Quantitative Analysis of α- and β-arbutin in *Artocarpus lacucha*, *A. thailandicus*, and Commercial Whitening Products

การวิเคราะห์ปริมาณสารแอลฟาและเบต้าอาร์บูตินจากมะหาด (*Artocarpus lacucha*) มะหาดไทย (*A. thailandicus*) และผลิตภัณฑ์เพื่อผิวขาว

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ABSTRACT

The research aimed to investigate α-arbutin and β-arbutin in *Artocarpus lacucha* and *A. thailandicus* hexane and ethanol extracts and in six commercial whitening creams and lotion, including F1, F2, F3, F4, F5.1 and F5.2, via high-performance liquid chromatography (HPLC). Arbutin was not detected in hexane plant extracts, because it is undissolved in non-polar solvent. While the ethanol plant extracts showed 1.485 g of β-arbutin in 25 g of dried leaves (or 5.94 g/100g of dried leaves), expecting sufficient amount to whiten skin and inhibit the formation of a black-brown color in post-harvest fruits. Some of the commercial samples were found to contain both β-arbutin and α-arbutin in F4 (4.83%), and F2 (0.22%) and F3 (0.11%), with ranges of safety for consumer as follows: 2-4% α-arbutin and up to 7% β-arbutin in order to use for skin whitening product complement.

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อวิเคราะห์หาสารแอลฟาและเบต้าอาร์บูตินจากใบมะหาด (*Artocarpus lacucha*) และมะหาดไทย (*A. thailandicus*) ที่สกัดด้วยตัวทำละลายเอทานอลและเฮกเซน และผลิตภัณฑ์เพื่อผิวขาว 6 ชนิด คือ F1, F2, F3, F4, F5.1 และ F5.2 ด้วย HPLC ผลการวิจัยพบว่ามีสารอาร์บูตินจากใบมะหาดและมะหาดไทย สารอาร์บูตินนี้ไม่ถูกตรวจพบในตัวทำละลายเฮกเซน แต่พบในตัวทำละลายเอทานอลในปริมาณ 1.485 กรัม/25กรัมใบแห้ง คิดเป็น 5.94 กรัมจากใบพืช 100 กรัม คาดว่าเป็นปริมาณที่เพียงพอต่อผลิตภัณฑ์เพื่อผิวขาวและนำไปใช้ยับยั้งการเกิดจุดสีน้ำตาลบนเปลือกผลไม้หลังการเก็บเกี่ยว การวิเคราะห์หาสารอาร์บูตินจากผลิตภัณฑ์เพื่อผิวขาว พบว่ามีการตรวจพบสารอาร์บูตินทั้งโครงสร้างแบบแอลฟาและเบต้าในบางผลิตภัณฑ์ ได้แก่ F4 (4.83%) F2 (0.22%) และ F3 (0.11%) และเป็นปริมาณที่ปลอดภัยต่อผู้บริโภค คือผลิตภัณฑ์เพื่อผิวขาวจะต้องมีสารแอลฟาอาร์บูตินเป็นส่วนประกอบปริมาณร้อยละ 2-4 และสารเบต้าอาร์บูตินจะต้องมีปริมาณไม่เกินร้อยละ 7

Keywords: Arbutin, *Artocarpus* species, High-performance liquid chromatography
คำสำคัญ: สารอาร์บูติน พืชสกุลอาร์โทคาร์ปัส เครื่องโครมาโทกราฟีแบบแรงดันสูง

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Introduction

Plants have long been used as food supplements and traditional medicines throughout the world to promote human health and well-being. Therefore, the uses, phytochemical types and quantities, and toxicity levels of plants are important to investigate.

