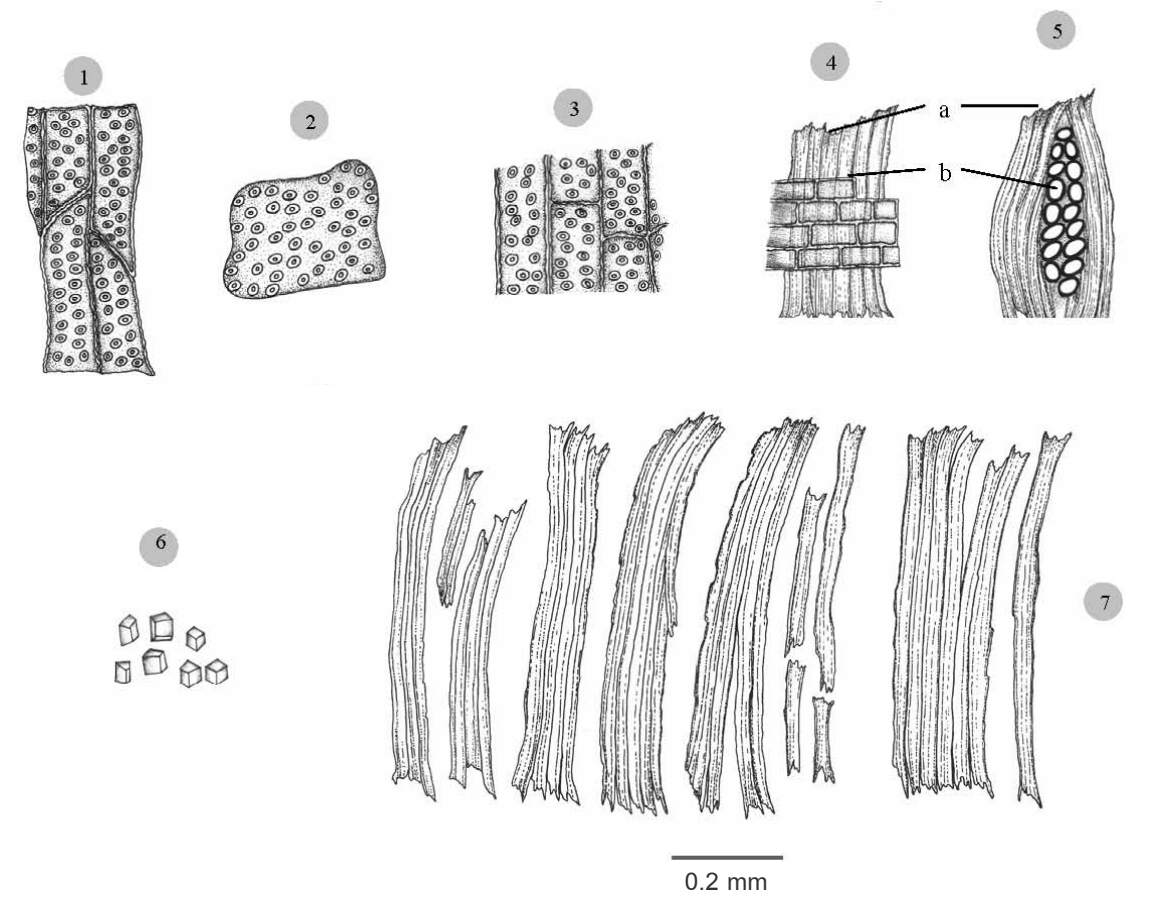

Figure 3 Microscopic characters (powdered) of *A. lakoocha* heartwood: (1) Bordered-pored tracheids; (2,3) Bordered-pored vessels; (4) Part of the xylem in radial longitudinal section showing wood fiber (a) and medullary ray cells (b); (5) Part of the xylem in tangential longitudinal section showing wood fiber (a) and medullary ray cells (b); (6) Calcium oxalate prism (7) Nonlignified and very slightly lignified wood fibers with brownish wall

