



## TLC-densitometry Profile of *Annona squamosa* and *Persea americana* as the Raw Material for Antihyperlipidemic Medicine

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### Abstract

Natural medicine products usually consist of two to five kinds of plants that have synergistic biological activity. The responsible constituents for the therapeutic activity in plants often involve diverse chemical components. The combination of ethanol extracts of *Annona squamosa* L. (EEAS) and *Persea americana* M. (EEPA) has been shown to have antihyperlipidemic activity. To ensure the correctness and consistency of the EEAS and EEPAs combination as the antihyperlipidemic treatments, it is necessary to characterize the plant's secondary metabolites. This study aimed to obtain the profiles of the secondary metabolites of *Annona squamosa* L. and *Persea americana* M. using the TLC-densitometry technique. Extract samples were applied to TLC plates and screened using densitometry at 254 nm. The obtained chromatogram peak was measured at the wavelength range of 200–700 nm to obtain the maximum wavelength of each peak. The results showed that EEAS had 5 peaks with *r<sub>f</sub>* values of 0.00, 0.61, 0.67, 0.73, and 0.87. The TLC-densitometry analysis results of EEPA showed eight peaks with *r<sub>f</sub>* values of 0.00, 0.11, 0.19, 0.49, 0.56, 0.71, 0.77, and 0.90. The extract chromatograms spectra showed that 2–3 peaks at the wavelength range of 300–350 nm. Chromatogram TLC-densitometry profile data on EEAS and EEPA can be used as the indicators in the raw material combinations standardization process of EEAS and EEPA as natural medicine products to treat antihyperlipidemic.

**Keywords:** *Annona squamosa* L., *Persea americana* M., Standardization, TLC-densitometry.

### 1. Introduction

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The use of plants to treat high-cholesterol-related conditions has a long history based on the empirical experience. *Allium sativum*, *Cinnamomum zeylanicum*, *Guazuma ulmifolia*, and *Glycine max* are the examples of plants used for the treatment of hypercholesterolemia. The approach used in herbal medicine relies on the diuretic

properties, laxative properties, and astringent effects of the used plant components [1].

The currently available natural medicine products in the market usually consist of two to five kinds of plants that have synergistic biological activity. The active ingredients in plants that are responsible for therapeutic activity often a diversity of components. The quality and safety of these natural ingredients-derived medicinal products are determined by the components and the concentrations of the active ingredients in the preparation step. To assure the truth and consistency of natural material products, standardization is needed. The analytical methods used to standardize the components of metabolite compounds are the thin-layer chromatography densitometry (TLC-Densitometer) technique, HPLC, IR, and others [2-3].

A previous study showed that the ethanol extract of *Annona squamosa* L. leaves was able to reduce levels of Low-Density Lipoprotein (LDL) of hyperlipidemic rats at the dose of 0.25 gram/body weight [4]. *A. squamosa*, also known as *Srikaya*, is a plant used for various applications, including medical treatment. The plant has been reported to be used as an insecticide, antitumor, and antidiabetic agent, as well as antioxidant, and as an antihyperlipidemic and an anti-inflammatory drug. Chemical compounds contained in *A. squamosa* including glycosides, alkaloids, flavonoids, saponins, phenolic compounds, acetogenins, diterpenes, lignin, hydroxyl ketone, and benzo quinazoline [5-6].

According to the previous study, ethanol extracts of *Persea americana* M. leaves were able to reduce levels of LDL of hyperlipidemic rats at a dose of 0.25 gram/Kg of body weight [7]. *P. americana*, or more commonly known as the avocado, is a member of the family *Lauraceae*. The compounds contained in *P. americana* include saponins, tannins, flavonoids, alkaloids, phenols, steroids, and cyanogenic glycosides [8]. Experimentally, the avocado (*P. Americana*) has been reported to have pharmacological actions, i.e. antidiabetic, hypotensive, antioxidant, hypolipidemic, and cardioprotective effect [9].