Arbutin (4-hydroxyphenyl D-glucopyranoside), which is a glycoside derivative of hydroquinone. This bioactive compound is a tyrosinase inhibitor, has been wildly used as a whitening agent in cosmetic products (Migas and Miroslawa., 2015). There are 2 anomeric form; α-arbutin (4-hydroxyphenyl α-D-glucopyranoside) and β-arbutin (4-hydroxyphenyl β-D-glucopyranoside) (Figure 1). While the tyrosinase inhibition acitivity of both arbutin, α-arbutin demonstrated a stronger tyrosinase suppression activity than β-arbutin in mammalian and mouse melanoma tyrosinase (Cho et al., 2011). α-Arbutin was enzymatically synthesized from hydoquinone and sugars. However, it is very difficult to manufacture the α-arbutin glycosides by synthesis and people in general have issues with synthetic compounds about side effect and safety. β-Arbutin has been found in species of several plant genera, such as *Arctostaphylos*, *Artocarpus*, *Bergenia*, *Lathyrus*, *Origanum*, *Pyrus*, and *Vaccinium* (Chang., 2009; Lee and Eun., 2012; Lukas et al., 2010; Pizzorno and Murray., 2013; Pop et al., 2009; Noikotr et al., 2018). Due to its function as a tyrosinase inhibitor, which inhibits mammalian melanogenesis, it is responsible for enzymatic browning reactions in damaged fruits during post-harvest handling and processing1. Thus, it is used in many cosmetics, particularly those aimed at lightening the skin, spot treatments, creams, lotions, soaps, serums and cleaners.

As reported of having arbutin, *Artocarpus lacucha* and *A. thailandicus* were screened for α- and β-arbutin via high-performance liquid chromatography (HPLC). Additionally, whitening products which are claimed to contain α- and β-arbutin, were purchased and investigated.

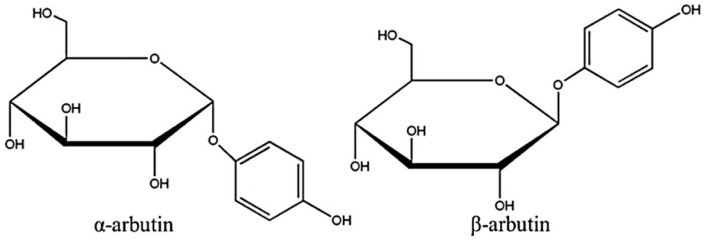

Figure 1 The two anomeric structure of arbutin, which consist α- and β-form (Garcia-Jimenez et al., 2017)

Objective of the study

The research aimed to investigate α-arbutin and β-arbutin in *Artocarpus lacucha* and *A. thailandicus* hexane and ethanol extracts and in six commercial whitening creams and lotion via high-performance liquid chromatography (HPLC).

Materials and methods

Plant materials

Artocarpus lacucha Roxb. ex Buch.-Ham. (voucher no. A. Chaveerach 956) and *A. thailandicus* C.C. Berg (voucher no. A. Chaveerach 1027) species were explored at the campus, altitude 200 m, of Khon Kaen University, northeastern Thailand, nearby Biology Department, Faculty of Science. The collected plants were identified by Prof. Dr. Arunrat Chaveerach using Flora of Thailand, volume 10, part four (Berg et al., 2011). The voucher specimens were kept at Department of Biology, Faculty of Science, Khon Kaen University, in September 2017. Then the mature leaves were washed with tap water, air dried and ground with an electronic blender at room temperature. The powder was subsequently used for the preparation of crude extracts via hexane and ethanol solvents and further used in HPLC analysis.

Commercial whitening creams and lotion

Six samples of whitening creams and lotion including 4 brands and 5 types (2 types were one brand, a day cream and a night cream) were bought and denominated F1, F2, F3, F4, F5.1 (day cream) and F5.2 (night cream). The product data was revealed as far as possible especially were said to contain α- or β-arbutin except for F5.2 as shown in the Table 1.

Table 1 The details of commercial whitening creams and lotion studied samples

Symbol	Product	Constituent
F1	Whitening cream	Alpha-arbutin, Licorice, *Centella asiatica*
F2	Serum cream	Glutathione, Arbutin (Beta-Arbutin), Vitamin C, Palmitate (Ascorbyl Palmitate)
F3	Facial whitening moisturizing cream	Glutathione, Ceramide, Vitamin C, Tourmaline, Arbutin
F4	Whitening lotion (liquid)	Arbutin from bearberry, cranberry, mulberry or blueberry, Vitamin C, Hyarulonic
F5.1	Day cream	N-acetyl-D-glucosamine, Alpha-arbutin, Niacinamide, Tranexamic acid, Vitamin C
F5.2	Night cream	Licochalcone A, Salicylic acid, Nano silver Pure, Yeast radiance, Tranexamic acid, Zinc PCA

Phytochemical extraction and preparation

Twenty-five grams of powdered leaves were extracted with 125 mL hexane and ethanol separately for 72 h in the dark at room temperature. The mixture was filtered through filter paper (WhatmanTM, 125 mm, Cat No. 1001 125). The solvents were eliminated from the remain filtrates via a vacuum concentrator (ScanVac LaboGene, Denmark) at -20°C, 200 rpm for 2 h or until completely evaporated. The crude extracts were gradually re-dissolved

with 100% DMSO and then maintained at -20 °C as stock concentrations. The 10% DMSO stock (prepared from 100% DMSO stock) were diluted with deionized water at 10-fold serial dilutions for 3 levels and filtrated with a nylon syringe filter (13×0.2 µm). The filtrates were maintained in vials for HPLC.

