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PHYTOCHEMICAL INVESTIGATION OF THE LICHEN PARMOTREMA SANCTI-ANGELII (LYNGE) HALE (PARMELIACEAE)

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ABSTRACT

Background: *Lichens are fungal and algal/cyanobacterial symbioses resulting in the production of specific metabolites with a great variety of effects such as antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic and antiproliferative. So far, lichens have been used in folk medicine in many countries. Objectives: As apart of searching bioactive compounds from lichens, a phytochemical investigation was conducted on a foliose lichen, Parmotrema sancti-angelii (Lynge) Hale, collected in Lam Dong province, Vietnam.* ***Methods:*** *Open column silica gel chromatography and preparative TLC techniques were employed to isolate compounds. Their structures were elucidated by HRMS, NMR analysis and compared with those in references.* ***Results:*** *Our present study led to the isolation of four compounds, atranol (1), methyl haematomate (2), orsellinic acid (3) and lecanoric acid (4).* ***Conclusion:*** *From 300 g of lichen P. sancti-angelii, four compounds were isolated and identified by spectroscopic methods and compared with literature data. This study contributed to investigate chemical constituents of lichens in Vietnam which have been ignored.*

Keywords: *Parmeliaceae, Parmotrema, atranol, orsellinic acid.*

I. INTRODUCTION

Lichens are symbiotic organisms, usually composed of a fungal partner (the mycobiont) and one or more photosynthetic partners (the photobiont) which is most often

either a green alga or cyanobacterium [1]. About 1035 secondary metabolites have been isolated from the 18500 lichen species described to date, and many more compounds remain to be characterized. Most of these compounds are polyketides, polyphenols, quinones or terpenoids, presumably of fungal origin and their biological activities remain largely underexplored [2,3]. However, a few have been shown to have antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic and antiproliferative activities [3]. Parmotrema is a large genera in the Parmeliaceae with approximately 350 species of foliose lichens and a high level of diversity in the tropical areas of the world. In Vietnam, investigation on chemical constituents of Parmotrema has not been noticed so far as only few studies have paid attention on it, especially Parmotrema sancti-angelii (Lynge) Hale. Recently, atranorin, lecanoric acid, α-collactolic acid, some monocyclic aromatic compounds and bicyclo compounds were reported in such lichen [4]. In 2016, chemical constituent study on its acetone extract led to the isolation of three compounds, methyl β-orcinolcarboxylate, salazinic acid and atranorin [5]. In this paper, we continue to show the isolation and identification of four compounds from the lichen P. sancti-angelii collected in Lam Dong province, Vietnam.

II. MATERIALS AND METHODS

2.1. Lichen material

Parmotrema sancti-angelii (Lynge) Hale was collected in Lam Dong province, Vietnam on November 2011. The scientific name was identified by Prof. Joël Boustie, Faculty of Pharmacy, University of Rennes 1, France. A voucher specimen (No Par-0913) was deposited in the herbarium of the Department of Chemistry, Can Tho Univeristy of Medicine and Pharmacy, Can Tho City, Vietnam.

2.2. Methods

Experimental

The NMR experiments were performed on a Bruker DMX 300 and 500 MHz spectrometer. HRMS-ESI were carried out on a MICROMASS ZabspecTOF spectrometer for electrospray ionization. Melting points were measured on a Melting Point Meter M5000 Krüss.

Column chromatography was performed on normal phase silica gel (40-63 μm, Keselgel 60, Merck 7667). TLC was performed on Kieselgel 60F254 plates (Merck) and spots were visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol), then heated. All solvents used were purchased from Chemsol, purity ≥ 99.0 %.

