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 (1H and 13C) were recorded in deuterated CDCl3 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 x 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 *Phyllosticta* 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.

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).

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**REFERENCES**