Whitening cream and lotion preparations

The six samples of whitening creams and lotion, 1 g. of each sample was weight and dissolved with 10 mL of ethanol. The samples were incubated for 24 h in the dark at room temperature. The extracts were filtrated through filter paper (WhatmanTM, 125 mm, Cat No. 1001 125). The ethanolic solutions were diluted for 10-fold serial dilutions for 3 concentration levels. They were then filtered with a nylon syringe filter (13×0.2 µm). The filtrates were maintained in vials for HPLC.

Reference standard preparations

The standard chemicals α-arbutin (0338, Sigma-Aldrich) and β-arbutin (Y0000806, Sigma-Aldrich) were prepared with deionized water in serial concentrations of 0.001, 0.01, 0.1, 1.0, 10 mg/mL. The solutions were filtered with nylon syringe filter (13×0.2 µm) and stored in vials for HPLC.

HPLC analysis of the plant extracts and commercial whitening creams and lotion

The crude extracts and commercial whitening creams and lotion were analyzed by HPLC using a Shimadzuu LC-20AD (Japan) model with a quaternary pump, PAD (SPD-M20A) detector, and column Inertsl ODS-3 C18, 4.6×250 mm, 5 microns (GLSciences Inc.). The 20-µl sample was injected. The mobile phase consisted of three solutions mixed in water: methanol: 0.1 M HCl at a proportion of 89:10:1 v/v/v. The elution was carried out at a flow rate of 1 mL/min. The detection wavelength was 222 nm, working in the range of 190-800 nm.

Analysis of standards and calibration curves

Linearity for the α- and β-arbutin standards derived from graph plotting between linear regression of the peak areas resulted from HPLC analysis and the five levels of concentration standards as 0.001, 0.01, 0.1, 1.0 and 10 mg/mL to create a linear curve, calibration equation and correlation factor (R^2) by Microsoft Excel. The calibration equation was used for α- and β-arbutin evaluation from the studied samples. The correlation factor was applied for the reliability of the calibration equation. The limit of detection (LOD) and limit of quantitation (LOQ) for verified substance were determined by the signal-to-noise (S/N) ratio. Analyzing a series of diluted standard solutions the S/N ratios 3 and 10, respectively. The precision of the method was evaluated by measurement in intra-day and inter-day variability tests from integration area. The intra- and inter-day precision were determined by estimating the corresponding response five time on the same day and on three different days. The recovery test was accomplished by adding standard solution at concentration of LOQ and over LOQ value.

Results

The correlation coefficient (R^2) values for α-arbutin 0.9998 and for β-arbutin 0.9995 were indicated good linearity in the examine concentration range. The limits of detection (LOD) and limits of quantitation (LOQ), defined

as signal-to-noise ratios of 3 and 10, respectively, were 1. 62x10^{-2}, and 4. 52 mg/ mL for α-arbutin and 1.55x10^{-2}, and 4.50 mg/mL for β-arbutin (Table 2).

The results shown in the Table 3 and 4 indicated that the developed analytical method was reproduced with good recovery and stability. The relative standard deviation (RSD) value of intra- and inter-day of both arbutin were between 0.2-1.7% for α-arbutin, and 0.41-1.24% for β-arbutin. The RSD value was less than 2% and exhibited precise method (Table 3).

Table 2 Linearity of standard curve (n=5) and limits of detection (S/N =3) and limits of quantitation (S/N=10)

Compound	Retention time (min)	Working rang (mg/mL)	Calibration equation[a]	R^2 (n=5)	LOD[b] (mg/mL)	LOQ[b] (mg/mL)
α-Arbutin	6.203	0.001-10.0	y = (2×10^7)x + 98492	0.9998	1.60x10^{-2}	4.52
β-Arbutin	5.602	0.001-10.0	y = (2×10^7)x + 43660	0.9995	1.55x10^{-2}	4.50

[a]In the calibration equation, x is concentration of the compounds solution in mg/mL and y is peak are of the compound.

