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Der Pharma Chemica, 2015, 7(2):43-45
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Chemical constituents from a Philippine mangrove endophytic fungi *Phyllosticta* sp.

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ABSTRACT

*Chromatographic purification on the crude EtOAc extract of Phyllosticta sp., an endophytic fungi identified from Philippine mangroves, led to the isolation of tyrosol C (**1**), cytosporone B (**2**), dothiorelone A (**3**), and dothiorelone C (**4**). This represents the pioneering work on the chemical constituents of Phyllosticta sp.*

Keywords: Phyllosticta, tyrosol C, cytosporone B, dothiorelone A, dothiorelone C

INTRODUCTION

The genus *Phyllosticta* (teleomorph: *Guignardia* Viala & Ravaz) was first introduced by Persoon in 1818 *with Phyllosticta convallariae* as the designated type species [1]. Species under this genus are known to cause diseases in many plants resulting in large economic losses worldwide. For example, the citrus black spot caused by *Phyllosticta citricarpa* resulted in large losses to farmers in Africa, Asia, South America and Australia [2,3]. *Phyllosticta musarum* was also known as a causative agent of freckle disease in banana [4]. Other plant pathogens reported under this genus were *Phyllosticta citriasiana* from China, Thailand and Vietnam [5] and *Phyllosticta citrichinaensis* from China [6]. However, *Phyllosticta* species are also known to exist as fungal endophytes. *Guignardia endophyllicola (*anamorph: *Phyllosticta capitalensis)* was found to exhibit an extensive host range, being isolated as an endophyte in many plants from the Kyoto Herbal Garden, specially in the leaves of monocotyledonous plants such as *Arundina chinensis* [7]. *Phyllosticta* was also one of the most commonly isolated fungal endophytes in shrubby medicinal plants in Southern India [8]. As endophytes, these *Phyllosticta* species were not observed to cause any disease in plants. Interestingly, other species of *Phyllosticta* were reported to have biological activities, e.g. *P. citricarpa* isolated from the leaves of *Citrus medica* was reported to be a source of taxol, a potent anticancer agent [9]. The crude extracts of *P. capitalensis*, *P. citriasiana*, and *P. cordylinophila* were found to have antimicrobial effect on *E. coli*, *B. cereus* and *P. aeruginosa* [10]. In this study, we report the secondary metabolites of a potentially new species of *Phyllosticta* isolated from Philippine mangroves.

MATERIALS AND METHODS

General experimental procedure

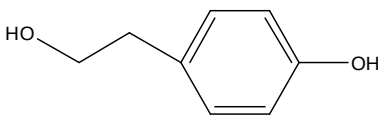
The NMR data (^{1}H and ^{13}C) were recorded in deuterated $CDCl_3$ on a Bruker Avance ARX 500 spectrometer. HPLC analysis was performed with a Dionex P580 system coupled to a photodiode array detector (UVD340s). The UV detection was set at 235, 254, 280, and 340 nm. The separation column (125 mm L x 4 mm ID) was prefilled with Eurospher-10 C18 (Knauer, Germany). Semi-preparative HPLC was performed on Lachrom-Merck Hitachi HPLC system (Pump L7100 and UV detector L7400) and a Eurospher 100-10 C18 column (300 mm 8 mm) (Knauer, Germany). Column chromatography was performed using Merck Silica gel 60 M (0.04–0.063 mm) as stationary phase. Thin layer chromatography (TLC) was performed on pre-coated Silica Gel 60 F254 plates and anisaldehyde as the visualizing agent.

Isolation of the secondary metabolites

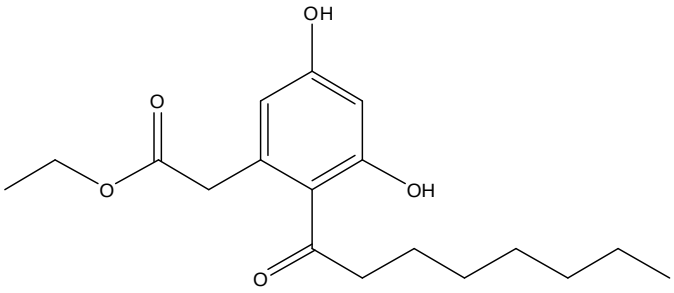
The crude EtOAc extract of *Phyllosticta* sp. was subjected to column chromatography using hexane/EtOAc (7:3, 1:1) and neat EtOAc as solvent systems. Five pooled fractions were obtained. Fraction 2 was purified using semi-preparative HPLC (MeOH-H2O as mobile phase) to afford tyrosol C (**1**, 2.5 mg) as colorless oil. Fraction 3 was also purified in the same manner to obtain cytosporone B (**2**, 3.2 mg) as light-yellow oil. Dothiorelone A (**3**, 3.1 mg) and C (**4**, 2.2 mg) were obtained from fraction 4 after purification by semi-preparative HPLC using MeOH-H2O as mobile phase.

