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To cite this article: E K Hayati *et al* 2019 *J. Phys.: Conf. Ser.* **1375** 012021

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The Separation Optimization of Berberine in Anting-Anting Plants (*Acalypha Indica* Linn) using High Performance Liquid Chromatography (HPLC)

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Abstract. Anting-Anting plants (*Acalypha Indica* Linn) is a plant widely found in Indonesia. This plant has potential as an antimalarial, antidiabetic, and antitoxic drug. Ethyl acetate extract of this plant contained isoquinoline alkaloid, berberine which confirmed by LC-MS spectra on m/z 321,2561. Then, this study was conducted to determine the optimum conditions of separation and determination the berberine level existed on ethyl acetate extract of Anting-Anting using HPLC. The separation was optimized by varying flow rate of eluent (0.6 mL/min, 0.8 mL/min, 1.0 mL/min), mobile phase (methanol: TFA 0.1% (50:50 v/v) and acetonitrile: TFA 0.1% (60:40 v/v)). The separation was using HPLC with C18 (ODS) 4.6 x 250 mm, 5 μ m column, isocratic method at various wavelengths (290 nm, 315 nm, and 345 nm). The optimum separation condition was obtained at flow rate 1 mL/min with mobile phase methanol: TFA 0.1% (50:50 v/v) and UV detector wavelength at 345 nm. The result showed that the berberine level of Anting-anting in ethyl acetate fraction was 11,82030 μ g/mL.

1. Introduction

Anting-anting (*Acalypha indica* L.) is weed that abundant in Indonesia but rarely used. It contained steroids, triterpenoids, flavonoids, tannins, and alkaloids [1,2]. Anting-anting can be used as antimalarials [2], antidiabetic [3], antibacterial [4], antioxidant and anticancer [5,6]. One type of alkaloid compounds contained in ethyl acetate fraction of ethanolic extract Anting-anting is berberin. Identification of the berberine compound was performed using HPLC/DAD/ESI/ToF which showed an absorption m/z 321.2 (Figure 1). This study was conducted to determine the berberine contained in Anting-anting quantitatively. Because of the secondary metabolite like berberine present as minor component, HPLC is ideal instrument to process the separation rapidly [7]. There is various condition to determine compound using High Performance Liquid Chromatography (HPLC) quantitatively. Separation with HPLC can be done using isocratic or gradient method with its benefit or drawback based on the separation condition [8,9]. Type of column/stationary phase, flow rate, mobile phase temperature, and pH will affect the separation condition [10-15].

There was several researches in optimizing HPLC separation in determining berberine in other source of plants. Separation of berberine compounds using HPLC from *Mahonia manipurensis* stem bark had been done by Srinivasan et al. [16]. The separations were performed using mobile phase acetonitrile: water (10:90 v/v), C-18 column (250 x 4.6 mm i.d, 5 μ m) with flow rate used 0.6 mL/ min and isocratic mode and detected at 266 nm, then berberine was retained at 8.6 min. In other cases, berberine was separated using mobile phase mixture of ammonium chloride and acetonitrile with a flow rate of 0.8



mL/min and C-18 (250 × 4.6 mm i.d, 5 μm) column type detected at 254 nm. Those condition caused retention time of berberine at 30,754 min [17]. Berberine could be separated by HPLC with mobile phase methanol: TFA buffer (0.1% v/v) with a flow rate of 1 mL/min using gradient mode and C-18 column (5 μm, 10 mm x 4.6 mm), with that condition the retention time of the berberine compound was 7,89 min [18]. So, by varied the operational condition of HPLC such as mobile phase and flow rate were able to optimizing the separation of berberine.

To determine berberine contained quantitatively, this research was conducted with isocratic method then to optimize the separation of berberine using HPLC by varied of flow rates (0.6, 0.8 and 1.0 mL/min) and mobile phases (methanol: TFA buffer (0.1% v / v) (50:50 v / v) and acetonitrile: TFA buffer (0.1% v / v) (40:60 v / v). Before optimizing the separation method, Anting-anting was extracted by maceration using ethanol 80% and fractionated using ethyl acetate.