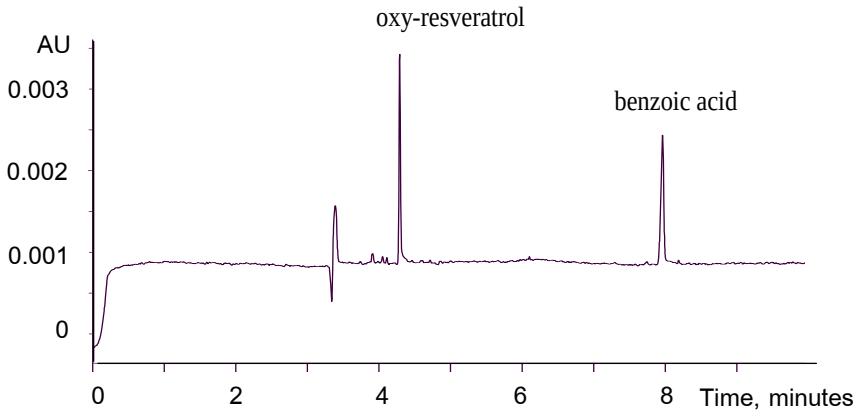

Figure 5 Chromatogram of methanolic extract of *A. lakoocha* heartwood by capillary zone electrophoresis

Table 2 Accuracy and precision of analytical method

No.	Oxy-resveratrol (mg/l)			
	Actual	**Estimated**	**Accuracy (%)**	**%CV**
1	20.0	19.5 ± 0.2	99.0	1.02
2	50.0	54.5 ± 1.5	106.0	2.75

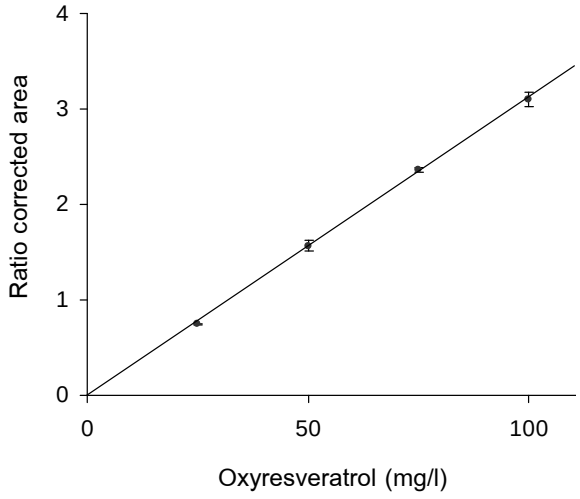

Figure 6 Calibration curve of oxyresveratrol by capillary zone electrophoresis

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คุณสมบัติทางเภสัชเวทของแก่นมะหาด

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บทคัดย่อ: ศึกษาคุณสมบัติทางเภสัชเวทของเครื่องยาแก่นมะหาดจากร้านขายยาแผนโบราณ 13 แห่ง ใน 5 พื้นที่ของประเทศไทย ตามคู่มือการจัดทำมาตรฐานสมุนไพรขององค์การอนามัยโลก ลักษณะทางจุลทรรศน์ของผงแก่นมะหาด พบเซลล์พาเรนไคมาและไฟเบอร์ของเนื้อไม้ ในขณะเดียวกัน พบท่อลำเลียงน้ำแบบรูที่มีขอบ ค่าเฉลี่ยของปริมาณสารปนเปื้อน เถ้าที่ไม่ละลายในกรด เถ้ารวม ความชื้น และสารออกซิเรสเวอราทรอล มีค่าร้อยละ 0.04, 2.06, 2.51, 9.57, และ 1.44 ตามลำดับ ขณะที่มีสารสกัดด้วยเอทานอล สารสกัดด้วยน้ำ และน้ำหนักที่หายไปเมื่อทำให้แห้ง ร้อยละ 7.93, 5.27 และ 9.79 ตามลำดับ วิเคราะห์สารออกซิเรสเวอราทรอลเชิงคุณภาพโดยวิธีทินเลเยอร์โครมาโตกราฟี และวิเคราะห์เชิงปริมาณโดยวิธีคาปิลลารีอิเล็กโตรโฟรีซิส

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