H_H0067: ATR-FTIR SPECTRAL ANALYSIS OF LICHENS USING AS FINGERPRINT FOR IDENTIFICATION OF LICHEN SPECIES

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Abstract: This study used Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopic imaging to detect secondary metabolites of lichen. It will be developed to simplify chemotaxonomy of lichen in the future. The study showed that ATR-FTIR spectra of five lichen species, *Relicina abstrusa*, *Relicinopis intertaxta, Cladonia submultiformis*, *Usnea undulata* and *Parmotrema tinctorum* were different. Spectra band provided detailinformation of the organic functional group of secondary metabolites, produced by lichen Therefore ATR-FTIR spectra can be efficiently used to detect lichen substances. Each species of lichen produces difference secondary metabolic products, which are used as the main criteria for taxonomic identification of lichen. This technique is less complicated and economically wise than TLC, HPLC or LC-MS, which are generally used in chemotaxonomy of lichen. We propose to examine ATR-FTIR spectra of taxonomic known lichens, and keep the information as reference like a fingerprint. Unknown lichen species can be identified easily by comparing ATR-FTIR spectrum. Most importantly, ATR-FTIR spectrum would assist tremendously in elucidating secondary metabolites from large numbers of lichen collections deposited in the herbarium. They are lack of taxonomic identity due to absence of information on lichen substances.

Introduction: Production of various unique extracellular secondary matabolites known as lichen substances or lichen acid are the consequences of symbiosis between fungi and algae or cyanobacteria. There are over 700 lichen substances reported to date and many are restricted to the lichenised state. Presently more than 20,000 species are known based on their morphology, anatomy and most importantly their chemical substances.Detection of lichen substances is usually performed by Thin Layer Chromatography (TLC), High performance Liquid Chromatography (HPLC) and Mass spectrometry (MS) etc., which are time consuming, complicated and costly. Large numbers of lichen collections in the herbarium are unidentified due to insufficient resources to detect lichen substances.

Attenuated Total Reflectance (ATR) is today the most widely used FTIR sampling tool. ATR generally allows qualitative or quantitative analysis of samples with little or no sample preparation. It is an efficient technique for analyzing functional group of organic substances for identify structure of compounds.

The goal of our work was to explore the possibility to use FTIR spectral as fingerprint of lichen substances found in each species. Different lichen species can be recognized by pattern of FTIR spectra. The information could be kept as reference materials for prompt identification of lichens.

Methodology: The lichens, *Relicina abstrusa*, *Relicinopis intertaxta*, *Cladonia submultiformis*, *Usnea undulata* and *Parmotrema tinctorum* were collected from Khao Yai National Park. The collected samples were air-dry at room temperature, foreign matters on thalli were manually removed under the light microscope. Samples were ground into powder with liquid nitrogen, and were then sieved through a 500 μm sieve.The fine powder samples

were kept in desiccator at least two days before analysis.The FT-IR spectra of the fine powder samples were record on Frontier FT-IR spectrometer (PerkinElmer, USA) using diamond/ZnSe UATR (universal attenuated total reflectance) through the wave number range4000-700 cm^{-1}. The FT-IR spectrum of five lichen species were compared in functional group region and fingerprint region. In order to confirm that the same species of lichen fromelsewhere give similar ATR-FTIR spectra. The lichen *Parmotrema tinctorum* was used for validation by collecting samples from Khao Yai National Park in Nakorn Rachasrima Province, Queen Sirikit Botanic Garden in Chiang Mai Province, Phohin Rongkla in Phitsanulok and Nahaew National Park in Loei Province. In order to confirm that the same family and same genus of lichens but different species give different ATR-FTIR spectra. The lichen family Parmeliaceae, genus *Parmotrema*, species: *tinctorum, gardneri, sulphuratum,* and *cristiferum* were collected to record ATR-FTIR spectra.

