# Oxyresveratrol Contents in Artocarpus Lakoocha Heartwood and Its Biological Activities

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## Abstract

*Artocarpus lakoocha* heartwood extract initiates a highly robust plant source to become a new natural ingredient for the Thai cosmetic industry. The plant harbors a potential skin-whitening agent namely oxyresveratrol. Five sources of *A. lakoocha* heartwood powder obtained from the Thai herbal markets were used as test materials. The study focuses on comparing the quality of the different source of plant materials against various solvents used for extraction including water, propylene glycol (PG), butylene glycol (BG) and 95% ethanol. The effective extract conditions were evaluated with quantitative correlation analysis of the active substance oxyresveratrol and total phenolic contents in comparison with their biological activities. The 95% ethanol, PG and BG extract exhibited high content of oxyresveratrol and correlating with anti-tyrosinase activity while total phenolic content in the extract was correlated with antioxidant activity. PG is commonly used in the cosmetics *industry* and the preparation of extracts with PG has the advantage of short time, convenient procedures for laboratory preparation, and cost reduction. PG influence on oxyresveratrol content was evaluated to promote the solvent and extraction method to be applied to the Thai cosmetic industry.

**Keywords:** *Artrocapus lakoocha* Roxb.; Oxyresveratrol; HPLC; Quantitative analysis; Tyrosinase activity

## 1. Introduction

*Artocarpus lakoocha* Roxb. is a tropical tree belonging to the family Moraceae. It is widely distributed in China, Nepal, India, Sri Lanka, Indonesia, Myanmar, Malaysia, Thailand, and Vietnam

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(Jagtap & Bapat, 2010; Palanuvej et al., 2007). *A. lakoocha* heartwood is rich in flavonoids, triterpenoids, steroids, and stilbenoids. The plant extract has a high content of a derivative of resveratrol namely oxyresveratrol (trans-2,4,3',5'-tetrahydroxystilbene). It is used in several medicinal applications and plays an important role as a raw material for producing an alternative whitening agent in natural cosmetics. (Likhitwitayawuid, 2008)

According to the chemical investigation of *A. lakoocha* heartwood, it showed that oxyresveratrol (2, 4, 3', 5'-tetrahydroxy stilbene) is a major active component that is responsible for the anthelmintic treatment, anti-herpetic activity, anti-inflammatory, antioxidant, neuroprotective effect and tyrosinase inhibitory activities which has a potentially used as a skin-whitening agent in Thailand. (Palanuvej et al., 2007)

The preparation of *A. lakoocha* heartwood extract for traditional anthelmintic treatment is mostly conducted by boiling dried plant material with water followed by removing the insoluble residues and finally drying the extract until the yellow-brown powder appears. The solvent extractions gain the benefit of lower production cost, simple and safe to operate compared with the water extraction methods. Therefore the solvent extraction method was submitted to the plant material to obtain ingredients for cosmetic products. The classical extracting solvents (methanol, ethanol, acetone, acetonitrile, and 50% propylene glycol) have been studied (Tengamnuay et al., 2006; Teeranachaideekul et al., 2013).

In order to introduce *A. lakoocha* heartwood extract to be used as a whitening agent, glycol solvents should be considered. These solvents exhibit an outstanding advantage of being directly added to cosmetic products and functioning as a skin conditioning agent (humectant). According to the previous study, there was only propylene glycol used for the maceration of *A. lakoocha* heartwood (Teeranachaideekul et al., 2013). In this work, four solvents widely used in cosmetic industries such as water, propylene glycol (PG), butylene glycol (BG), and 95%ethanol were studied. The heartwood extracts were determined for oxyresveratrol contents by HPLC and were correlated analyzed with their biological activities. The heartwood extract of *A. lakoocha* was proved to possess *in vitro* tyrosinase-inhibitory activity and the *in vivo* melanin-reducing efficacy in human volunteers. The extract was dissolved in PG and was tested in female human volunteers using a parallel clinical trial with self-control for 12 weeks. It was found that *A. lakoocha* extract was the most effective agent, giving the shortest onset of significant whitening effect after only 4 weeks of application ($P<0.05$), followed by 3% kojic acid (6 weeks) and 0.25% licorice extract. (Teeranachaideekul et al., 2013)