The previous study has been conducted to see the potential use of a combination of ethanol extracts of *A. squamosa* (EEAS) and *P. americana* (EEPA) as the raw materials for cholesterol drugs. The results of anti-hypercholesterol activity testing showed that the combination of EEAS and EEPA was able to reduce LDL levels at a dose of 125 mg/kg BW [4]. Based on the previous research, it is necessary to characterize the secondary metabolites of traditional medicine materials using a TLC-densitometry technique. The purpose of this study was to obtain a profile of secondary metabolites of EEAS and EEPA as anti-hyperlipidemic agents. The chromatogram profile can be used as one of the indicators to guarantee the quality in the development of combinations of EEAS and EEPA as the anti-hyperlipidemic drug materials.

## 2. Materials and Methods

### 2.1. Materials

The leaves of *A. squamosa* and *P. americana* were obtained from the plants that grew in the cities of Pasuruan and Malang, Indonesia, respectively, and were processed at the Balai Materia Medika Batu. The other materials used in this study were KLT F 254 plates (Merck), hexane solvent (technical grade), and ethyl acetate solvent (technical grade).

### 2.2. Instruments

The instruments used in this study were TLC Scanner (CAMAG), UV lights at 254 nm and Vis 365 nm, and TLC Visualizing Chamber (CAMAG).

### 2.3. Extract Preparation

The extracts were obtained from *A. squamosa* and *P. americana* leaf powder by a maceration method. The leaf powder was soaked in the ethanol solvent for 24 h, then filtered, and then separated from the filtrate. The residue was given the same treatment, repeated three times. The filtrate obtained was concentrated using a rotary evaporator at a temperature of 50 °C until a thick extract was obtained.

### 2.4. Profiling with TLC-Densitometry Technique

The obtained 0.050 g ethanol extract of *Annona squamosa* L. and *Persea americana* M. was dissolved in 1.0 mL. A total of 5 µL of each extract solution was applied to the TLC F254 plate. The TLC plate was developed

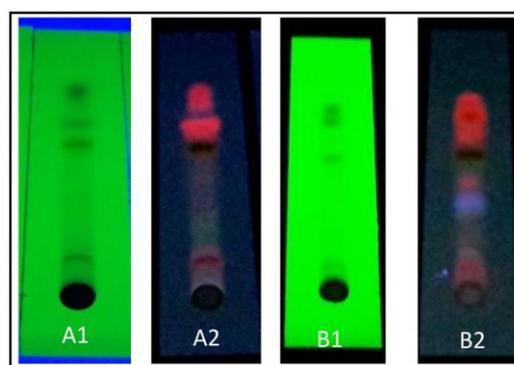
using hexane: ethyl acetate mobile phase in a ratio of 4:6. Then the KLT plate was loaded and examined at the wavelengths of 254 nm and 365 nm. Each chromatogram peak detected was reviewed at a wavelength range of 200–700 nm.

### 2.5. Analysis Technique

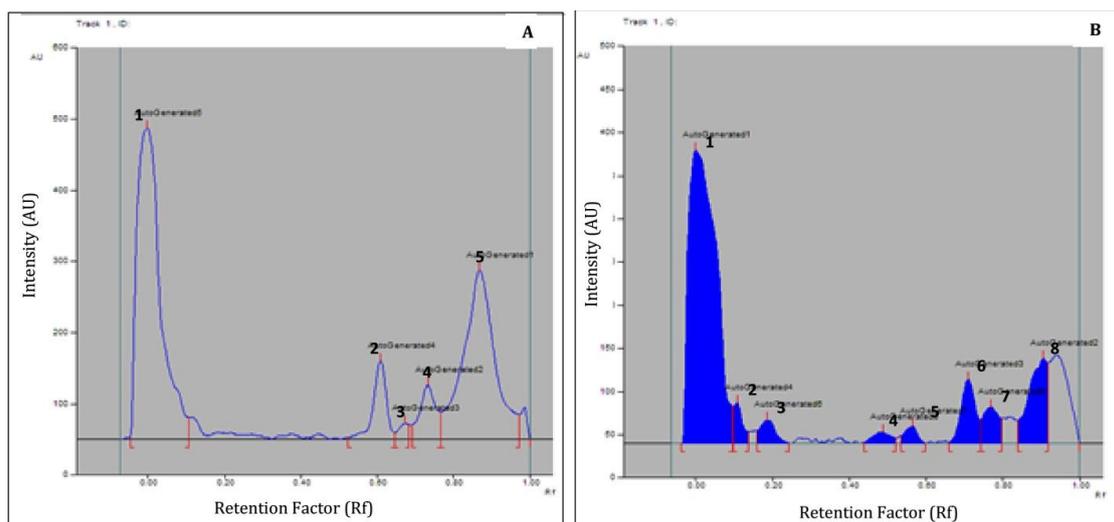
The data obtained from the TLC analysis were the stained profiles, which indicated by the color and *rf* value of each stain. Data from densitometry analysis were in the forms of chromatogram data profiles and spectra of each stain. The data were analyzed descriptively.