Results and Discussion: As expected, all infrared spectrum of lichen *Parmotrema tinctorum* collected from different habitats show the same pattern of FTIR spectrum as shown in figure 1. It can be concluded that same species of lichen from elsewhere had the same pattern of FTIR spectra. Whereas same genus *Parmotrema* with different species had different pattern of FTIR spectra as shown in figure 2. It can be concluded that different species of lichen hadthe different pattern of FTIR spectra. Five lichens species, *Relicina abstrusa*, *Relicinopis intertaxta*, *Cladonia submultiformis*, *Usnea undulata* and *Parmotrema tinctorum*, produce different lichen substances as shown in Table 1, of which chemical structure of these substances is shown in Figure 3. FTIR analysis of five lichen species illustrated different pattern of FTIR spectrum as shown in Figure 4 and 5.

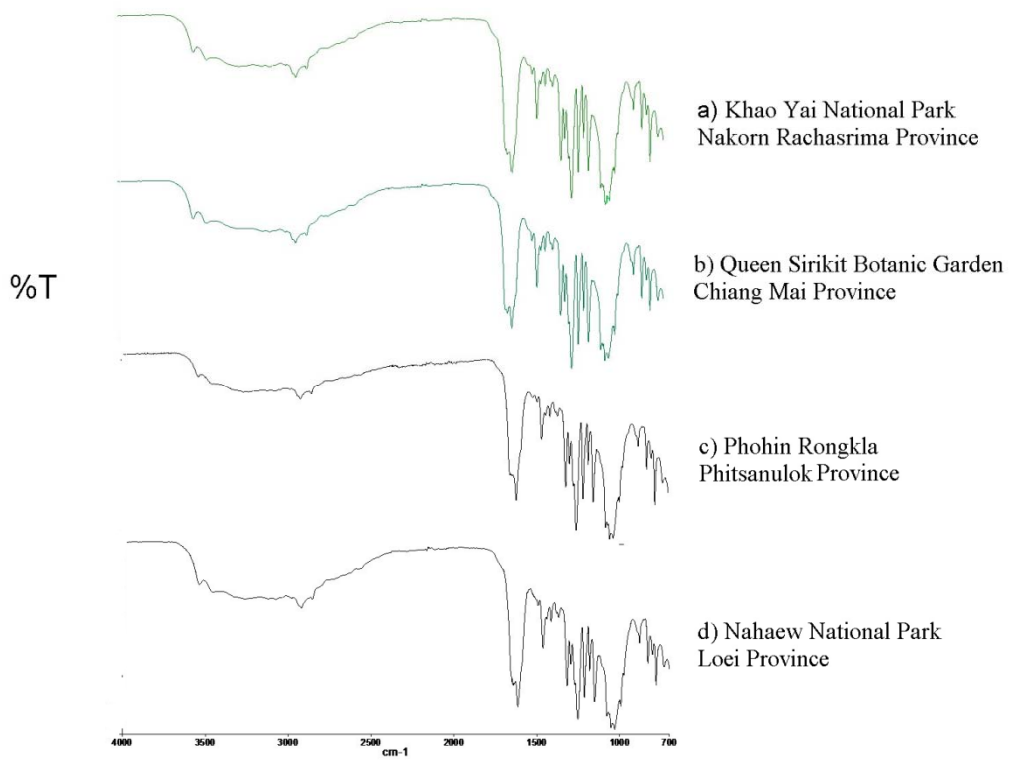


Figure 1.FTIR spectra of the lichen *Parmotrema tinctorum*: a) KhaoYai National Park in Nakorn Rachasrima Province, b) Queen Sirikit Botanic Garden in Chiang Mai Province,c) Phohin Rongkla in Phitsanulok and d) Nahaew National Park in Loei Province.