The appropriate solvent and the extract condition were selected to extract five samples of *A. lakoocha* heartwood obtained from the herbal market. The extracts were determined for oxyresveratrol contents and biological activities. This research was performed in order to determine quantitative HPLC analysis of the active constituents including method validation, biological activities of *A. Lakoocha* extracts and also to investigate the quality of the plant materials in the herbal market. The results should be beneficial for the quality control of raw materials and their extract which could promote improvements in plant extraction and standardization.

## 2. Methods

### 2.1 Material

#### 2.1.1 Plant materials

Five samples of dried powder of *A. lakoocha* heartwood (CM1- CM5) were purchased from a traditional drugstore in Bangkok. The samples were ground into a fine powder, and then the particle size measurement of the samples was conducted. The powder passing a 60-mesh sieve and ranging in 250 μm was collected and subjected to the study.

#### 2.1.2 Chemical and reagents

Methanol, acetonitrile, absolute ethanol, and formic acid were purchased from Merck KGaA (Darmstadt, Germany). Propylene glycol (PG) and butylene glycol (BG) were USP grade and purchased from Shell Chemicals (Houston, USA) and OXEA Corporation (Texas, USA) respectively. Oxyresveratrol, 2,2-Di (4-tert-octylphenyl)-1-picryl hydrazyl (DPPH), 3-(3,4-Dihydroxyphenyl-2,5,6-d3)-L-alanine (L-DOPA), tyrosinase from mushroom (EC1. 14. 18. 1), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), L-ascorbic acid and kojic acid, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were HPLC grade or analytical grade.

### 2.2 Sample preparation

*A. lakoocha* heartwood powder (0.50 g) was weighed in an Erlenmeyer flask and separately extracted with 25 ml water, PG, BG, and 95% ethanol for 1 hour in an ultrasonic bath (Branson 3200, Connecticut, USA). Portions of extract were initially filtered through a 0.45 μm PTFE syringe filter for chemical quantitative and biological evaluations. The filtered extract was diluted 10 times with methanol before filtering with a 0.45 μm PTFE syringe filter into a vial and subsequently injected into the HPLC system.

### 2.3 HPLC analysis

#### 2.3.1 Apparatus and chromatographic conditions

A Waters Alliance e2695 equipped with a binary pump, Waters 2707 autosampler and Waters 2998 photodiode array detector controlled by software Empower 3 (Waters Corporation, Milford, MA, USA) was used for separation and analysis. Chromatography was carried out on a Symmetry C18 column (5 µm, 150 mm x 3.9 mm, I.D.) (Waters Corporation, Milford, MA, USA). The mobile phase was a gradient prepared from 1% formic acid in water (Solvent A) and acetonitrile (solvent B). The elution gradient was modified (Zhou et al., 2013) started from 0 min, 5% B to 35 min, 25% B. The flow rate was set at 1.0 ml/min, the injection volume was 10 µl, and the column temperature was 40 °C. The peak of oxyresveratrol was detected at the maximum wavelength of 320 nm.

**2.3.2 Method validation**

The developed HPLC method was validated in terms of linearity, accuracy, precision on oxyresveratrol contents of *A. lakoocha* heartwood extracted by water, PG, BG, and 95% aqueous ethanol.

**2.3.3 Preparation of standard solutions**

A standard solution with an accurate concentration of 4.00 mg/ml was prepared by dissolving 40 mg of oxyresveratrol in methanol 10 ml. From this solution, seven working standard solutions within a range of 0.02 - 0.14 mg/ml were prepared by dilution with methanol. All working standard solutions were filtered through 0.45 µm PTFE syringe filters prior to HPLC analysis.

**2.3.4 Linearity**

Linearity was determined over the range of 0.02 and 0.14 mg/ml. Seven working standard solutions with the concentrations of oxyresveratrol at 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 mg/ml in methanol. The calibration curve was constructed from the average area under curve (AUC) versus concentration (mg/ml). All standard solutions were analyzed in triplicate. Linearity was determined by regression analysis and was expressed as the correlation coefficient ($r^2$).