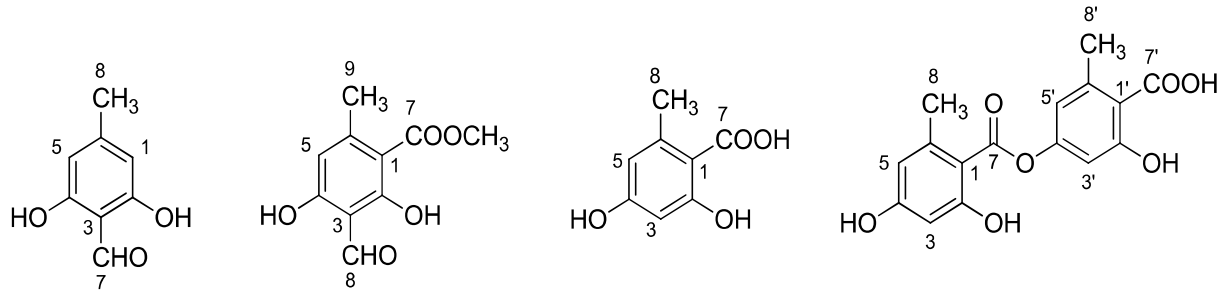
Extraction and isolation

Air-dried crushed thalli of the lichen P. sancti-angelii (300 g) were successively extracted with acetone by maceration 24 h at room temperature (3 times x 2 L) to give acetone extract (60 g). The acetone extract was subjected to silica gel column chromatography and eluted by the solvent system of petroleum ether–ethyl acetate with increasing ethyl acetate ratios to obtain nine fractions from Ac1 to Ac9. The fraction Ac2 was subjected to preparative TLC using n-hexane–chloroform 8:2 as eluent to afford compound 1 (6.5 mg) and compound 2 (4.2 mg). The fraction Ac5 was silica gel

rechromatographed, eluting with n-hexane–ethyl acetate–acetic acid (9:1:0.5) to give two compounds, 3 (11.0 mg) and 4 (7.8 mg).

III. RESULTS

Figure 1: Structures of four isolated compounds from *P. sancti-angelii*



Atranol (**1**): yellow needle (in chloroform); M.p 124°C; ^{1}H NMR (acetone-d_6, 500 MHz): δ_H 10.69 (2H, *s*, 2-O**H**, 4-O**H**), 10.27 (1H, *s*, H-7), 6.26 (2H, *s*, H-1, H-5), 2.23 (3H, *s*, H-8); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 108.4 (C-1), 163.0 (C-2), 109.2 (C-3), 163.0 (C-4), 108.4 (C-5), 151.5 (C-6), 194.1 (C-7), 22.2 (C-8); ESI-HRMS *m/z* 175.0373 $[M+Na]^+$ (calcd. for $C_8H_8O_3Na$).

Methyl haematomate (**2**): colorless powder; M.p 146-147°C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 12.89 (1H, *s*, 2-O**H**), 12.42 (1H, *s*, 4-O**H**), 10.34 (1H, *s*, H-8), 6.29 (1H, *s*, H-5), 3.97 (3H, *s*, 7-OC**H$_3$**, 2.53 (3H, *s*, H-9); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 103.7 (C-1), 168.2 (C-2), 108.3 (C-3), 166.5 (C-4), 112.0 (C-5), 152.3 (C-6), 171.9 (C-7), 193.8 (C-8), 25.2 (C-9), 52.3 (7-OC**H$_3$**); ESI-HRMS *m/z* 233.0430 $[M+Na]^+$ (calcd. for C10H10O5Na).

*Orsellinic acid (**3**):* white needle (in diethyl ether); ^{1}H NMR (MeOD, 500 MHz): δ_H 6.14 (1H, *brs*, H-3), 6.09 (1H, *brs*, H-5), 2.43 (3H, *s*, H-8); ^{13}C NMR (MeOD, 125 MHz): δ_C 105.5 (C-1), 163.7 (C-2), 101.6 (C-3), 166.9 (C-4), 112.3 (C-5), 145.3 (C-6), 175.1 (C-7), 24.3 (C-8).

Lecanoric acid (**4**): pale yellow needle (in acetone); ^{1}H NMR (DMSO, 300 MHz): δ_H 10.31 (1H, *s*, 2-O**H**), 9.99 (1H, *s*, 2'-O**H**), 6.62 (1H, *d*, 2.1, H-3'), 6.59 (1H, *d*, 2.1, H-5'), 6.22 (2H, *s*, H-3, H-5), 2.37 (3H, *s*, 8-C**H$_3$**), 2.35 (3H, *s*, 8'-C**H$_3$**); ^{13}C NMR (DMSO, 75 MHz): δ_C 108.1 (C- 1), 160.0 (C-2), 100.4 (C-3), 161.0 (C-4), 109.8 (C-5), 139.4 (C-6), 166.6 (C-7), 21.37 (C-8), 116.3 (C-1'), 158.8 (C-2'), 107.3 (C-3'), 152.1 (C-4'), 114.6 (C-5'), 140.2 (C-6'), 170.4 (C-7'), 21.4 (C-8').

IV. DISCUSSION

Compound **1** appeared as yellow needles and the ESI-HRMS showed an ion peak at *m/z* 175.0373 $[M+Na]^+$ corresponding the molecular formula of $C_8H_8O_3$. The ^{1}H-NMR spectrum exhibited six singlets for two chelated hydroxyl groups at δ_H 10.69 ppm, a formyl proton at δ_H 10.27 ppm, two aromatic protons at δ_H 6.26 ppm and a methyl group at δ_H 2.23 ppm. The ^{13}C NMR spectrum showed eight carbon signals including a methyl group (δ_C 22.2), two aromatic methines (δ_C 163.0), a formyl group (δ_C 194.1), and four quaternary

aromatic carbon signals (δ_C 108.4, 108.4, 109.2 and 151.5). The obtained spectroscopic data were suitable with the published ones [6,7] therefore compound **1** was atranol.