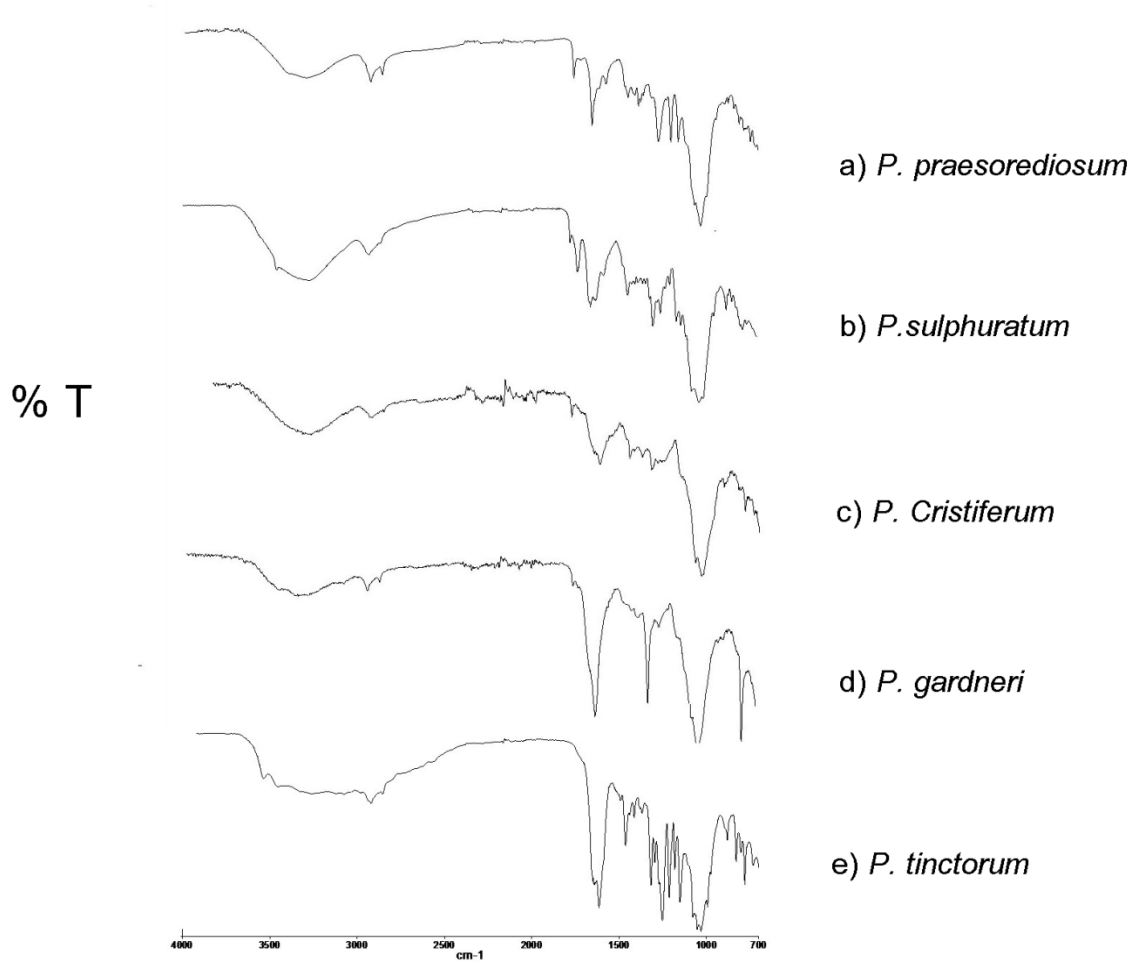
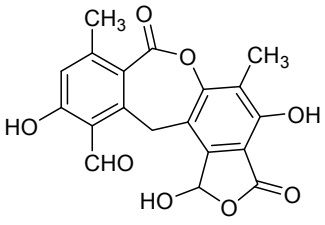


Figure 2.FTIR spectra of the lichens in genus *Parmotrema* : a) *P. praesorediosum*,b) *P. sulphuratum* c) *P. cristiferum*, d) *P.gardneri* and e) *P. tinctorum*

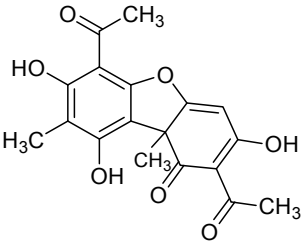
Table 1. The five lichens used for analyzing FTIR spectra and their secondary metabolites, from Khao Yai National Park[5]