**2.3.5 Accuracy**

Determination of accuracy was performed using the recovery method. Five hundred milligrams of the *A. lakoocha* heartwood powder was separately spiked with 2, 6, and 10 ml of 4.00 mg/ml oxyresveratrol standard solution. The fortified sample was extracted with PG as described in sample preparation. Each of these solutions was then analyzed. The accuracy was expressed as the percentage recovery of oxyresveratrol calculated from the equation:

A : Concentration found = {($Area_{sample}$ - $Area_{blank}$) – Intercept} /Slope

B : Concentration Add ed = Wu/dilution factor

Where Wu means weight of oxyresveratrol

%Recovery = (Concentration found/ Concentration Added) x 100

The percentage of recovery for triplicate preparation of 3 concentrations should be within 95.0 – 105.0%.

**2.3.6 Precision**

The precision of the analysis was measured according to repeatability (intra-day) and intermediate precision (inter-day). Intra-day precision was performed by preparing 6 replicates of the *A. lakoocha* heartwood extract in PG as described in sample preparation and analyzed by HPLC within 1 day while the inter-day precision was determined for 3 independent days by different person. Precision was expressed as relative standard deviation (RSD) as follows:

$$\% \text{ Oxyresveratrol (w/w)} = \left[\frac{C \times V}{m}\right] \times D \times 10^{-1}$$

Where; C = Calculated concentration of calibration curve (mg/ml)

V = Volume of extract solvent (ml)

*m* = Weight of sample (g)

D = Dilution factor

%RSD = SD/X x 100

**2.4 Effect of solvent on oxyresveratrol content in *A. lakoocha* heartwood extracts**

The influence of solvents on oxyresveratrol content was examined by separately extracting *A. lakoocha* heartwood powder (CM1) with water, PG, BG, 95% ethanol as described above. One milliliter of each *A. lakoocha* heartwood extract was dissolved in 10 ml methanol. The obtained solution was then analyzed for oxyresveratrol content using validated conditions. The result was shown as mean ± S.D. and R.S.D. The solvents that gave extract with high oxyresveratrol content would be selected and the *A. lakoocha* heartwood powder would be studied.

**2.5 Determination of total phenolic content (TPC)**

The total phenolic content (TPC) was determined with Folin–Ciocalteu reagent adapted from Ahmad et al. with some modifications (Ahmad et al., 2017). Twenty microliters of the standard solutions were mixed with 100 µl of 10% (w/v) Folin–Ciocalteu reagent in 96 wells microplate and then 80 µl of 20% (w/v) $Na_2CO_3$ were added. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 740 nm using the microplate reader (Multi Read 400, Biochrom, UK). The standard solution of gallic acid at the concentration of 1000 µg/ml was prepared by dissolving 1 mg of gallic acid in 1 ml of distilled water. The standard solution was serially diluted with distilled water and seven standard solutions within a range of 9.765 - 625 µg/ml were obtained. The standard solutions were analyzed, and the calibration line was constructed. Total phenolic contents were expressed as mg Gallic Acid Equivalents (GAE) per g of extract. The sample of *A.*

*lakoocha* heartwood extract was prepared in triplicate for each analysis and the mean value of absorbance was calculated.

**2.6 Determination of DPPH radical scavenging activity (DPPH)**

The free radical scavenging activity was determined by 2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay according to the method of Que et al. with some modifications (Que et al., 2006). Different sample concentrations were prepared in ethanol, 6.25 – 100 µg/ml for the standards (Ascorbic acid and Trolox) and 200 – 2,000 µg/ml for the extracts. Briefly, an aliquot of 100 µl sample was added to each well in a 96-well microplate (3 wells/each sample) followed by addition of 100 µL of DPPH solution (0.3 mM in ethanol) into the well. The mixture was incubated in the dark at room temperature for 30 min. Absolute ethanol was used as the control solution. The absorbance of the reaction mixture was measured at 517 nm using microplate reader (Multi Read 400, Biochrom, UK). The activity was calculated according to the following equation.

$$\% \text{ DPPH radical Inhibition} = \frac{A-(B-C)}{A} x100$$

A is the absorbance of control

B is the absorbance of the sample or standard with DPPH

C is the absorbance of the sample or standard without DPPH

The sample concentration that provides 50% inhibition (IC50) was calculated from the curve between the percentage of inhibition and the concentration of the samples.