[b]LOD = limit of detection. LOQ = limit of quantification.

Table 3 The intra-day and inter-day precision analysis of α- and β-arbutin standard solutions

Compound	Concentration (mg/mL)	Inter-day variability (n=5)		Intra-day viability (n = 5)	
		Mean±S.D. (AU)	% RSD[a]	Mean±S.D. (AU)	%RSD[a]
α-Arbutin	0.01	230643±446.32	0.20	230623±446.41	0.20
	0.1	2204252±4004.2	0.20	2204241±4004.1	0.20
	1	19519442±229695.4	1.17	19519422±229690	1.17
β-Arbutin	0.01	318668.4±3943.27	1.24	318667±3943.20	1.24
	0.1	2461474±24532.09	1.00	2461474±24532.09	1.00
	1	27876297±113801.9	0.41	27878288±113800.02	0.41

[a]RSD = relative standard deviation

The accuracy of the proposed method was determined by sample extract solution analyzing spiked with two different concentrations of both standard arbutin solution . Recovery rates for α-arbutin between 99.77% and 100.22%, for β-arbutin between 99.55% and 100.22% were obtained, their RSD values were 0.022% (Table 4). The excellent recoveries of the added standard suggested that the method is accurate.

Table 4 The analytical results of recoveries of α- and β- arbutin in the extract of commercial whitening cream (F4) and *Artocarpus lacucha*

Compound	Initial concentration of the extract (mg/mL)	Concentration added (mg/mL)	Concentration found: Mean±S.D. (mg/mL)	Recovery (%)	%RSD
F4	1	4.52	4.51±0.001	99.77	0.022
(α-Arbutin)		9.04	9.06±0.002	100.22	0.022
A. lacucha	3.6	4.50	4.48±0.001	99.55	0.022
(β-arbutin)		9	9.02±0.002	100.22	0.022

The peak and retention time (RT) of α- and β-arbutin standard were used to confirm this organic compound detection in leaves of *Artocarpus lacucha*, *A. thailandicus*, and commercial whitening products, and 10% DMSO was as a control. Appropriated concentration of 3.4 mg/ml ethanol *A. lacucha* extract, 3.4 mg/ml ethanol *A. thailandica* extract and 1 mg/ml of commercial studied samples were used for HPLC analysis.

The RT of 10% DMSO, α-and β-arbutin were detected at 3.764, 6.203, and 5.602 min, respectively. The results demonstrated the arbutin was detected in ethanolic extract of *Artocarpus lacucha* and *A. thailandicus* at 5.615 and 5.637 min. Moreover, this compound not detected in hexanolic laeves extract of two *Artocarpus* species (Figure 2). The facial whitening creams was labeled F1, F2, F3, F4, and F5.1 and F5.2. The arbutin compound was detected in F1, F2, F3, and F4 at 6.134, 6.094, 6.076, and 5.745 min, respectively (Figure 3). No arbutin was detected in whitening creams and lotion F5.1 and F5.2.

The quantity of arbutin was applied peak area of the samples and calculated by standard equation ; α-arbutin was $y = (2\times10^7)x + 98492$, whereas that for β-arbutin was $y = (2\times10^7)x + 43660$. The quantity of arbutin were evaluated using peak area and standard equation showing amounts of 0.11 mg/mL for two *Artocarpus* species, and 0.023, 0.013, and 0.38 mg/mL for commercial products; F2, F3, and F4, respectively. The RSD values was between 0.03% and 0.26%. The peak area of sample F1 could not be used for arbutin quantification folliwung the α-arbutin calibration equation (Table 5).

The peak areas were released with specific retention times, revealing the type of β-arbutin at the times of 5.77-5.79 in the two *Artocarpus* extracts and the sample F4, and α-arbutin at the time of 6.13-6.20 in the samples F1-F3. Finally, both arbutin quantities were applied to evaluate arbutin by weight (g) and percentages from the total of extracts and the samples. The 25-g extracts and sample F4 contained 1.485 and 1.450 g of β-arbutin, respectively, representing 5.5% and 4.83%, respectively (Table 6).

