ความสัมพันธ์ระหว่างกรดลีคานอริกและอะทาโนรินในไลเคน *Parmotrema tinctorum*

The Correlation of Lecanoric Acid with Atranorin in Lichen *Parmotrema tinctorum*

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บทคัดย่อ

งานวิจัยนี้ต้องการศึกษาสหสัมพันธ์ของกรดลีคานอริกและอะทาโนรินโดยใช้วิธีการคำนวณสหสัมพันธ์เพียร์สัน ทำการเก็บตัวอย่างไลเคนมากกว่า 25 ตัวอย่างในกรณีต่างเวลาซึ่งมีผลให้สภาพแวดล้อมต่างกันจากอุทยานแห่งชาติเขาใหญ่ ใช้วิธีโครมาโทกราฟีของเหลวสมรรถนะสูงวิเคราะห์สารไลเคน คอลัมน์ที่ใช้คือไฮเปอร์ซิล C18 ขนาด 250 x 4.0 mm, 5 µm ทำการชะด้วยระบบแกรเดียนท์ ตรวจวัดด้วยเครื่อง ยูวี ที่ความยาวคลื่น 254 นาโนเมตร เฟสเคลื่อนที่ 2 ชนิดที่ใช้คือเมทานอลเป็นตัวทำละลาย B และสารละลายบัฟเฟอร์ของ 1% กรดฟอสฟอริก เป็นตัวทำละลาย A โปรแกรมการชะของระบบแกรเดียนท์คือเริ่มต้นด้วย 30% B ที่อัตราการไหล 0.7 มิลลิลิตรต่อนาที จากนั้นเพิ่มตัวทำละลาย B เป็น 70% ภายในเวลา 14 นาที แล้วเพิ่มเป็น 100 % ภายในเวลา 30 นาที ผลที่ได้จากการวิเคราะห์คือ กรดลีคานอริกและอะทาโนรินไม่มีความสัมพันธ์กันและพบว่าปริมาณเฉลี่ยของกรดลีคานอริกและอะทาโนรินในช่วงฤดูฝนมีปริมาณมากกว่าในช่วงฤดูแล้ง

คำสำคัญ: อะทาโนริน โครมาโทกราฟีของเหลวสมรรถนะสูง กรดลีคานอริก สารทุติยภูมิ

ABSTRACT

In this research investigation, the researchers study the correlation of lecanoric acid and atromarin using Pearson's correlation. The collection of more than twenty-five lichen samples was conducted at different times with different environments at Khao Yai National Park. The technique of high performance liquid chromatography was used to analyze lichen substances. The hypersil C18 column (250 x 4.0 mm, 5µm) under gradient elution and UV detection at λ 254 nm was employed. The two mobile phases used were methanol as solvent B and buffer solution of 1 percent phosphoric acid as solvent A. The program of gradient elution started with 30 percent B at the flow rate of 0.7 milliliter per minute. Then, solvent B was increased at 70 percent within fourteen minutes and at 100 percent within thirty minutes. The analysis

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showed that lecanoric acid and atranorin did not exhibit correlation. It was also found that the average amount of lecanoric acid and atranorin in the rainy season was higher than in the dry season.

Keywords: atranorin, HPLC, lecanoric acid, secondary metabolites

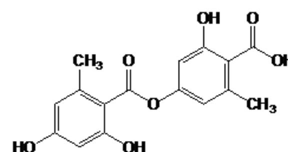
Introduction

Lichen is a symbiotic partnership of two separate organisms, fungi and algae and synthesize numerous secondary metabolites, known as the lichen substances. Lichens and their metabolites have manifold biological activities: antiviral, antibiotic, antitumor, plant growth inhibitory, antiherbivorous, and enzyme inhibitory activities. Lecanoric acid and atranorin are the main secondary metabolites of the lichen Parmotrema tinctorum. These substances can vary among habitats due to different environmental stresses.