**2.7 Tyrosinase inhibition assay**

Tyrosinase inhibition assay was modified from Batubara's research (Batubara et al., 2010). Kojic acid and oxyresveratrol were used as the positive control. Different sample concentrations were prepared in 0.10 M phosphate buffer pH 6.8 as follows, at 12.5 - 200 µg/ml for kojic acid, 0.097 - 3.125 µg/ml for oxyresveratrol, and 1.562 – 50 µg/ml for the extracts. Each well contained 25 µl of sample or standard solution with 25 µl of phosphate buffer (0.1 M, pH 6.8), 25 µl of tyrosinase (223.29 units/ml). The mixture was incubated for 10 min at room temperature and then 25 µl of L-Dopa (2.5 mM) was added to each well. The mixture (100 µl) was incubated for another 10 min at room temperature, and the absorbance was measured at 492 nm using a microplate reader (Multi Read 400, Biochrom, UK). The percentage inhibition of tyrosinase activity was calculated using the following equation.

$$\% \text{ Tyrosinase Inhibition} = \frac{A-(B-C)}{A} x100$$

A is the absorbance of control

B is the absorbance of the sample or standard with tyrosinase

C is the absorbance of the sample or standard without tyrosinase

The activities were evaluated by determination of the IC50 values, which corresponds to the concentration of samples that are able to scavenge 50% of dopachrome formation in the reaction mixture.

**2. 8 Comparison of oxyresveratrol content, total phenolic content, and biological activities of *A. lakoocha* heartwood from five commercial sources**

To investigate the quality of herbal raw materials in the herbal market, five sources namely, CM1, CM2, CM3, CM4 and CM5, respectively. *A. lakoocha* heartwood powders were extracted with PG, and the extracts were determined for oxyresveratrol content, total phenolic content, and biological activities including antioxidant activity, and anti-tyrosinase activity.

**2.9 Statistical Analysis**

Experimental results were means ± standard deviations of three parallel replicates. ANOVA, correlation analysis was performed using the IBM SPSS Statistics, version 20. 0 for Windows. Significant differences between means were determined by Duncan's multiple range tests at a level of $P<0.05$.

## 3. Results and Discussion

**3.1 Effect of solvent on oxyresveratrol content in *A. lakoocha* heartwood extracts**

The oxyresveratrol content in *A. lakoocha* heartwood extracted with water, PG, BG and 95% ethanol, was examined for oxyresveratrol content by HPLC without evaporation. The result showed that the highest oxyresveratrol content was found in 95% ethanol extract, while the lowest was found in the water extract and PG and BG extracts contained oxyresveratrol contents close to that of 95% ethanol extract as shown in Table 1. The percentage R. S. D. values of the oxyresveratrol content in all extracts were found in the range of 0. 2000 – 1. 6488 %, which indicated that the analysis method was capable to perform with various extracting solvents. PG is commonly used in cosmetics formulation to dissolve substances in product to get the ingredients work together properly. It also acts as the carrier for active ingredients. The preparation of extracts with PG has the advantage of short time, convenient procedures for laboratory preparation, and cost reduction. Therefore, PG was chosen for this study.