Compound **2** was a monocyclic aromatic compound and had the molecular formula $C_{10}H_{10}O_5$ as determined by ESI-HRMS. The ^{1}H-NMR spectrum displayed signals of one methyl group at δ_H 2.53 ppm (3H, *s*), one methoxy group at δ_H 3.97 ppm (3H, *s*), one aromatic methine proton at δ_H 6.29 ppm (1H, *s*), one formyl group at δ_H 10.34 ppm (1H, *s*), two phenolic –O**H** at δ_H 12.42 ppm (1H, *s*) and 12.89 ppm (1H, *s*). The ^{13}C-NMR spectrum showed the resonances of 10 carbons including one carbonyl ester group (δ_C 171.9), one methoxy group (δ_C 52.3), one aromatic methyl group (δ_C 25.2), one formyl group (δ_C 193.8), six aromatic carbons. Comparison with previously reported data [4,6] confirmed the structure of **2** as methyl haematomate.

Compound **3** obtained as white needles. The ^{1}H-NMR spectrum displayed signals of one methyl group at δ_H 2.43 ppm, two aromatic methine protons at δ_H 6.14 and 6.09 ppm. The ^{13}C NMR spectrum revealed eight carbon signals, including one carboxyl group, two aromatic methines, one methyl group and four quaternary aromatic carbons. The spectroscopic data were compatible with the published ones in the literatures [4], so compound **3** was orsellinic acid.

Structure of compound **4** was confirmed by the ^{1}H NMR spectrum (DMSO, 300 MHz) as two chelated hydroxyl groups at δ_H 10.31 and 9.99 ppm, four aromatic methine protons at $\delta_{H\ ppm}$ 6.62 (1H, *d*, 2.1), 6.59 (1H, *d*, 2.1) and 6.22 (2H, *s*), two methyl groups at δ_H 2.37 and 2.35 ppm. The ^{13}C NMR spectrum showed signals due to 16 carbons corresponding to two methyls, four aromatic methines and eight quaternary carbons and two carboxyl carbons. Comparison of these data with the ones in literature [4] suggested that compound **4** was lecanoric acid.

VI. CONCLUSIONS

From the lichen *Parmotrema sancti-angelii* (Lynge) Hale collected in Viet Nam, four compounds were isolated and identified as atranol (**1**), methyl haematomate (**2**), orsellinic acid (**3**) and lecanoric acid (**4**). Their structures were confirmed unambiguously by spectroscopic data and compared with those in references. Further studies on its chemical constituents are in progress.

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SOME APPLICATIONS OF CELL-BASED PHARMACOLOGICAL SCREENING FOR MEDICINAL PLANTS IN VIETNAM

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ABSTRACT

Background: *Vietnam has a wide variety of plants traditionally used as treatment for mild to severe diseases. In order to add up scientific evidences for utilization of medicinal plants in disease prevention and treatment, cell based-assay has gradually been applied to speed up drug discovery process in terms of screening a large amount of sample in a short time with reliable results, while investigating drug's action mechanism at molecular and cellular levels.* ***Objectives:*** *This article reviewed the existing cell-based assays that could be applied to screen anticancer, hepatoprotective and renoprotective effects of medicinal plants in Vietnam.* ***Methods:*** *Bioassays used to screen for anticancer activity consist of anti-proliferation test, apoptosis investigation, inflammation and oxidative stress evaluation of in cancer cells. Hepatoprotective and renoprotective effects are evaluated through several assays including cell proliferation, oxidative stress, necrosis, apoptosis activation and Western blotting.* ***Results:*** *In Vietnam, Annona glabra, Ehretia longifolia, Uvaria cordata, Stephania glabra, Morus alba, Paramignya trimera… have been studied for their anticancer effect. Some medicinal plants that have hepatoprotective effects are Eclipta alba, Phyllanthus amarus, Ocimum sanctum whole plant, Andrographis peniculata, Houtyna cordata ariel part, Curcuma longa rhizome, Taxaracum officinale, Glycyrrhiza glabra root, Moringa oleifera, Camelia sinensis, Cynara scolymus leaves… However, there are few researches on screening the renoprotective effect of medicinal plants.* ***Conclusions:*** *Because the rate of hepatic or renal diseases and cancer has increased in recent years in Vietnam, cell-based screening should be applied in order to best utilize the rich sources of medicinal plants.*

Keywords: *anticancer, cell-based screening, hepatoprotective, medicinal plant, renoprotective.*