Lichens have received special attention in recent years because of their ability for use as a bioindicator of air pollution and for the production of secondary metabolites (Plinio Cesar Pinto et al., 2009; Bialonska and Dayan, 2005) which have the potential to be used commercially. Some of the main compounds of secondary metabolites, known as the lichen substances, were produced for survival in extreme environments, for example lecanoric acid, parietin, emodin, atranorin, gyrophoric acid, fumarprotocetraric acid, rhizocarpic acid, pulvinic dilactone and usnic acid. (Huneck, 1999; Luo et al., 2009) These substances could vary among habitats because of different environmental stresses. It was found that these substances have manifold biological activities including antiviral, antibiotic, antitumor, plant growth inhibitory, antiherbivorous, and enzyme inhibitory activities (Luo, 2009). Lecanoric acid and atranorin, the phenolic compounds, are the main secondary metabolites of the lichen Parmotrema tinctorum. Both of them have antioxidant activity and biological activities. The most powerful tool for the separation and identification of secondary metabolites in lichens is high performance liquid chromatography (HPLC) (Feige, 1993). HPLC is widely used for the quantitative determination of secondary metabolites in lichen. Precise determination of lichen products is essential to enhance our understanding of the production and roles of lichen novel products, which have extensive implications for sustainable utilization in several aspects.

This work aimed to study the correlation of lecanoric acid with atranorin by using the Pearson correlation coefficient. The aim was to prove that the production of secondary metabolites in lichen has no correlation, the production of each substance depending on its environmental surrounding.

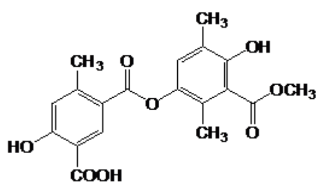
Lecanoric acid $C_{16}H_{14}O_7$ (MW 318.27)



UV(EtOH): [λ_{max} nm (logε)] 214 (4.63), 270.5(4.30), 305.5(4.09)

Potential: antioxidant activity, antifungal activity, enzyme inhibitory activity

Atranorin $C_{19}H_{18}O_8$ (MW 374.33)



UV(MeOH): [λ_{max} nm (logε)] 210 (4.20), 252(4.16), 312(3.57)

Potential: antioxidant activity, wound healing activity, allergenic activity

Experiment

Chemicals and reagents

Methanol was HPLC grade, while ortho-phosphoric acid, and acetone were of analytical reagent grade. All of them were bought from Merck.

The deionization water with a specific resistance of more than 18.0 M□-cm-1 used in this experiment was prepared by an Easy Pure RF Compact ultrapure water system from Barnstead.

The standard substances of lecanoric acid and atranorin were prepared by extracting and purifying them from the lichen Parmotrema tinctorum. They were prepared by Phiphatpong Thepnuan (Thepnuan, 2014).

Analysis

Chromatographic analyses were performed by using the HP 1100 series consisting of a HP G1312A binary pump and a HP G1314A UV variable wavelength detector. Separation was achieved on an ODS Hypersil 250 x 4 mm I.D., 5 µm column.

The lichen samples were collected from Khao Yai National Park at 14° 24' 52.67" N and 101° 22' 36.70" E from August 2015 to June 2016. Each collection collected had five samples (N = 5).

The lichen samples were manually cleaned to remove foreign debris on thalli, and then ground into powder with liquid nitrogen using a ceramic mortar and pestle. The fine powder samples were kept frozen in a refrigerator until analysis. 10.0 mg of ground samples were accurately weighed and extracted with acetone after soaking overnight. The extracts were filtered and evaporated until dry. The residues were then dissolved with a small amount of methanol and diluted to the exact volume of 70:30 of methanol:water. The solution samples were filtered through 0.45 µm syringe membrane before being injected into HPLC.