Lichen species	Collected from	secondary metabolites
Relicina abstrusa	DEF (dry evergreen forest)	1.Norstictic acid **(1)** 2.Usnic acid **(2)**
Relicinopsis intertaxta	TRF (tropical rain forest)	1.Menegazziaic acid **(3)** 2.Protocetraric acid **(4)** 3.Usnic acid **(2)**
Cladonia submultiformis	LMF(lower montane forest)	1.Fumarprotocetraric acid **(5)** 2.Homosekikaic acid **(6)** 3.Atranorin **(7)**
Usnea undulata	LMF (lower montane forest)	1.Salazinic acid **(8)** 2.Galbinic acid **(9)** 3.Norstictic acid **(1)** 4.Usnic acid **(2)**
Parmotrema tinctorum	DDF (dry dipterocarp forest)	1.Orsellinic acid **(10)** 2. Methyl orsellinate **(11)** 3. Lecarnoric acid **(12)** 4. Atranorin **(7)**



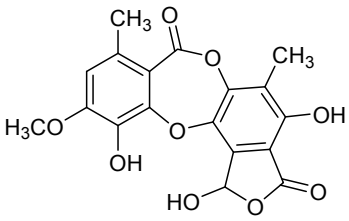
Norstictic acid

(1)



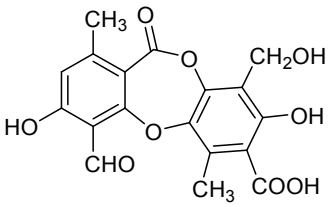
Usnic acid

(2)

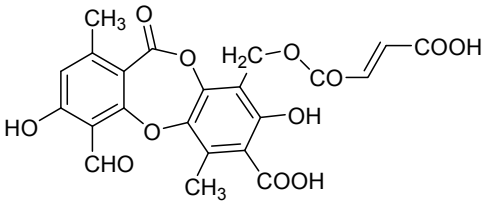


Menegazziacid acid

(3)(4)

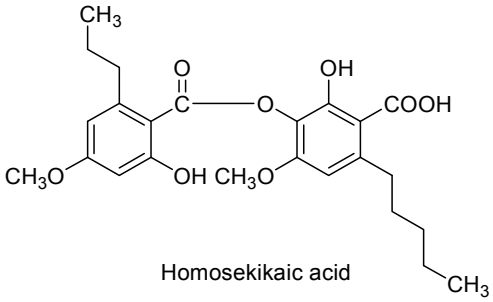


Protocetraric acid



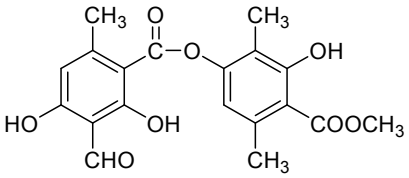
Fumaprotocetraric acid

(5)



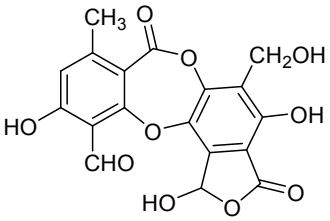
Homosekikaic acid

(6)



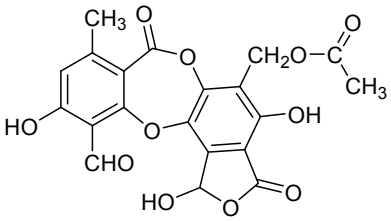
Atranorin

(7)



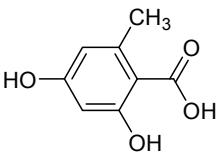
Salazinic acid

(8)

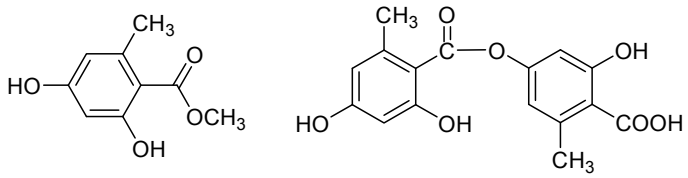


Galbinic acid (α-Acetylsalazinic acid)

(9)(10)