## 3. Results and Discussion

The chromatogram profile of EEAS and EEPA can be seen in Figure 1. The mobile phase used in the TLC analysis was selected based on the optimization results from previous studies. Based on the results of the TLC analysis, EEAS had four spots that can



**Figure 1.** Chromatogram profile of *A. squamosa* ethanol extract at (A1) 254 nm and (A2) 365 nm and *P. americana* ethanol extract at (B1) 254 nm and (B2) 365 nm.



**Figure 2.** The chromatogram peaks of *A. squamosa* (A) and *P. americana* M (B) at a wavelength of 254 nm with a densitometer.

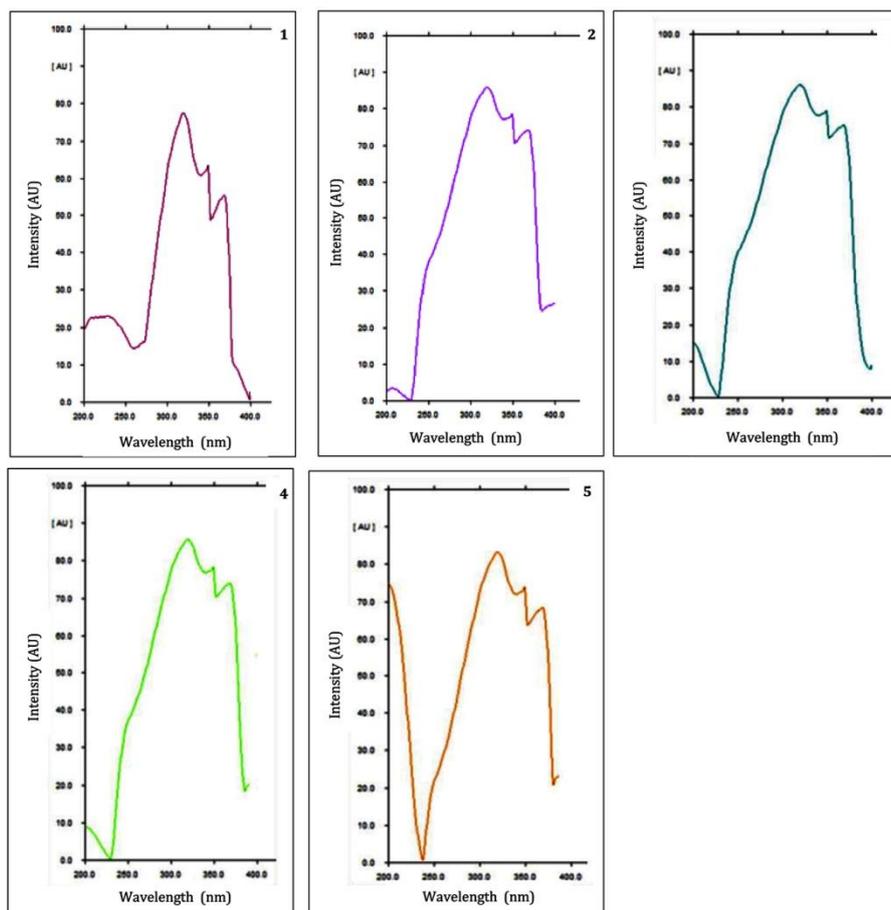
reduce the emission of UV light at a wavelength of 254 nm. Observations using the Vis 365 showed eight spots were compounds that could fluoresce in red at Vis 365. These results were in agreement with previous research, namely that there were spots that can reduce UV 254 at rf 0.662 and 0.712 and there were spots that were able to fluoresce Vis 365 at Rf 0.544, 0.619 and 0.683 [10].