Two mobile phases were methanol as solvent B and 1% phosphoric acid as solvent A (pH = 2.3 - 2.7). The run started with 30% B at a flow rate of 0.7 ml/min. Solvent B was increased to 70% within 14 minutes, and then up to 100 % in 30 minutes (Feige et al., 1993). At the end of the run time, the post time was set to 10 min before a new run was started. The compounds were detected at a wavelength of 254 nm. The injection volume was 20 µl.

Results and Discussion

The HPLC chromatogram of the separation of secondary metabolites from the lichen Parmotrema tinctorum is shown in Figure 1. Table 1 shows the amount of lecanoric and atranorin of the thirty samples taken at different times. The results revealed that lecanoric acid had no correlation with atranorin. The Pearson correlation coefficient was 0.260 at $p < 0.01$ as shown in Figure 2.

Table 1. The Sampling Time and Amount of Lecanoric acid and Atranorin

Sampling time	*Amount*		*Sampling time*	*Amount*	*(µg/g)*
	Lecanoric acid	Atranorin		Lecanoric acid	Atranorin
	2827.56	418.25		2239.99	201.87
	2604.17	382.20		1645.45	143.25
Aug-15	2467.18	328.09	Feb-16	2244.16	270.51
	2701.70	411.36		1919.73	245.94
	3018.76	375.63		1579.58	285.39
	2760.18	301.19		1883.89	185.28
	2325.47	248.43		2496.13	185.26
Oct-15	2611.19	283.36	Apr-16	2443.53	167.73
	2393.80	266.43		2333.13	214.60
	2352.38	263.27		861.08	192.41
	2483.85	112.66		2304.03	163.05
	2693.00	72.54		2585.18	169.55
Dec-15	1468.34	328.29	Jun-16	2969.87	185.26
	1747.31	143.89		2173.51	165.45
	2210.01	47.92		2023.69	132.35

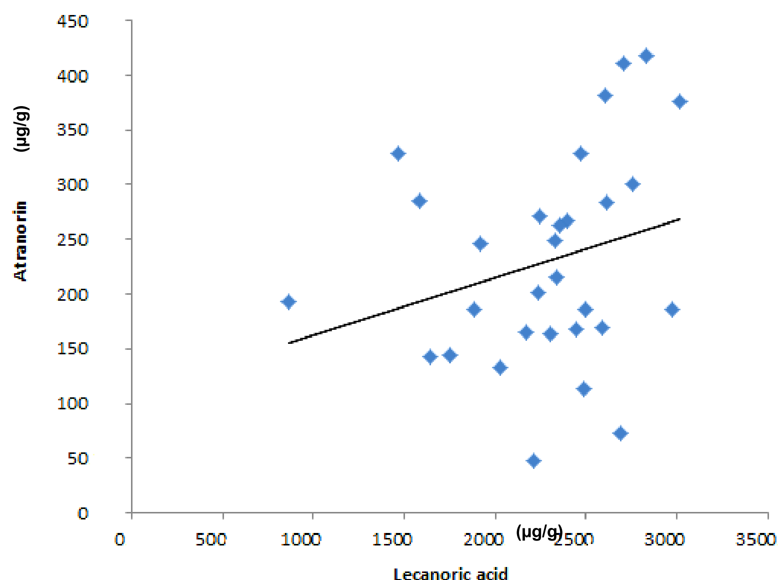


Figure 2. The Pearson correlation coefficient of lecanoric acid and atranorin was 0.260 at $p < 0.01$

Conclusion

The amount of secondary metabolites, lecanoric acid and atranorin can be quantified by using the external standard calibration curve of the HPLC technique. It can be concluded that when lichen produce a high amount of lecanoric acid, it does not mean that atranorin is high as well. Some environments may be suitable for the production of lecanoric acid and other environments may be suitable for the production of atranorin. Another reason is that both substances are produced from different parts of lichen; lecanoric is produced in the medullar layer and atranorin is produced in the cortex layer (Noicharoen, 2002). When comparing the average amount of lecanoric and atranorin produced over time, it was found that during August 2015 – October 2015, which is rainy season, lecanoric acid and atranorin were higher than those during December 2015 – April 2016, which is dry season as shown in Figure 3. This suggests that the different seasons which have diverse environmental conditions, such as sunlight, moisture and temperature, significantly affect the production of lichen secondary metabolites.

