

Original Research Article

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Vinca alkaloids Produced by Endophytic Fungi Isolated from *Vinca* plants

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ABSTRACT

Keywords

Vinca plants, endophytic fungi, cytotoxicity, bio-active natural products, vinca alkaloids

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Seventeen strains of endophytic fungi have been isolated from three species of *Vinca* plants - *V. minor*, *V. erecta* and *V. rosea* collected from different areas of Uzbekistan. These endophytic fungi represented different genera and were screened for vinca alkaloids production. Out of the seventeen endophytic fungal cultures screened, extracts of 5 strains contain compounds of alkaloid nature (vinblastine and vincristine).

Introduction

Endophytic fungi are recognized as one of the richest sources of natural products displaying a broad spectrum of biological activities. The reported natural products from endophytes includes antibiotics, antipathogens, immunosuppressants, anticancer compounds, antioxidant agents and other biologically active substances (Aly *et al.*, 2008; Guo *et al.*, 2008; Gutierrez *et al.*, 2012; Hazalin *et al.*, 2009; Padhi *et al.*, 2013; Pimentel *et al.*, 2011; Wang *et al.*, 2014; Zhang *et al.*, 2006). The phytochemistry of endophytic microbes continues to increase in significance and there 400 natural products are known to date, of which most have novel structures and/or useful biological activities, from 128 plant-

associated microorganisms. Over the last ten years more than 100 compounds with significant cytotoxic activity including camptothecin, diosgenin, hypericin, paclitaxel, podophyllotoxin, vincristin, which have pharmaceutical importance have been obtained from endophytic fungi of various plants (Joseph and Priya, 2011). Some endophytes produce certain phytochemicals, originally characteristic of the host, due to a genetic recombination of the endophyte with the host that occurred in evolutionary time. Several endophytes producing plant metabolites isolated from *Vinca* (family *Apoceaceae*) plants. For example, vinca alkaloid vincamine, known under the trade name oksibral, originally received from *Vinca minor* (Yin and Sun, 2011). This compound

has been detected in endophytes isolated from leaves of *V. minor* growing in Iran (Farahanikiaa *et al.*, 2011). Vincristine, also known as leurocristine, is a vinca alkaloid originally isolated from endophyte *Fusarium oxysporum* inhabiting the leaves of periwinkle *Catharanthus roseus* (syn. *Vinca rosea*) (Kumar *et al.*, 2013).

From *V. minor*, *V. erecta* and *V. rosea* (syn. *Catharhantus roseus*) grown in Uzbekistan we isolated 17 strains of fungal endophytes and 5 of which showed pronounced cytotoxic activity against Hep-2, HBL-100 and HeLa cell lines (Abdulmyanova *et al.*, 2015; Abdulmyanova *et al.*, 2016). Phytochemical studies of *Vinca* plants are quite well developed, and their medical use, in particular, *V. rosea* and *V. minor* due to antitumor, hypoglycemic, hypotensive, cerebral effects of their metabolites (Kharwar *et al.*, 2005; Singh *et al.*, 2001). Since microbial fermentation has several advantages over the plant raw materials use for obtaining of medicinal preparations and substances, study of cytotoxic compounds from endophytic fungi of *Vinca* plants can create an alternative way for their production. In this context, the aim of the present work was the study of the nature of secondary metabolites produced by endophytic fungi associated with *Vinca* plants.

Materials and Methods

Study area and material sampling

V. minor, *V. erecta* and *V. rosea* plants were collected in March 2016 and 2017 on the territory of Tashkent city neighborhoods (Uzbekistan). Plant samples were identified and stored in a herbarium.

Isolation of endophytic fungi

Endophytic fungi were isolated by the method as described previously by Hazalin *et al.*,

(Hazalin *et al.*, 2009). Roots, stems and leaves were respectively washed in tap water, sterilized in 70% ethanol for 1 min followed by 0.1% HgCl₂ for 7 min, rinsed three times in de-ionized water, cut into segments approximately 5 mm in diameter and placed in 90 mm Petri dishes containing Czapek-Dox agarized medium with 50 mg/ml chlortetracycline and 250 mg/ml streptomycin sulfate to inhibit bacterial growth.

The plates were incubated for 7-14 days at 28 °C. Different mycelia growing out of the segments were sub-cultured and individually maintained on antibiotics-free Czapek-Dox-agar medium. Colony morphology and growth and spore formation of the isolates were then studied on Potato-Dextrose-agar medium.

Endophytic fungi identification

Isolated strains were identified by classical methods on the basis of morphology using pertinent monographs (Litvinov, 1967). Isolated strains were deposited at the Institute of Microbiology of the Uzbekistan Academy of Sciences where they were maintained at +4 °C.

Fermentation

To accumulate biomass for further extraction and determination of biological activity, endophytes were grown by submerged fermentation in 500 ml flasks containing 100 ml of Chapek-Dox liquid medium for 5 days at 28 °C.

Results and Discussion

A total of 17 morphologically distinct fungal isolates were isolated and identified from three species of *Vinca* plants - *V. minor*, *V. erecta* and *V. rosea* obtained from different areas of Uzbekistan. It was found that extracts of 5 strains contain compounds of alkaloid nature.