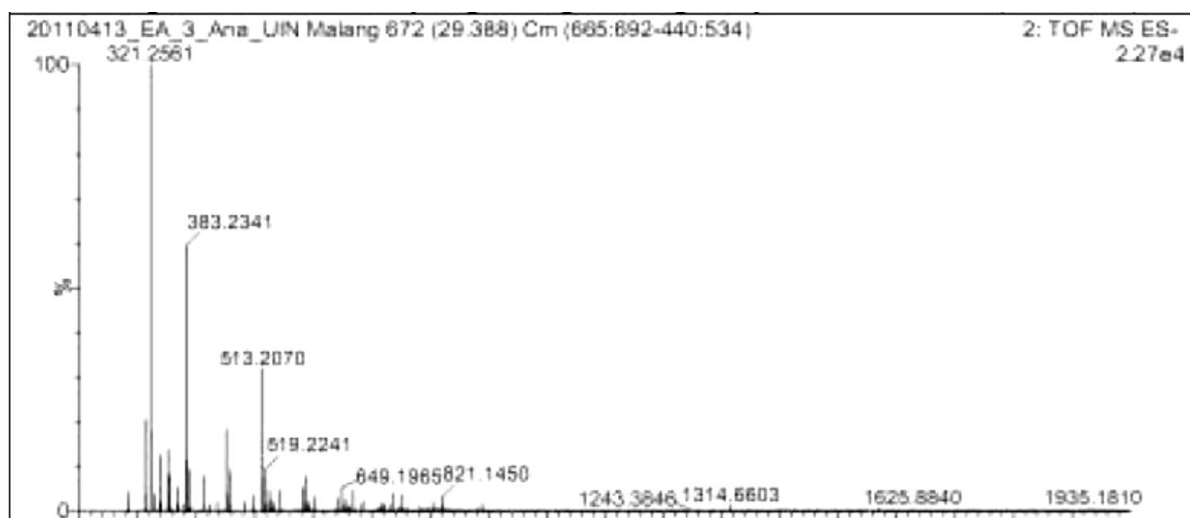


Figure 1. MS spectra of berberine in Anting-anting.

2. Materials and method

2.1. Chemicals and apparatus

Berberine amount was measured using Agilent 1200 Series HPLC; Zorbax ODS column 4.6 x 250 mm, 5 μm (880952-702). The source of Anting-anting was collected from Malang, East Java, Indonesia. Berberin standard was purchased from Wako Pure Chemical Industries, LTD, Japan Ethanol, ethyl acetate, HCl, methanol, acetonitrile, Trifluoroacetic acid (TFA) was purchased from Merck. All materials were used of analytical grade except methanol and acetonitril was HPLC grade.

2.2. Sample preparation

5 kg anting-anting was collected, cleaned and dried. Then it was ground and sieved (90 mesh). 100 g powder of anting-anting was macerated with 500 mL 80% ethanol for a day and shook for 3 hours at 120 rpm. Macerating process was repeated 4 times with 200 mL ethanol each. Then, the solvent was removed by vacuum rotary evaporator at 60°C. The crude ethanol extract was fractionated using ethyl acetate: water (50:50) using separatory funnel, repeated for 6 times and followed by removing the solvent.

2.3. Optimization HPLC operational system

20 μL berberine standard 2 μg/mL (methanol as solvent) was injected into the HPLC with varied of flow rate (0.6, 0.8 and 1.0 mL/min) for each mobile phase (methanol: TFA buffer (0.1% v / v) (50:50 v / v) and acetonitrile: TFA buffer (0.1% v / v) (40:60 v / v) then it was measured at varied of wavelength at

290 nm; 315 nm; and 345 nm. HPLC was operated at room temperature. The mobile phase was sonicated for 20 mins before use.

2.4. Determination berberine contained in Anting-anting

A series of berberine standard (1 μ g/mL, 5 μ g/mL, 10 μ g/mL, 20 μ g/mL and 30 μ g/mL) and ethyl acetate fraction of anting-anting was injected into HPLC with optimum HPLC operational system. The berberine amount contained in Anting-anting was calculated based on the peak area calibration curve.

3. Results and discussion

3.1. Ethyl acetate fractioned of Anting-anting

Brownish-green Anting-anting powder was macerated using ethanol and collected 16.420 g crude extract (%yield 8.2 %). From fractioning step using ethyl acetate, the crude extract was reduced. The amount of ethyl acetate fractioned was 5.56 g or 37.07% of the crude extract.

3.2. Optimization HPLC separation system

The optimum condition of HPLC separation system for measuring berberine was at flow rate 1 mL/min, mobile phase methanol: TFA buffer 0.1 % (50:50) at 345 nm. It was an optimum condition because of the fastest retention time with good pressure column (under <210 Kg/cm² [19] and with fair theoretical plate value than other condition (table 1). Chromatogram of berberine with optimum condition was showed at Figure 2.

Table 1. Optimization HPLC operational result.