The TLC results of EEPA showed that UV 254 depicted five spot stains showed the presence of compounds that capable of reducing UV 254. Vis 365 described nine spot stains showed compounds that could fluoresce in red and blue at Vis 365 nm.

EEAS and EESA were analyzed using TLC techniques and then analyzed using a densitometer. To detect the presence of secondary metabolites, the TLC plate was analyzed at the wavelengths of 254 nm and 365 nm. Analysis of these two types of wavelengths aimed to detect all secondary

metabolites. The results of the EEAS analysis showed five chromatogram peaks with rf values of 0.00, 0.61, 0.67, 0.73, and 0.87. The results of the EEPA analysis showed eight peaks with rf values of 0.00, 0.11, 0.19, 0.49, 0.56, 0.71, 0.77, and 0.90. The images of the chromatogram using a densitometer can be seen in Figure 2.

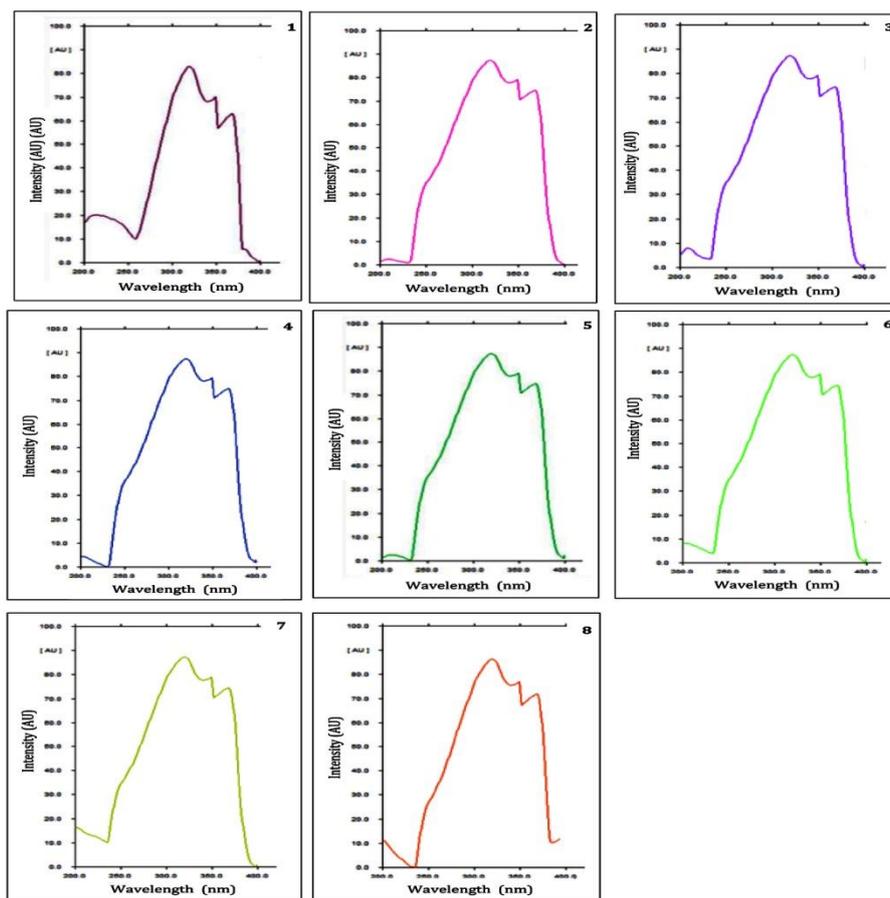
The spectral data of the secondary metabolites of each extract can be obtained from each chromatogram peak at a wavelength within 200–700 nm. The results showed that the secondary metabolites in EEAS and EEPA had multiple components. This was shown at each peak on the chromatogram at 254 nm wavelength having the different spectrum data. The analysis results of each chromatogram peak for EEAS and EEPA showed a spectrum with two to three peaks with a maximum wavelength within 300–350 nm. The spectrum can be seen in Figures 3 and 4.



