QUANTITIES OF SECONDARY METABOLITES FROM THE LICHEN *Parmotrema tinctorum* BEFORE AND AFTER TRANSPLANTED TO POLLUTED AREAS IN BANGKOK, THAILAND

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Abstract: The amounts of secondary metabolic products from the lichen *Parmotrema tinctorum* before and after transplanted from Khao Yai National Park to nine public parks in Bangkok, Thailand, were analyzed by using HPLC method. Lichen substances were isolated through Hypersil C18 column (250 mm x 4.0 mm, 5 μm) under gradient elution and UV detection at λ 254 nm using methanol and 1% phosphoric acid as mobile phases. Lichen samples before and after transplantation were ground in liquid nitrogen and extracted by acetone. Benzoic acid was used as internal standards to control HPLC system. The lichen compounds including orsellinic acid, methyl orsellinate, lecanoric acid, atranorin and chloroatranorin were quantified by using peak area for comparing the amount before and after transplantation. Transplantation period was 7 months lasted from 19 September 2010 to 20 April 2011. The average peak area of orsellinic acid, methyl orsellinate, lecanoric acid, atranorin and chloroatranorin before transplantation were 657.6±179.5, 47.6±17.5, 17140.7±1219.0, 1465.8±101.9, and 266.6±39.2, respectively. After transplantation these amounts were 753.3±265.1, 34.8±22.7, 11066.5±1679.9, 1502.5±161.0 and 271.0±43.5, respectively. By using Paired Samples T-Test, the amounts of lichen substances before and after transplanted to polluted sites are compared. The results revealed that lecanoric acid was significantly decreased after transplanted (P < 0.05), whereas the other substances were not different statistically.

1. Introduction

Lichens contain a great number of organic compounds including primary and secondary metabolites [1,2]. Secondary metabolic products were produced from biochemical pathways known as lichen substances [3]. They provide protective roles against adverse environment and vary as a result of environmental stress [4]. Many hypotheses concerning their biological role have been proposed [5-7]. The lichen *Parmotrema tinctorum* is a widely distributed species globally. It produces polyphenolic lichen acid such as orsellinic acid, methyl orsellinate, lecanoric acid, and atranorin which commonly occurs with chloroatranorin [8,9]. Their structure are shown in figure 1. Lecanoric acid is the main–product of the lichen *Parmotrema tinctorum* which has an important role in modern medicine. It has inhibitory function of histamine decarboxylase, potential agent against allergy, microcirculatory hemostasis, gastric secretion, inflammation and some neutral functions [10]

The objective of this study was to compare the amount of secondary metabolic products of lichen *Parmotrema tinctorum* before and after transplantation from Khao Yai National Park to nine public parks in Bangkok, Thailand.

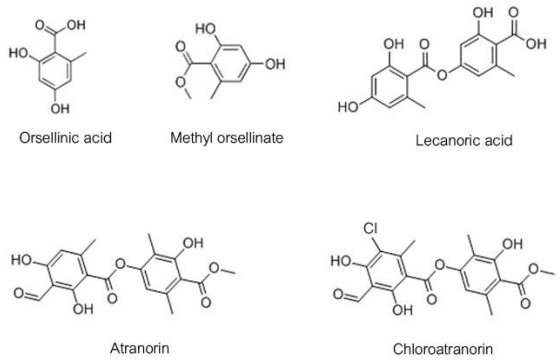

Figure 1. The structures of five secondary metabolic products from lichen *Parmotrema tinctorum*

2. Materials and Methods

2.1 Chemicals and reagents

All chemicals are analytical grade except methanol is HPLC grade from Merck. Deionized water (DI) with specific resistance > 18.0 MΩ-cm was prepared by an Easy Pure RF Compact ultrapure water system from Barnstead, was used for preparation 1% phosphoric acid as mobile phase A.