Optimization Parameter	λ (nm)	Result of optimization parameter of each variation					
		Acetonitril:TFA buffer 0.1% (40:60)			Methanol:TFA buffer 0.1% (40:60)		
		0.6 mL/min	0.8 mL/min	1.0 mL/min	0.6 mL/min	0.8 mL/min	1.0 mL/min
Retention time tR	290	4.263	3.184	2.282	4.807	3.387	2.668
	315	4.270	3.186	2.284	4.601	3.364	2.652
	345	4.668	4.407	2.743	4.524	3.264	2.518
Theoretical plate (N)	290	204.30	213.09	238.96	435.63	453.62	347.89
	315	255.76	233.76	219.83	1287.00	882.33	779.29
	345	327.94	267.65	244.07	2832.7	1565.2	1055.6
Column pressure (kgf/cm ²)		70	93	116	138	182	193

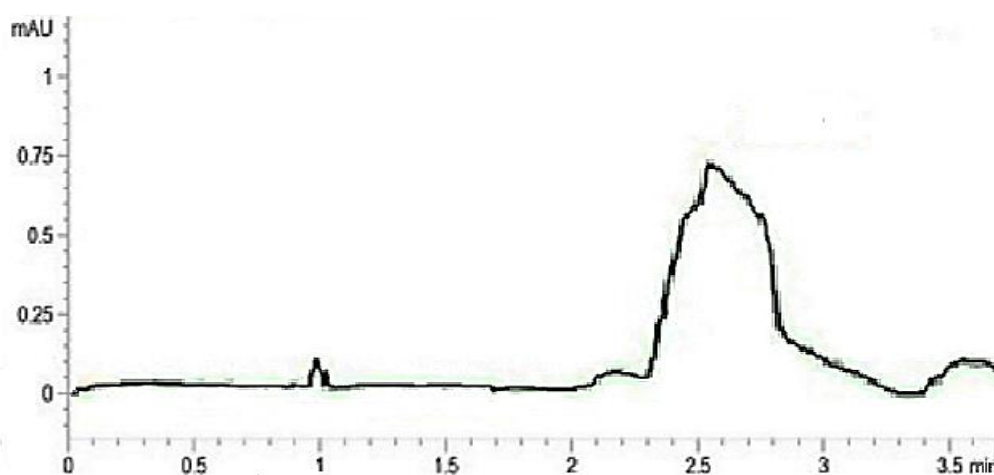


Figure 2. Chromatogram of berberine standard in optimum HPLC separation system.

3.3. Berberine amount of Anting-anting

Calibration curve of a series of Berberine standard was produced a correlation equation of relation between concentration (X) and absorbance (Y). The equation was $Y = 0.018524 X + 3.54490$ with linearity $R = 0.95871$. The chromatogram of ethyl acetate fraction of anting-anting was showed at Figure 3. The retention time was slightly delayed compared from berberine standard due to its interaction with other secondary metabolites. This slight delay was also happened in Utami, et al, 2017 [20] So, based on the peak area of fraction chromatogram, the result of this research show that in each 100 ppm ethyl acetate fraction of Anting-anting contained 11.8203 $\mu\text{g/mL}$.

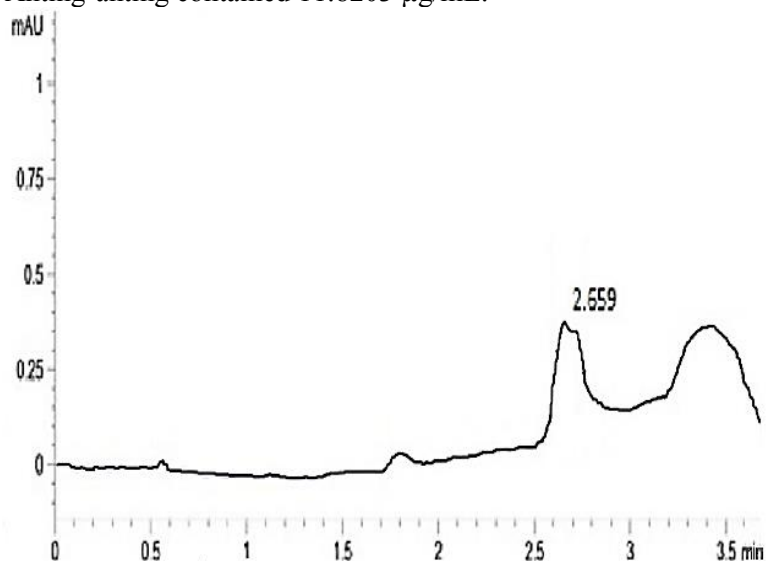


Figure 3. Chromatogram of ethyl acetate fraction of Anting-anting.

4. Conclusion

The optimum separation condition was obtained at flow rate 1 mL/min with mobile phase methanol: TFA 0.1% (50:50 v/v) and UV detector wavelength at 345 nm. The result showed that the berberine level of Anting-anting in ethyl acetate fraction was 11.82030 $\mu\text{g/mL}$.

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