**Table 1** Oxyresveratrol contents in *A. lakoocha* heartwood extract

| Solvent | Oxyresveratrol content (mg/ml) | R.S.D. (%) |
|---|---|---|
| water | 0.3313 ± 0.0055 | 1.6488 |
| propylene glycol (PG) | 0.7104 ± 0.0072 | 1.0189 |
| butylene glycol (BG) | 0.7103 ± 0.0014 | 0.2000 |
| 95% ethanol | 0.8318 ± 0.0137 | 1.6476 |

### 3.2 HPLC analysis

#### 3.2.1 Method validation

The method was validated for linearity, accuracy and precision, and intra-day and inter-day reproducibility with AOAC guidelines (AOAC, 2002), an important assessment of method quality. Representative HPLC chromatograms of standard oxyresveratrol solution and *A. lakoocha* heartwood extracts are shown in Figure 1. For the calibration curve, a linear correlation was obtained for AUC and the oxyresveratrol concentration in the range of 0.02 – 0.14 mg/ml and characterized by the equation: $y = 5.45x10^{7} - 134{,}666$ ($r^2 = 0.9997$), where y is AUC and x is concentration of oxyresveratrol (mg/ml) (Figure 2). The result showed that the correlation coefficient ($r^2$) indicates a good linearity of the modified method. The sample CM1 was represented for method validation.

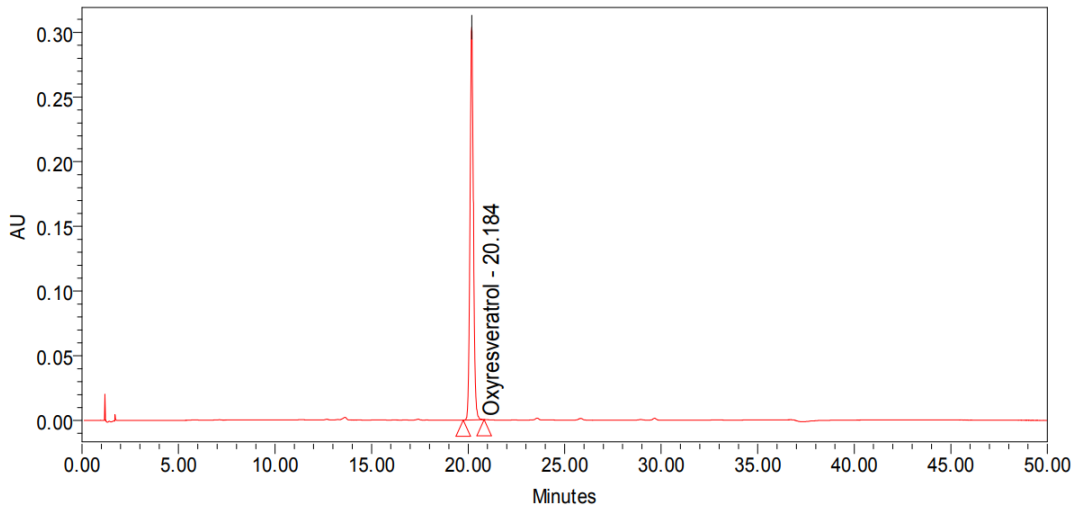


**Figure 1** HPLC chromatograms of *A. lakoocha* heartwood extract (CM1) (2 mg/ml)

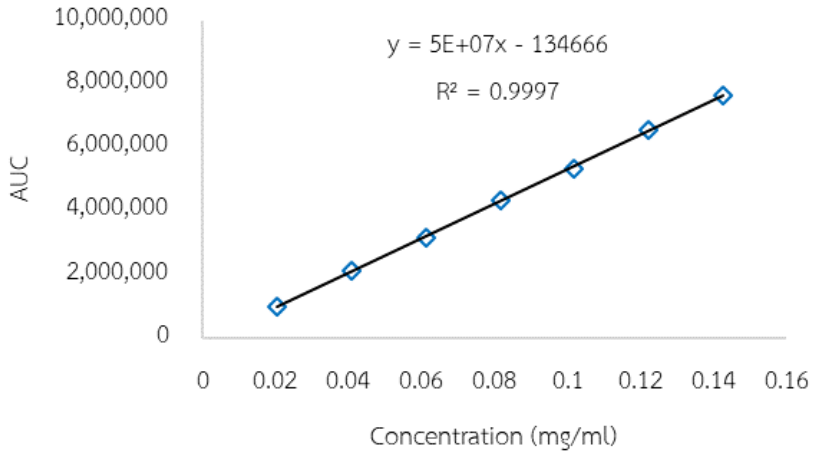


**Figure 2** Calibration curve for standard solution of oxyresveratrol.

### 3.2.2 Accuracy

The accuracy was indicated by the percentage recovery of 3 known amounts of individual standard (oxyresveratrol) at 0.02, 0.04, and 0.05 mg/ml spiked in *A. lakoocha* heartwood extracted with PG. The percentage of recovery for triplicate preparation of 3 concentrations should be within 95.0 – 105.0%. The result from Table 2 showed the recovery values of 101.0, 102.6, and 99.0 % with R.S.D. < 2.9%.