Table 5 Content of arbutin in leaves of *Artocarpus lacucha*, *A. thailandicus* and commercial whitening creams: F1, F2, F3, F4, F5.1 and F5.2

Sample	Concentration (mg/mL)	Retention time (min)	Arbutin content	
			X±S.D. (mg/mL)[a]	%RSD
A. lacucha (ethanol extract)	3.6	5.615	0.11±0.005	0.045
A. thailandicus (ethanol extract)	3.4	5.637	0.11±0.011	0.1
F1	1	6.134	-	-
F2	1	6.094	0.023±0.006	0.26
F3	1	6.076	0.013±0.002	0.15
F4	1	5.745	0.38±0.012	0.03
F5.1	1	nd	-	-
F5.2	1	nd	-	-

[a]Mean arbutin content in the extract ±SD (standard deviation) in mg/mL (n=3); nd = peak not detected.

Table 6 Contents of α- or β-arbutin evaluated in plant extracts and commercial whitening creams.

Sample	Weight of sample (g)	Arbutin content		
		Amount (g)	Amount (%)	Type
A. lacucha (ethanol extract)	25	1.485	5.50	β-arbutin
A. thailandicus (ethanol extract)	25	1.485	5.50	β-arbutin
F1	10	-	-	α-arbutin
F2	15	0.033	0.22	α-arbutin
F3	30	0.033	0.11	α-arbutin
F4	30	1.450	4.83	β-arbutin
F5.1	10	-	-	-
F5.2	10	-	-	-