2.2 Lichen samples

This experiment quantified the amount of secondary metabolites in the lichen *Parmotrema tinctorum* before and after transplanted to public parks in Bangkok known as polluted areas. The thalli of *Parmotrema tinctorum* were collected and then exposed to ambient air at the lichen's camp at KaoYai National Park before fixing on 40 x 50 cm plastic nets Five nets were left at KhaoYai National Park as control lichen (CL). The others were brought to nine public parks in Bangkok and attached on trees as show in figure 2. Five plastic nets, with fixed lichens, were placed in each public parks. The nine public parks

were described by Polyium, et al., 2009 [11] which included: (1) Thawiwanarom (TW), (2) Thonburirom (TR), (3) Lumphini (LN), (4) Suanluang Rama IX (R9), (5) Nong Chok (NC), (6) Phra Nakhon (PK), (7) Santiphap (SP), (8) Seri Thai (ST), (9) Rommani Thongsikan (RT). The transplanted period lasted for 7 months from 19/9/2010 to 20/4/2011. The lichen samples were brought to analysis at the laboratory of Chemistry Department, Ramkhamhaeng University.

Figure 2. The lichen *Parmotrema tinctorum* on plastic nets were transplanted to public parks in Bangkok.

2.3 Samples preparation

Lichen samples were air-dry at room temperature for two days. Foreign debris on thalli were manually removed. Samples were ground into powder with liquid nitrogen using a ceramic mortar and pestle, and were then sieved through a 500 µm filter. The fine powder samples were kept frozen in refrigerator before analysis. A 10 mg of grounded lichen samples was accurately weighed and extracted with acetone by soaking overnight. The extracted samples were filtered and evaporated to dryness. The residues were then dissolved by small amount of methanol and diluted to exact volume by 70:30 of methanol : water using benzoic acid as an internal standard. The sample solutions were filtered through 0.45 µm syringe membrane before injection to HPLC. The analysis include three replicate and the average was taken from two or three readings that had similar values. Quality control of the analytical procedure was carried out by analyzing the same procedure.

2.4 HPLC analysis

Lichen extracts were analyzed by a HP 1100 series consisting of HP G1312A binary pump, HP G1314A UV variable wavelength detector. Separation was achieved on an ODS Hypersil 250 x 4 mm I.D., 5 µm column. The solvent A consisted of 1% phosphoric acid in water (pH = 2.3-2.7) and solvent (B) was 100 % methanol. The run start with 30% B at flow rate 0.7 ml/min solvent B was increased to 70% within 14 min, then up to 100 % in 30 min. The programmed run was modified from G.B. Feige [12]. 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 wavelength 254 nm and the identification of compounds were based on retention times. .

3. Results and Discussion

The chromatogram of the lichen *P. tinctorum* was shown in figure 3. The order of retention times were orsellinic acid, benzoic acid, methyl orsellinate, lecanoric acid, atranorin and chloroatranorin. The precision values of retention times and peak areas of compounds were analyzed from seven replicates as shown in table 1. The results showed that %RSD were less than 5%. Since peak area was a direct proportion of concentration, therefore they represented the amount of lichen substances and can be used to compare quantities of lichen substances before and after transplantation.

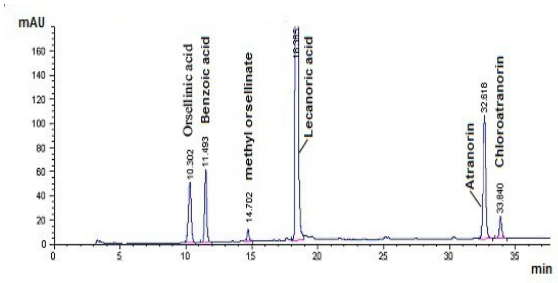

Figure 3. The HPLC chromatogram of lichen *Parmotrema tinctorum*

Table 1. Precision data of retention time and peak area of lichen substances in seven replicate of analyses

Compound	Retention time		Peak area	
	$\bar{X}$ ± SD	%RSD	$\bar{X}$ ± SD	%RSD
Orsellinic acid	10.28 ± 0.05	0.5	687.2 ± 13.6	2.0
Benzoic acid	11.48 ± 0.04	0.4	677.6 ± 8.6	1.3
Methyl orsellinate	14.69 ± 0.04	0.3	37.7 ± 1.1	2.9
Lecanoric acid	18.35 ± 0.05	0.3	19953.6 ± 138.8	0.7
Atranorin	32.55 ± 0.11	0.3	1427.7 ± 36.7	2.6
Chloroatranorin	33.77 ± 0.12	0.4	236.8 ± 5.8	2.4

The amount of secondary metabolites before and after transplantation to nine public parks are shown in Table 2. The average amounts of orsellinic acid, methyl orsellinate, lecanoric acid, atranorin and chloroatranorin from the nine public parks before transplantation were 657.6±179.5, 47.6±17.5, 17140.7±1219.0, 1465.8±101.9, and 266.6±39.2 respectively. After transplantation these amounts were 753.3±265.1, 34.8±22.7, 11066.5±1679.9, 1502.5±161.0 and 271.0±43.5 respectively. The average amount of lichen substances from the nine sites before and after transplantation were compared by Paired Samples T-Test using SPSS 17.0 for window. The results revealed that lecanoric acid in the lichens after transplantation was significantly lower ($P < 0.05$) than those before transferring to Bangkok. Other substances were not different significantly.