**Table 2** The recovery of oxyreveratrol in *A. lakoocha* heartwood extracts with PG

| Sample No. | Oxyresveratrol added (mg/ml) | Oxyresveratrol found (mg/ml) | Recovery (%) | R.S.D. (%) |
|---|---|---|---|---|
| 1 | 0.02 | 0.021 ± 0.001 | 101.0 | 2.9 |
| 2 | 0.04 | 0.042 ± 0.000 | 102.6 | 1.1 |
| 3 | 0.05 | 0.050 ± 0.001 | 99.0 | 1.1 |

**Table 3** Intra-day and inter-day precision of oxyresveratrol in *Artocarpus lakoocha*.

| Intra-day (n = 6) | | | | | | Inter-day (n= 18) | |
|---|---|---|---|---|---|---|---|
| Day 1 | | Day 2 | | Day 3 | | | |
| oxyresveratrol (mg/ml) | R.S.D. (%) | oxyresveratrol (mg/ml) | R.S.D. (%) | oxyresveratrol (mg/ml) | R.S.D. (%) | oxyresveratrol (mg/ml) | R.S.D. (%) |
| 0.7104 ± 0.0072 | 1.02 | 0.7300 ± 0.0040 | 0.5430 | 0.7146 ± 0.0047 | 0.6526 | 0.7183 | 1.4366 |

### 3.2.3 Precision

Precision was expressed as repeatability (intra-day) and intermediate precision (inter-day), which was derived from R.S.D. in six replicates of *A. lakoocha* heartwood extracted with PG. The percentage of R.S.D. values for intra-day and inter-day precisions were found to be 0.543-1.02 % and 1.4366%, respectively (Table 3). The values were less than 3% according to the recommendation of AOAC Guidelines (AOAC, 2002) so the result showed good repeatability (intra-day) and intermediate precision (inter-day).

### 3.3 Evaluation of total phenolic content

The total phenolic content in *A. lakoocha* heartwood water, PG, BG, and 95% ethanol extract, was examined using the Folin-Ciocalteu's reagent. The total phenolic content is expressed in terms of gallic acid equivalent (the standard curve equation: y = 4.4819X + 0.0683, $r^2$ = 0.997). The results represented that the 95% ethanol extract exhibited the highest total phenolic content, followed by PG, BG and water extract at 0.120, 0.084, 0.073, and 0.034 mg GAE/ml, respectively. The statistical analysis indicated that the total phenolic content of *A. lakoocha* heartwood extracts in various solvents was significant difference ($P<0.05$). Phenolic compounds have redox properties, which allow them to act as antioxidants (Soobrattee et al., 2005). As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity.

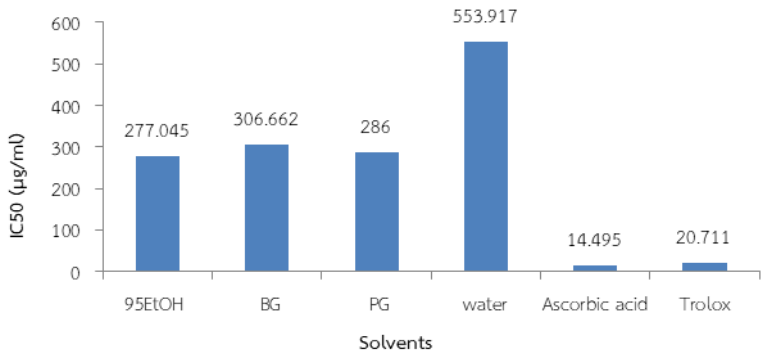


**Figure 3** Scavenging activity (IC50) of water, PG, BG and 95% ethanol extracts from *A. lakoocha* heartwood compare with ascorbic acid and trolox determined by DPPH radical scavenging activity