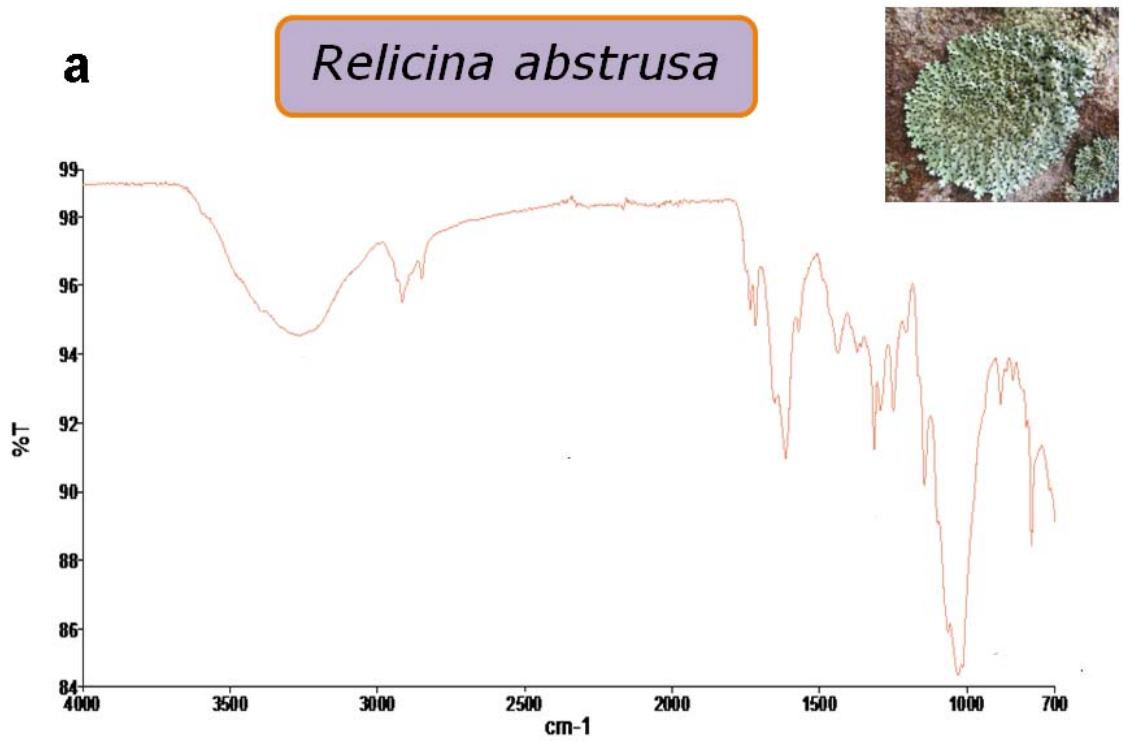
Orsellinic acid

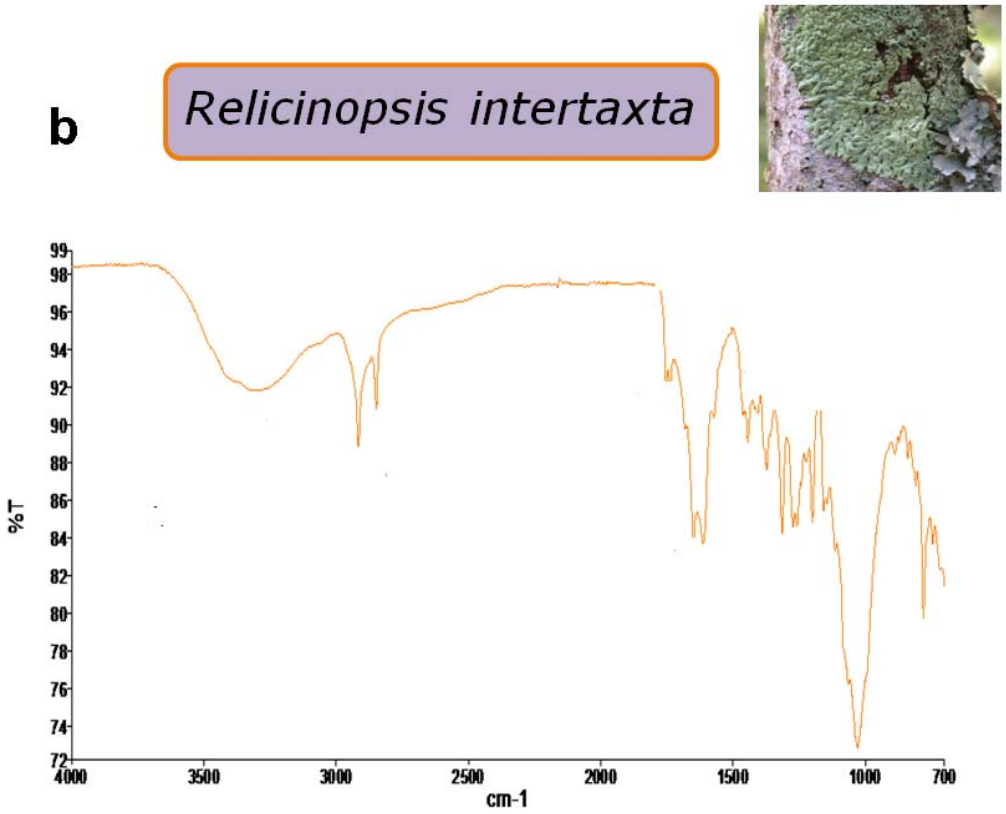


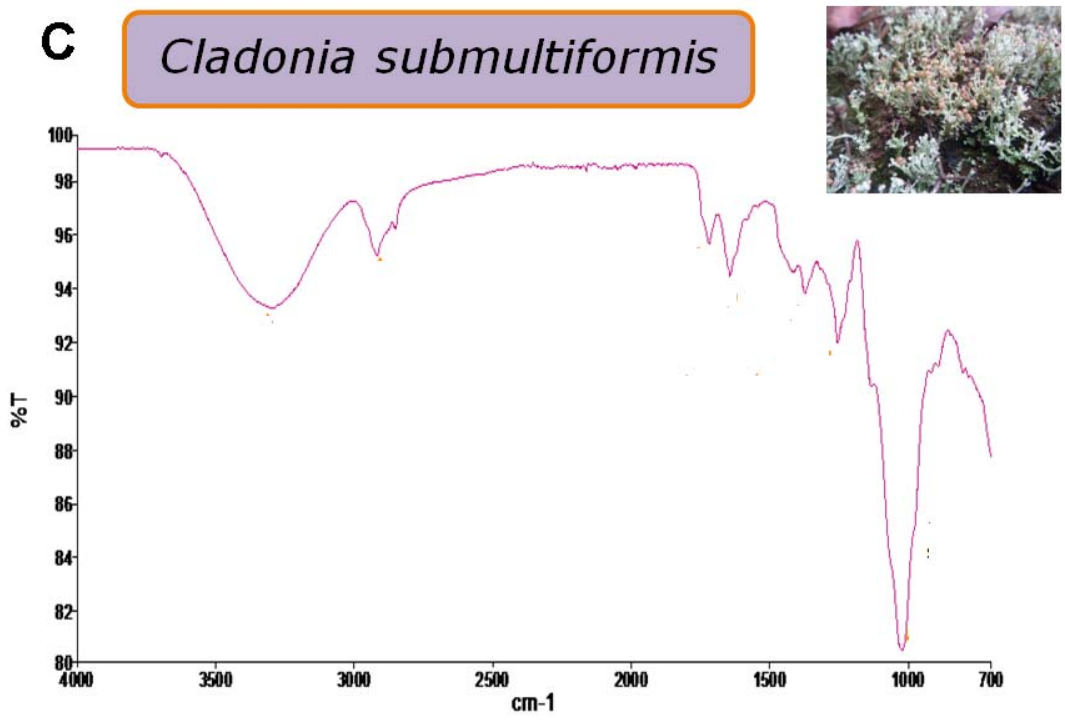
Methyl orsellinate Lecanoric acid

(11)(12)

Figure 3. Chemical structures of secondary metabolites in the study lichens (1)Norstictic acid (2) Usnic acid(3) Menegazziaic acid (4) Protocetraric acid (5) Fumarprotocetraric acid (6) Homosekikaic acid (7) Atranorin (8) Salazinic acid(9) Galbinic acid (10) Orsellinic acid (11) Methyl orsellinate (12) Lecarnoric acid