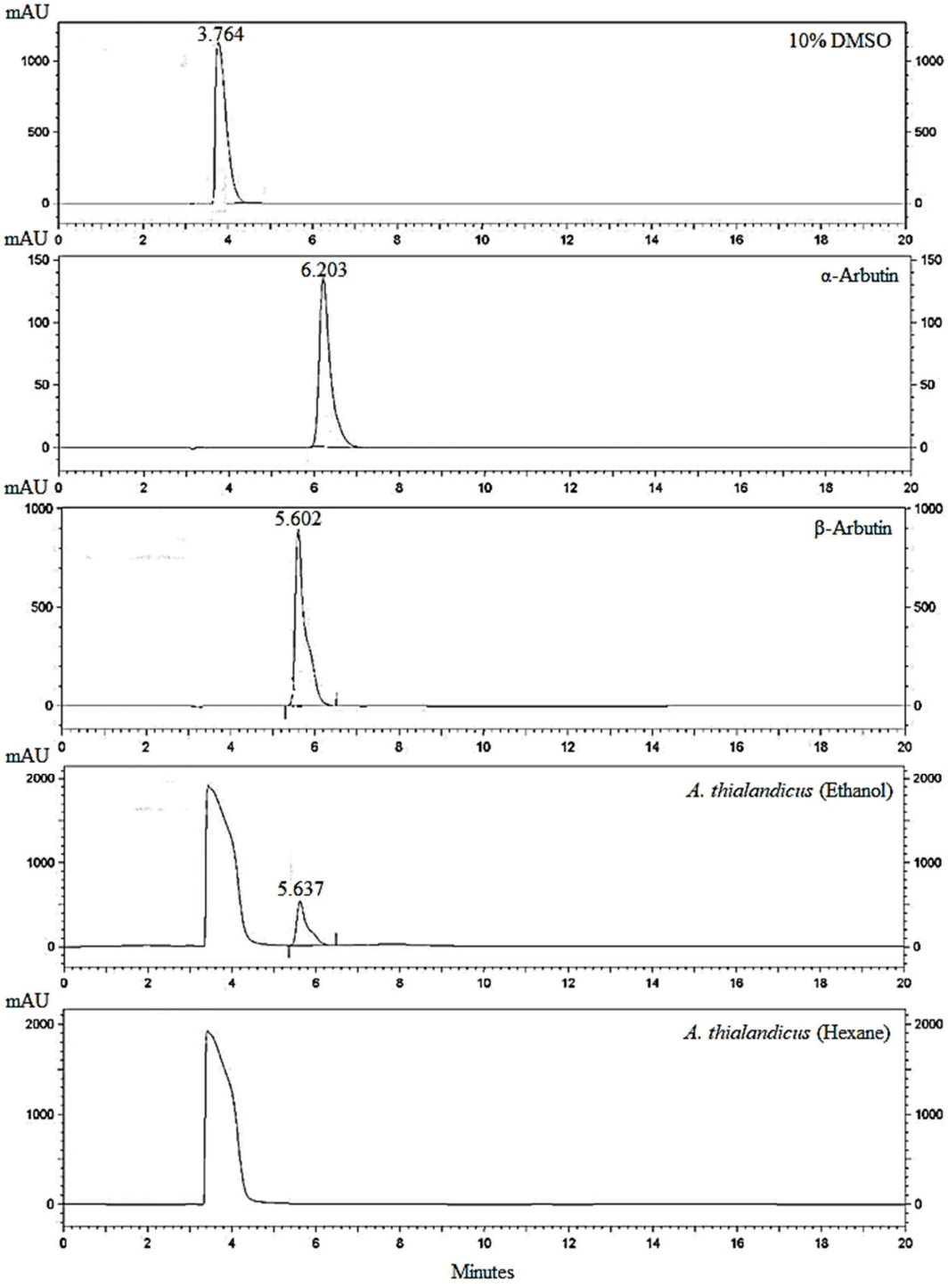

Figure 2 The HPLC chromatogram of the peak areas of the 10% DMSO (control), standards of α- and β-arbutin,; α-arbutin (0.1 mg/ml) and β-arbutin (0.1 mg/ml), and the ethanol and hexane leaves extract of *Artocarpus thailandicus* (3.4 mg/mL).