RESULTS AND DISCUSSION

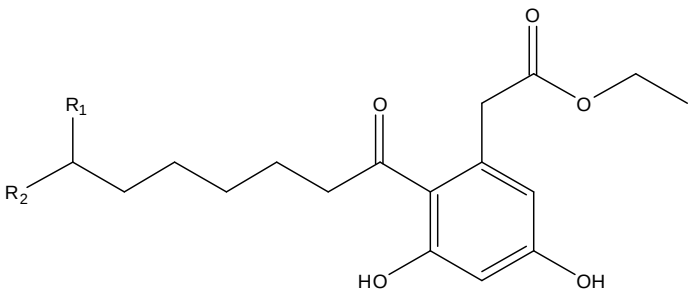
Chromatographic purification of the crude EtOAc extract of *Phyllostica* sp. obtained from Philippine mangrove led to the isolation of compounds **1**-**3**. These were identified as tyrosol C (**1**) [11], cytosporone B (**2**) [12], dothiorelone A (**3**) [13] and dothiorelone C (**4**) [13] based on spectroscopic analyses (1H and 13C NMR and MS) and in comparison with the literature data.



Tyrosol C (**1**)



Cytosporone B (**2**)



Dothiorelone A (**3**) R_1= CH_3, R_2= OH
Dothiorelone C (**4**) R_1= CH_2OH, R_2= H

Fig. 1. Chemical constituents from *Phyllostica* sp.

Previous studies reported that **1** exhibited antioxidant and anticancer activities [14]. Compound 2 was also reported to show a moderate antibacterial activity against *E. coli* [15].

CONCLUSION

This study represents the pioneering work on the identification of the chemical constituents from *Phyllosticta* sp. isolated from Philippine mangroves. Chromatographic purification led to the isolation of tyrosol C (**1**), cytosporone B (2), dothiorelone A (**3**), and dothiorelone C (**4**).

Acknowledgement
M.A. Tan thanks the German Academic Exchange Service (DAAD) for the fellowship grant.

REFERENCES

[1] C.H. Persoon, **1818**. Traitésur les champignons comestibles, contenantl'indication des especesnuisibles; al'histoire des champignons. Belin-Leprieur, Paris.
[2] R.P. Baayen, P.L.M. Bonants, G. Verkley, G.C. Carroll, H.A. Van der Aa, M. De Weerdt, I.R. Van Brouwershaven, G.C. Schutte, W. Maccheroni, C. Glienke de Blanco, J.L. Azeved, *Phytopathology*, **2002**, 92, 464-477.
[3] J.M. Kotzé, **2000**. Black spot. In: Timmer, L.W., Garnsey, S.M., Graham, J.H. (Eds.), Compendium of Citrus Diseases, second ed. The American Phytopathological Society, St. Paul, MN, pp. 23-25.
[4] D.R. Jones, **2000**. Fungal diseases of the foliage. In: Jones DR. (ed) Diseases of Banana, Abaca and Enset. Wallingford, UK, CABI Publishing, CAB International, pp 37–141.
[5] N.F. Wulandari, C. To-Ann, K.D. Hyde, L.M. Duong, J. De Gruyter, J.P. Meffert, J.Z. Groenewald, P.W. Crous, *Fungal Diversity*, **2009**, 34, 23-39.
[6] X. Wang, G. Chen, F. Huang, J. Zhang, K.D. Hyde, H. Li, *Fungal Diversity*, **2011**, 52, 209-224.
[7] I. Okane, S. Lumyong, A. Nakagiri, T. Ito, Mycoscience, 2003, 44, 353-363
[8] B. Naik, J. Shashikala, Y. Krishnamurthy, *Fungal Ecology*, **2008**, 1, 89-93.
[9] R. Kumaran, J. Muthumary, B. Hur, *Journal of Bioscience and Bioengineering*, **2008**, 106, 103-106.
[10] S. Wikee, P. Jaidee, S. Wongkam, E. McKenzie, K. Hyde, E. Chukeatirote, *Mycology: An International Journal on Fungal Biology*, **2013**, 4, 112-117.
[11] S. Christophoridou, P. Dais, *Analytica Chimica Acta*, **2009**, 633, 283-292.
[12] S. Brady, M. Wagenaar, M. Singh, J. Janso, J. Clardy, *Organic Letters*, **2000**; 2, 4043-4046.
[13] U. Sommart, V. Rukachaisirikul, Y. Sukpondma, S. Phongpaichit, N.H. Towatana, P. Graidist, Z. Hajiwangoh, J. Sakayaroj, *Archives of Pharmacal Research*, **2009**, 32(9), 1227-1231.
[14] E.Y. Ahn, Y. Jiang, Y. Zhang, E.I. Son, S. You, S. Kang, J. Park, J. Jung, B. Lee, D. Kim, *Oncology Report*, **2008**; 19, 527-534.
[15] M. Bungihan, M.A. Tan, H. Takayama, T. dela Cruz, M.G. Nonato, *Philippine Science Letters*, **2013**, 6(1), 57-61.