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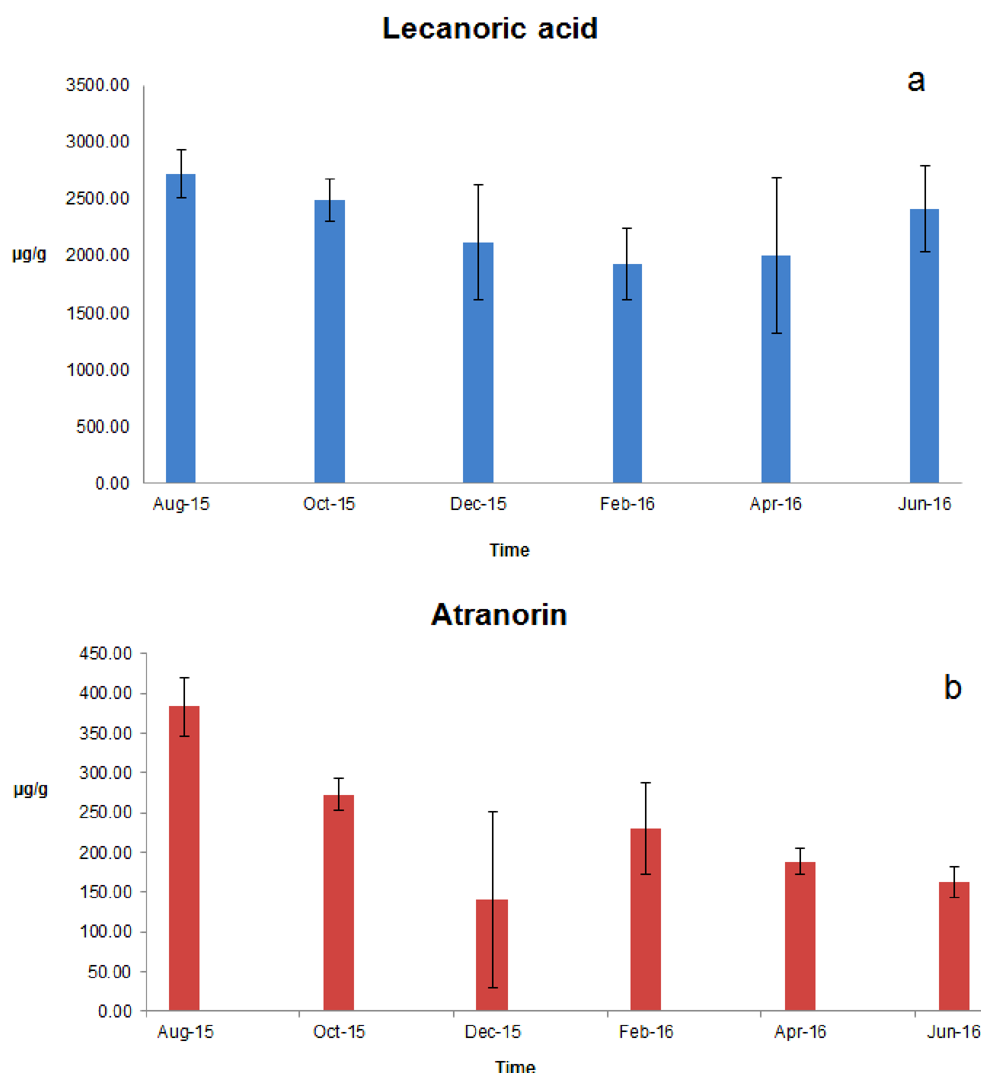

Figure 3. The amount of a) lecanoric acid b) atranorin in lichen *Parmotrema tictorum* collected on August 2015 - June 2016

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