b
Relicinopsis intertaxta
%T
cm-1


C
Cladonia submultiformis
%T
cm-1

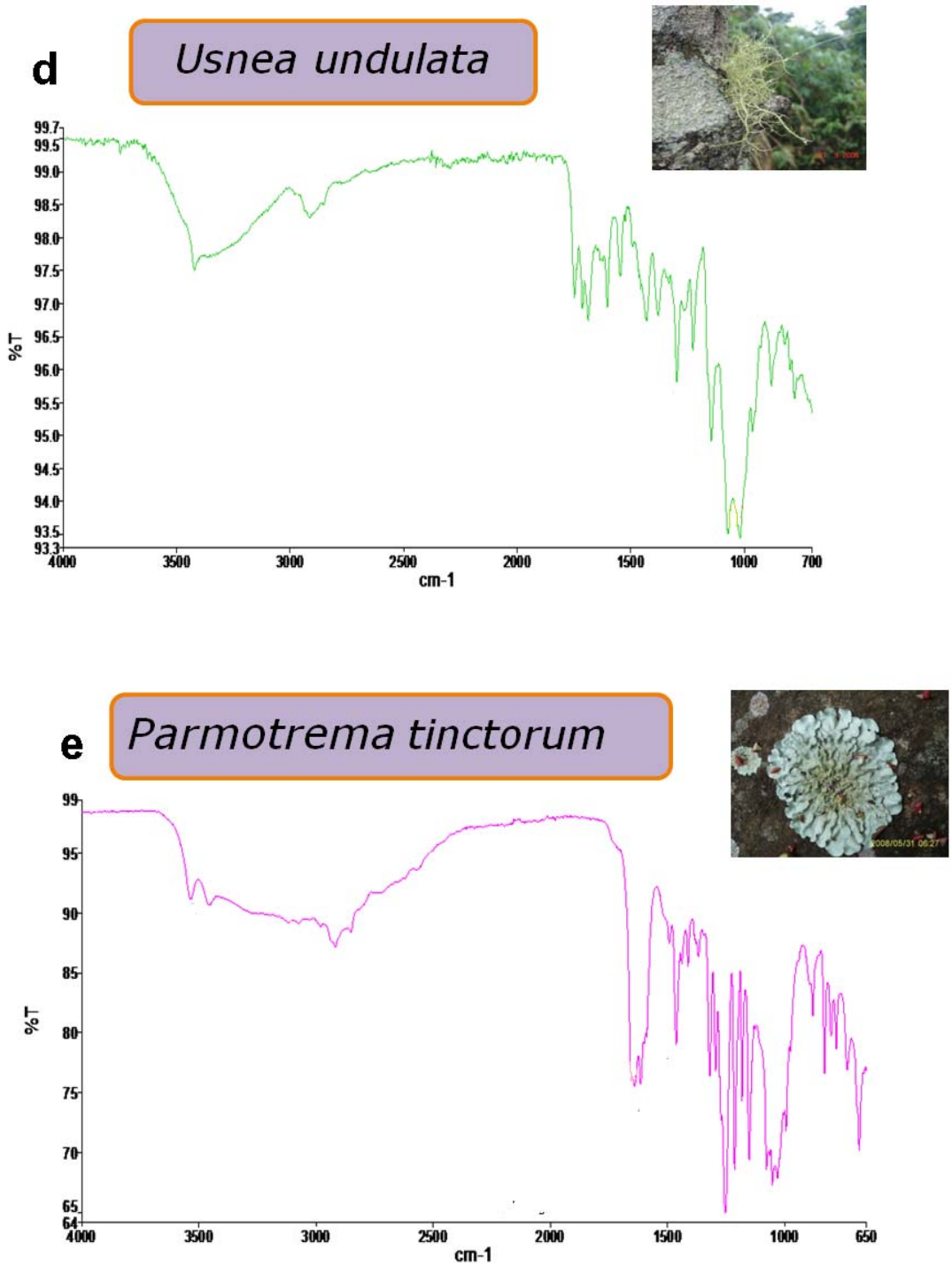


Figure 4.FTIR Spectra of Lichens: a) *Relicina abstrusa*, b) *Relicinopis intertaxta*,c) *Cladonia submultiformis* ,d) *Usnea undulata* and e) *Parmotrema tinctorum* analyzing by using ATR-FTIR Spectrometer.