**3.4 Determination of DPPH radical scavenging activity**

Free radical scavenging activities of the plant extracts were assayed by using the stable DPPH radical. Their capacities were expressed as the IC50 value, the concentration of the sample required to inhibit 50% of radical (Figure 3). A lower IC50 value is related to a good antioxidant activity (Li et al., 2009). The results showed that IC50 values obtained from the 95% ethanol extract exhibited the highest antioxidant activity followed by PG, BG, and water extract with IC50 277.045 ± 1.858, 286.000 ± 0.423, 306.662 ± 3.610, and 553.917 ± 1.186 µg/ml, respectively. The statistical analysis indicated that scavenging activities were significant differences ($P<0.05$) among different solvent.

**3.5 Evaluation of anti-tyrosinase activity by the DOPA-chrome method**

Tyrosinase inhibitory activities of water, PG, BG and 95% ethanol extracts from *A. lakoocha* heartwood were measured by the DOPA-chrome method. The results showed that the 95% ethanol extract exhibited the highest capacity of the tyrosinase inhibitory activity followed by PG, BG, and water extract with IC50 4.746 ± 0.098, 5.199 ± 0.012, 5.860 ± 0.076, and 21.722 ± 0.246 µg/ml, respectively. The attention was focused on IC50 of CM1 extract in various solvents which were found to be better than the IC50 of kojic acid (32.178 ± 1.293 µg/ml) ($P<0.05$) but lower than that of standard oxyresveratrol (0.363 ± 0.004 µg/ml), the major compounds of *A. lakoocha* heartwood extract.

**3. 6 Comparison of oxyresveratrol content, total phenolic content, and biological activities of *A. lakoocha* heartwood from five commercial sources**

The oxyresveratrol content of CM1 to CM5 extracts with PG was determined by HPLC. The results showed that CM1 extract contained the highest oxyresveratrol content at 0.7104 ± 0.0072 mg/ml, followed by CM4, CM3, CM5, and CM2 at 0.5806 ± 0.0054, 0.0666 ± 0.0012, 0.0542 ± 0.0003, and 0.0251 ±0.0008 mg/ml, respectively. The statistical analysis indicated that the oxyresveratrol content of 5 sources of *A. lakoocha* heartwood was significantly different ($P<0.05$). Therefore, it can be concluded that oxyresveratrol content also depended on the origin of *A. lakoocha* heartwood raw materials.

The total phenolic content of CM1 to CM5 extracts with PG was determined in terms of gallic acid equivalent with the standard curve equation : $y = 5.3703X + 0.0207$, $r^2 = 0.9995$. The results showed that the CM5 extract exhibited the highest total phenolic content followed by CM4, CM3, CM2, and CM1. Their gallic acid equivalent values were 0.162 ± 0.004, 0.146 ± 0.009, 0.099 ± 0.003, 0.084 ± 0.002, and 0.078 ± 0.004 mg GAE/ml, respectively.

The antioxidant activity of CM1 to CM5 extracts with PG was measured by DPPH radical scavenging activity method. The result revealed that CM5 extract showed the highest activity in

scavenging DPPH radicals following by CM4, CM3, CM1, and CM2. Their IC50 values were 141.637 ± 2.360, 198.016 ± 5.015, 235.662 ± 1.505, 286.000 ± 0.423, and 311.602 ± 4.316 µg/ml, respectively.

This study focused on anti-tyrosinase activity, the main purpose of skin whitening. From Table 4, it is obvious that the CM1 extract exhibited the highest capacity of the tyrosinase inhibitory activity followed by CM4, CM5, CM3, and CM2. The IC50 value were 5.199 ± 0.012, 6.279 ± 0.052, 21.278 ± 0.812, 27.279 ± 1.069, 93.377 ± 0.098 µg/ml, respectively. The anti-tyrosinase activity of four out of five extracts was found to be significantly higher than kojic acid ($P<0.05$).