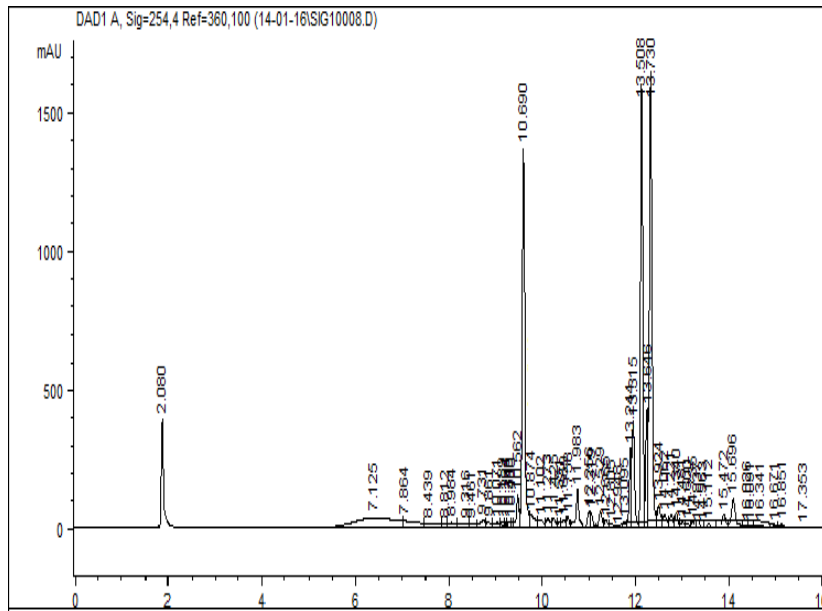
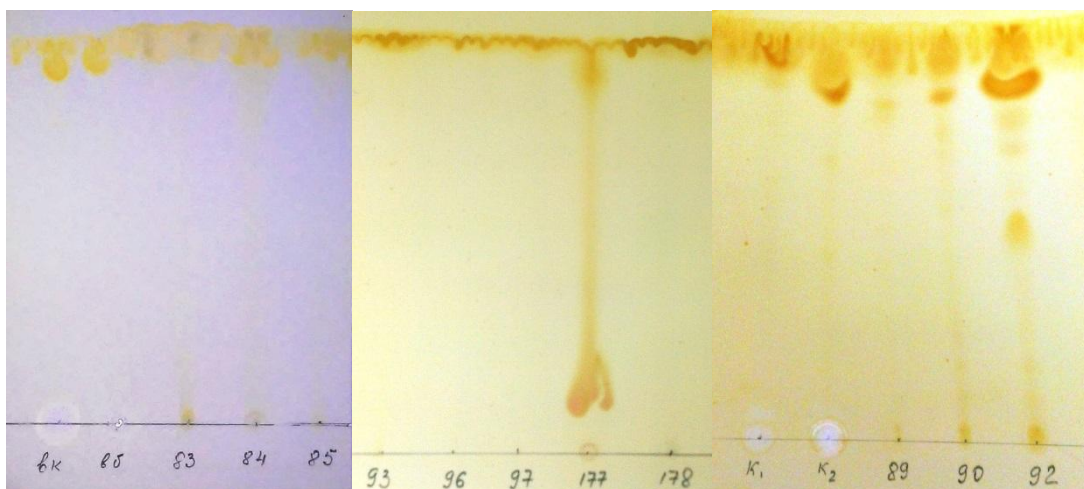


Fig.1 TLC analysis of extracts of endophytic fungi isolated from *Vinca* plants



Thin-layer chromatography of these extracts determined the presence of compounds corresponding to vinblastin and vincristine in extracts of two strains *Penicillium sp.-VE89L* and *A. terreus-VE90R*, and the presence of compound corresponding to vinblastin in extracts of three strains identified as *Alternaria sp.-VM84L*, *Cladosporium sp.-VE92L* and *A. amstelodami-VR177L* (Fig. 1).

However, comparative analysis of chromatograms of the total extracts of endophytic fungi and commercial products cytocristine (vincristine) and cytoblastine (vinblastine) by HPLC showed the presence of peaks which coincides with the time of release in extracts of all studied strains: *Penicillium sp.-VE89L*, *A. terreus-VE90R*, *Cladosporium sp.-VE92L*, *Alternaria sp.-VM84L* and *A. amstelodami-VR177L*.

In quantitative terms, the highest content of vincristine is defined in the extract of endophyte *Penicillium sp.-VE89L* isolated from leaves of *V. erecta* (Fig.2).

In previous studies we have shown that extracts of endophytic fungi *Alternaria sp.-VM84L*, *Penicillium sp.-VE89L*, *A. terreus-VE90R*, *Cladosporium sp.-VE92L*,

A.amstelodami-VR177L as well as *Penicillium sp.-VM86S*, *Penicillium sp.-VE93R*, *Alternaria tenuis-VE98L* and other have cytotoxic activities. Pronounced growth inhibition was showed by incubation of extracts with cultured cells of HBL-100.

Presented in this report, the results indicate that the cytotoxic effect of the extracts may be due to the presence of vinblastine and vincristine.

The production of these compounds by endophytes of *Vinca* plants, which grows on the territory of Uzbekistan, can serve as a basis for the development of study of these endophytic fungi as source of new anticancer drugs.

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