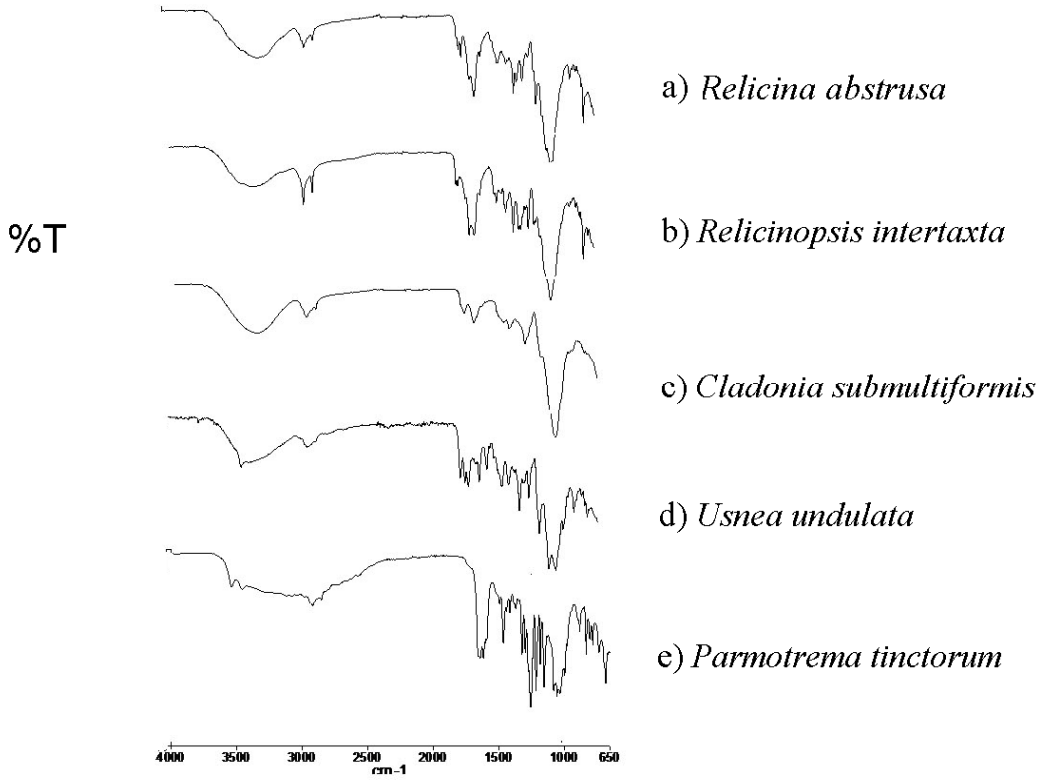


Figure 5. Comparison ofFTIR Spectra of lichen in different genus**:** a) *Relicina abstrusa*,b) *Relicinopis intertaxta*,c) *Cladonia submultiformis* ,d) *Usnea undulata*and e) *Parmotrema tinctorum*

Lichen species can well be distinguished by their secondary metabolites, which are often present in considerable quantities. Lichen substance play an important role in the taxonomy known as chemotaxonomy. Lichens product is one the main criteria used to identify lichen, especially those that are difficult to recognize morphologically. The FTIR spectra discloses functional group of chemical substances of lichen. Therefore, it can be used for identification lichen species.

Conclusion:FTIR spectral analysis of five lichen species demonstrated different spectrum.Although each species of lichen had the similar band of functional group, but the pattern of spectrum was completely different. So it is for the first time, possible to identify lichen species by comparing the ATR-FTIR spectrum. In the future, taxonomically known lichens should be analyzed for ATR-FTIR spectrum. These information should be used as references for fast identification of other unknown lichens.

References:

1. Huneck, Yoshimura. SPINGER. 1996;493p
2. Vattem AD, Vaden M, Jamison BY, Martin J. Pharmacol Toxicol. ISSN 1816-496x, DOI10.3923/jpt.2012.
3. Edwards HGM, Newton EM, Wynn-William DD, Lewis-Smith RI. Spectrochimicacta A. 2003;59:2301-2309.

4. Meysurova AF, Khizhnyak SD, Pakhomov PM. J Appl Spectrosc. 2011;78(5):711-718.
5. Pohjaroen W. Master Thesis Ramkhamhaeng University, Bangkok. 2012.

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