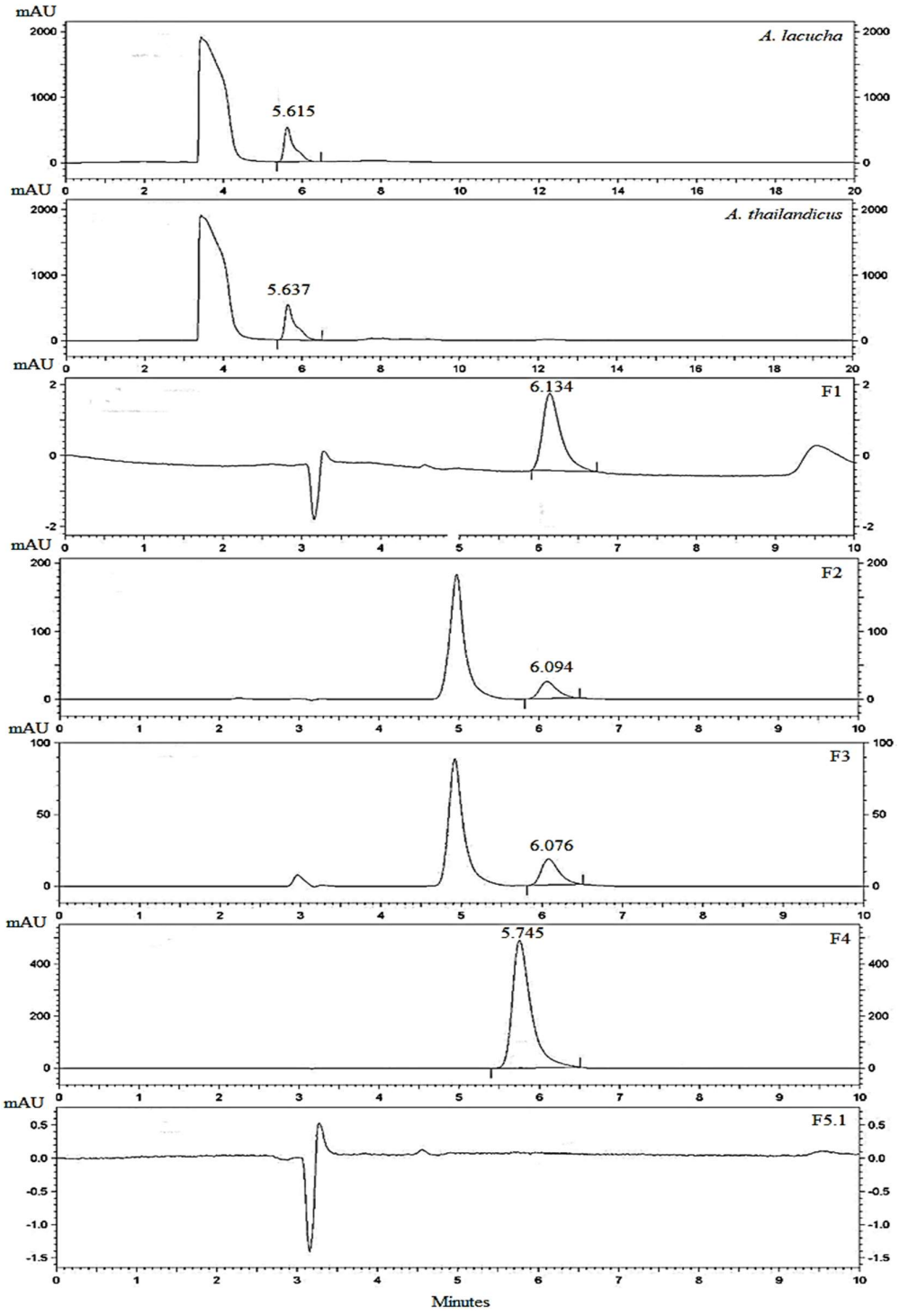

Figure 3 HPLC chromatogram of studied samples, including ethanol extracts of *Artocarpus lacucha* and *A.thailandicus* and the facial whitening creams F1, F2, F3, F4, and F5.1.

Discussion and conclusion

Important phytochemicals include skin-lightening ingredients, which are included in cosmetics worldwide. Many types of skin-lightening substances are found both in natural and synthetic sources. The mechanism of skin lightening is related to tyrosinase inhibition. Tyrosinase is a copper-containing enzyme present in melanocytes that catalyses melanogenesis. Several chemicals have been frequently used in whitening cosmetics, namely, retinoids, hydroquinone and ester derivatives, mercury salts, arbutin, kojic acid, azelaic acid, glutathione, vitamin C, E and B3, alpha hydroxyl acids (AHAs or fruit acids) and corticosteroids. Many of these substances have been banned due to their possible hazardous effects (Burger et al., 2016).

Arbutin is a glycosylated and copper-containing oxidase that catalyzes mammalian melanogenesis and is responsible for the enzymatic browning reaction in damaged fruits during post-harvest handling and processing (Chang., 2009). Therefore, it is normally used as a skin whitening agent in cosmetic creams and lotions, particularly those aimed at lightening the skin, spot treatments, creams, lotions, soaps, serums and cleaners. They are found in percentages of 7.5-27.9% (Pop et al., 2009). Due to patent specifications, most skin care products contain plant extracts containing arbutin, such as bearberry (*Arctostaphylos uva-ursi*), pear (*Pyrus pyrifolia*) and lingonberry (*Vaccinium vitis-idaea*) rather than pure arbutin. Additional applications of arbutin are related to its tyrosinase function, so it is used in fruit preservation (Chang., 2009; Lee and Eun., 2012; Pizzorno and Murray., 2013; U.S. Department of health and human services., 2015).

The previous research showed that α-arbutin, a synthetic form, is a higher-quality skin whitening agent than β-arbutin, which is a natural product. It also has the greatest inhibitory activity against mammalian tyrosinase (Couteau and Coiffard., 2016). Many plant species have been found to contain β-arbutin, as previously mentioned, and latterly *A. lacucha* and *A. thailandicus* in Thailand. Additionally, β-arbutin in high enough quantities is included in many products, such as lotions and facial creams. α-Arbutin is used in cosmetic formulations to lighten skin pigmentation. For this purpose, up to 2% α-arbutin is used in finished cosmetic products for face/neck care and up to 0.5% for body lotions (SCCS and Degen., 2016). According to the studied commercial samples, F2-F3 contained 0.22 and 0.11% α-arbutin in safe limits, even though the F2 advertisement label revealed to contain β-arbutin. Moreover, the SCCS considers a concentration of β-arbutin up to 7% in face creams to be in the range of safety (Ibrahim et al., 2017), and the studied sample F4 contained β-arbutin at a concentration of 4.83%. However, the studied sample F1 had too low of a concentration to attain whitening. The studied samples F5.1 and F5.2 also showed no detectable arbutin according to HPLC processes. In small amounts, this substance may be oxidized by high temperatures (Couteau and Coiffard., 2016). It is reasonable for F5.2 that it did not contain arbutin. Our research found that 1.485 g of β-arbutin can be extracted from 25 g dried leaves of *A. lacucha* and *A. thailandicus*, which are commonly found on the Khon Kaen University campus and in other areas of Thailand. Extracts of these two species can potentially be further used to whiten skin and inhibit or slow the black-brown reaction in post-harvest fruits.

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