**Figure 3.** The spectrum of secondary metabolites of *A. squamosa* ethanol extract: (1) peak 1 (rf 0.00); (2) peak 2 (rf 0.61); (3) peak 3 (0.67); (4) peak 4 (rf 0.73); (5) peak 5 (rf 0.87).

Compounds that have been isolated from the leaves of *A. squamosa* were isoquinoline alkaloids, namely anonaine, aporphine, corydine, isocorydine, norcorydine, norisocorydine, glaucine, norlaureline, and roemerine [11-12]. The spectrum obtained showed that anonaine and roemerine compounds had peaked at wavelengths of 231–235, 270–272 and 310–315 nm; norlaureline and aporphine compounds at wavelengths 273–276 and 304–316 nm; norcorydine compounds, corydine, norisocorydine at wavelengths 220, 268–270, and 302–308 nm; and glaucine compounds at wavelengths 218, 280–282, and 302 nm [13-

14]. Isoquinoline alkaloids can activate the adenosine monophosphate-activated protein kinase (AMPK), which is responsible for controlling cellular energy balance, metabolism, and defense responses to oxidative and inflammatory stress [15]. The isoquinoline alkaloid group inhibited ox-LDL-induced proliferation of HUVEC by decreasing the PCNA, NF- $\kappa$ B and LOX-1 expression, and suppressing the PI3K/Akt, ERK1/2, and p38MAPK pathways. This showed that the isoquinoline alkaloid group is a latent candidate for anti-atherosclerosis clinically [16].



**Figure 4.** Spectrum of secondary metabolites in *P. americana* ethanol extract: (A) peak 1 (rf 0.00); (B) peak 2 (rf 0.11); (C) peak 3 (0.19); (D) peak 4 (rf 0.49); (E) peak 5 (rf 0.56); (F) peak 6 (rf 0.71); (G) peak 7 (0.77); (H) peak 8 (rf 0.90).

Whereas for the isolated compounds from *P. americana* leaves were flavonoids, namely isorhamnetin, luteolin, rutin, quercetin, and apigenin. The resulting spectra showed isorhamnetin compounds had a maximum wavelength of 260, 272, 322, and 367 nm [17]. Flavonoid compounds could increase the ability of LDL to bind the receptors, so they are metabolized into cholesterol esters in the tissues. Furthermore, high-density lipoprotein (HDL) will bind cholesterol ester to be excreted into the small intestine [18]. Besides, flavonoid compounds can reduce LDL oxidase through lipid peroxidation, reduce the

oxidative stress of macrophages by inhibiting the cellular oxygenase, and activating cellular antioxidants [18].

#### 4. Conclusion

Based on the results of the study, it can be concluded that the EEAS chromatogram profile had five peaks, where each chromatogram peak had a three-peak spectrum at a wavelength of 300–350 nm. The results of the analysis of EEPA showed eight peaks, where each peak of the chromatogram had a spectrum with a wavelength of 300–350 nm. The peaks may be anonaine and roemerine,

norlaureline, aporphine, norcorydine, corydine, norisocorydine, and glaucine compounds in the *A. squamosa* leaves and isorhamnetin compounds in the *P. americana* leaves. The TLC-densitometry chromatogram profile data on EEAS and *EEPA* can be used as an indicator in standardizing the combination process of ethanol extracts of *Annona squamosa* and *Persea americana* as natural ingredients for antihyperlipidemic treatments.

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