Table 2. The amount (peak area) of lichen substances at nine public parks in Bangkok city before and after transplantation (the value are the average of three replicate analyses)

site	Orsellinic acid		methyl orsellinate		Lecanoric acid		Atranorin		Chloroatranorin	
	before	after	before	after	before	after	before	after	before	after
(1)TW	456.8	726.7	36.7	36.2	16852.6	8699.0	1536.9	1568.3	237.3	288.4
(2) TR	796.0	750.1	64.0	56.8	14993.2	12130.5	1349.5	1584.4	201.2	308.2
(3) LN	1008.1	576.5	78.9	47.5	17970.3	12273.2	1590.5	1734.9	279.7	322.7
(4) R9	580.8	614.4	32.2	46.0	17935.4	12472.2	1583.2	1447.8	316.1	273.0
(5) NC	785.4	1290.7	41.6	64.7	18418.2	13467.1	1420.5	1656.3	272.7	309.3
(6) PK	568.0	307.4	63.5	ND	15572.9	9366.6	1464.5	1184.0	240.1	182.0
(7) SP	570.1	805.7	37.1	ND	17793.5	9218.6	1406.8	1393.5	286.7	240.7
(8) ST	466.2	867.4	26.4	33.4	16572.3	11389.7	1307.4	1500.5	245.0	251.5
(9) RT	686.6	840.4	48.1	29.1	18157.8	10581.9	1533.0	1452.8	320.6	263.3
$\overline{X}$	657.6	753.3	47.6	44.8	17140.7	11066.5	1465.8	1502.5	266.6	271.0
SD	179.5	265.1	17.5	12.9	1219.0	1679.9	101.9	161.0	39.2	43.5
CV	27.3	35.2	36.8	28.8	7.1	15.2	7.0	10.7	14.7	16.0

Seven months after transplantation lichen at Khao Yai National Park, the control samples, were also corrected and determined the amount of secondary substances. The results show that the amounts of all substances from these lichens increased as showed in table 3.

Table 3. The amount of control lichens at control sites (Khao Yai National Park)
A = starting time before transplantation
B = after time past to 7 months

Lichen substances	A	B
Orsellinic acid	546.0	739.9
Methyl orsellinate	61.5	67.3
Lecanoric acid	14853.6	20154.2
Atranolin	1619.5	1757.4
Chloroatranorin	288.7	332.9

It was reported that concentration of the secondary metabolites is higher in older thalli, larger size, than the younger ones [7]. However, this study choose lichens which had similar thallus sizes. Pangpet and Boonpragob, 2005 [13] revealed that growth rate of the lichen *P. tinctorum* varied among ecosystems. Lichens produced secondary metabolites to protected them against adverse environment, which different among ecosystems. *P. tinctorum* transplanted to nine public parks in Bangkok accumulated atmospheric pollutants and heavy metals about 0.3- 19.5 folds higher than those before transplantation reported by Boonpeng et al. [14]. These pollutants affected lichen primary metabolism such as photosynthesis [15,16], and thus lichen secondary metabolites were declined.

4. Conclusions

Lichen substances including orsellinic acid methyl orsellinate, lecanoric acid, atranorin and chloroatranorin, from the lichen *P. tinctorun* were quantified before and after transplanted to nine public parks in Bangkok. Lecanoric was the main substance with concentration of over 10 folds higher amount than the other substances. This substance had declined amounts in all parks after transplantation, whereas the other three substances, of small quantities, had lower amounts in some parks. It is reasonable to conclude that air pollutants in public parks caused the decline of the major lichen substance, lecanoric acid. Whilst declining and increasing of the other three minor substances might be the effects of human, methodological and instrumental errors due to very small quantities of these substances.

Acknowledgements: This work was supported by the National Research Council of Thailand (NRCT). Spectial gratitude goes to Khao Yai National Park under the department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment of Thailand.

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