**Table 4** Bioactive activity of A. *lakoocha* heartwood extracts with propylene glycol from five commercial sources and positive control.

| Sample | Oxyresveratrol (mg/ml) ± SD | Total phenolic (mg GAE/ml) ± SD | DPPH IC50 (µg/ml) ± SD | Anti-tyrosinase IC50 (µg/ml) ± SD |
|---|---|---|---|---|
| CM1 | 0.7104 ± 0.0072 $^{e}$ | 0.078 ± 0.004$^{a}$ | 286.000 ± 0.423$^{d,*,**}$ | 5.199 ± 0.012$^{a}$ |
| CM2 | 0.0251 ± 0.0008 $^{a}$ | 0.084 ± 0.002$^{b}$ | 311.602 ± 4.316$^{e,*,**}$ | 93.377 ± 0.098$^{d}$ |
| CM3 | 0.0666 ± 0.0012 $^{c}$ | 0.099 ± 0.003$^{c}$ | 235.662 ± 1.505$^{c,*,**}$ | 27.279 ± 1.069$^{c}$ |
| CM4 | 0.5806 ± 0.0054$^{d}$ | 0.146 ± 0.009$^{d}$ | 198.016 ± 5.015$^{b,*,**}$ | 6.279 ± 0.052$^{a}$ |
| CM5 | 0.0542 ± 0.0003 $^{b}$ | 0.162 ± 0.004$^{e}$ | 141.637 ± 2.360$^{a,*,**}$ | 21.278 ± 0.812$^{b}$ |
| Ascorbic acid | - | - | 14.495 ± 0.067 | - |
| Trolox | - | - | 20.711 ± 0.240 | - |
| Oxyresveratrol | - | - | - | 0.363 ± 0.004 |
| Kojic acid | - | - | - | 32.178 ± 1.293 |

Mean in the same column followed by different letters (a-e) represent statistical significance ($p<0.05$) according to the Duncan's multiple range test of CM1, CM2, CM3, CM4 and CM5

$^{*}$ Demonstrate the significant difference between extracts and ascorbic acid.

$^{**}$ Demonstrate the significant difference between extracts and Trolox.

From the results showed total phenolic content correlated with antioxidant activity in agreement with two previous studies (Akinmoladon et al., 2010; Chatatikun & Chiabchalard, 2017) but did not correlate with anti-tyrosinase activity because polyphenolic compounds consist of

phenols, flavonoids, and tannins (Singhatong et al., 2010) and oxyresveratrol is the compound in stilbenes group that is a small amount in flavonoid. Oxyresveratrol did not affect total phenolic contents and antioxidant activity but directly affected anti- tyrosinase activity. Therefore, oxyresveratrol from *A. lakoocha* extracts could be a good source of natural whitening agent for cosmeceutical products substituting for kojic acid which has recently been banned in many countries. (Likhitwitayawuid, 2008)

## 4. Conclusion

Oxyresveratrol is an active ingredient in *A. lakoocha* heartwood with skin whitening activity. The qualified extract should contain significant amounts of the active substance with biological activities. In this study, the oxyresveratrol content of *A. lakoocha* heartwood extracts (CM1) with 4 kinds of solvents was quantitated by HPLC. The analytical method was validated in terms of linearity, accuracy and precision were obtained. The extracts were tested for total phenolic content, antioxidant activity, and anti-tyrosinase activity. The results demonstrated that the oxyresveratrol content was correlated with the anti-tyrosinase activity. Moreover, glycol solvents, PG and BG, were practical solvents for *A. lakoocha* heartwood extract.

To investigate the quality of herbal raw materials in the herbal market, the PG extracts from five commercial sources were analyzed for oxyresveratrol content, phenolic content, and biological activities. The results revealed the variance among the commercial extracts. It can be concluded that raw material was the most important factor for the quality of cosmeceutical products. The information from this study might be of benefit to a specification development guideline for *A. lakoocha* heartwood extract in Thailand.

## 5. References

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