Larvicidal, Antimycobacterial and Antifungal Compounds from the Bark of the Peruvian Plant Swartzia polyphylla DC

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The 95% ethanol extract of the bark of Swartzia polyphylla DC (Fabaceae) possesses important larvicidal, antimycobacterial and antifungal activity in vitro. Bioassay-guided studies performed on the crude ethanol extract afforded T-cadinol as the larvicidal and anti-Mycobacterium tuberculosis principle, while the antifungal activity of the extract is due to the presence of the flavonoids biochanin A and dihydrobiochanin A.

Key words: antimycobacterial; antifungal; biochanin A; dihydrobiochanin A; larvicidal; Swartzia polyphylla

Perú is a country with a large number of medicinal plants, many of which are used for the treatment of infectious diseases,1) although only few studies have been conducted to prove their efficacy and safety.2—3) Due to the emergence of micro-organism resistance to the common antibiotics4) and its worldwide impact on health, our research aims to identify new natural products that may lead to the discovery of new antibacterial agents with higher efficiency and lower toxicity.

As part of our continuing work on bioactive compounds from Peruvian medicinal plants,5—7) the in vitro antimycobacterial activity of 102 ethanol extracts from 84 plants—used traditionally in Perú for the treatment of inflammatory or infectious disorders—was screened using a tetrazolium microplate assay (TEMA).8) In a separate screening, the antifungal and larvicidal activities of over 100 plants, including those previously tested for antimycobacterial activity, were bio-assayed.

As a result of these screenings, Swartzia polyphylla DC (Fabaceae) was found to exhibit powerful antimycobacterial action against the sensitive H37Rv and multidrug-resistant Mycobacterium tuberculosis. It also exhibited the in vitro growth of the dermatophyte Trichophyton mentagrophytes, and was active against the larvae of the mosquito Culex quinquefasciatus. We are now pleased to report the isolation of the larvicidal, antimycobacterial and antifungal principles present in the crude extract of Swartzia polyphylla.

A solvent-partition of the 95% ethanol extract showed that the larvicidal and antimycobacterial activities were concentrated in the hexane fraction, while the 90% methanol fraction was active only in the antifungal assay. The hexane fraction (28 g) was chromatographed on a silica gel column using a hexane–chloroform–methanol gradient. Each fraction (F1—F7) was evaluated for larvicidal and antimycobacterial activity in vitro. The most active fraction (F6, 8.7 g) was purified by column chromatography using a hexane–dichloromethane–ethanol gradient and then by MPLC (Lobar Lichroprep silica gel RP-8, 40—63 μm, 310×25 mm, Merck) with acetonitrile–methanol–water (3 : 2 : 2) yielding the most active fraction F-6-4-2 (51.4 mg). This fraction was finally purified by HPLC (Waters Nova-pak H R silica 6 mm, 3.9×300 mm, Waters Model 600E with Waters 2996 PDA detector) using hexane–chloroform gradient (0 to 70%) to obtain T-cadinol (1, 9 mg) (Fig. 1).9,10)

The 90% methanol fraction (25.5 g) was subjected to column chromatography (silica gel, 0.063—0.200 mm) using a hexane–chloroform–methanol gradient. Each fraction (F1—F9) was tested for antifungal activity in vitro. The most active fraction (F3) yielded fraction F3-7-5 (756 mg) after repeated column chromatography using hexane–ethylacetate–methanol as eluent. This fraction was finally purified by MPLC with methanol–water (6 : 4) to afford biochanin A (2, 15 mg) and dihydrobiochanin A (3, 59 mg) (Fig. 1).11)

The bark of S. polyphylla contains various flavonoids and isoflavones, some of them with strong activity against carcinogenic bacteria.11—13) A bioassay-guided isolation of the 95% ethanol extract of S. polyphylla afforded the compound T-cadinol, which showed a moderate anti-Mycobacterium tuberculosis activity (MIC = 50 μg/ml for the sensitive and multidrug-resistant strains) and strong larvicidal activity. T-cadinol, at a concentration of 300 μg/ml, produces 100% mortality of the larvae of C. quinquefasciatus after 1 h exposure. Biochanin A (2) and dihydrobiochanin A (3) are responsible for the antifungal activity present in the ethanol extract of S. polyphylla (Table 1). Both compounds are very active especially against filamentous fungi. The remaining fractions obtained through the bioassay-guided isolation studies were devoid of larvicidal, antimycobacterial or antifungal activities.

Fig. 1. Structures of Compounds 1—3

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Bioassays. Antifungal Activity The yeast Candida albicans ATCC 90028 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, U.S.A.), while Trichophyton mentagrophytes var. interdigitatum HEM 0584 was provided by the Belgian Coordinated Collection of Microorganisms (BCCM/HEM, Brussels, Belgium). Microsporum gypseum IMTAVH 36836 was a clinical isolate obtained from the Laboratorio de Microbiología of the Instituto de Medicina Tropical “Alexander von Humboldt” (IMTAHV, Lima Perú). M. gypseum was isolated from a patient with tinea corporis and was identified by one of us (B.B.) by classical microbiology techniques.14)

The antifungal activities of the extracts or pure compounds were evaluated by means of the agar-well diffusion assay. The assay was carried out according to the method of Hufford et al.15) with some modifications. The media used was Sabouraud Dextrose agar (Difco). Molten agar (20 ml) at 45 °C was aseptically mixed with 1 ml fungal suspension (1×10⁸ CFU/ml) and poured into 100 mm×15 mm sterile Petri dishes. For the preparation of the inocula, colonies of fungi were suspended in sterile saline. The suspensions were adjusted turbidimetrically to 0.5 for C. albicans and by using a hemacytometer cell counting chamber for T. mentagrophytes and M. gypseum. The concentration of the suspensions was corroborated by serial dilution plate counts. Once the agar was hardened, 11 mm wells were bored using a wooden dowel. Control experiments consisted of adding 0.2 ml of solvent controls (95% ethanol and DMSO) or pure compounds (1 mg/ml) were placed into the wells and their mortality was recorded after 1, 3 and 5 days. Solutions of the extract or pure compound were dissolved in 0.2 ml DMSO–ethanol (1 : 1) at different concentrations. The activities were measured as the diameter (mm) of clear zone of growth inhibition. Solvent controls (95% ethanol and DMSO) were included in every experiment as negative controls. Final amounts of isolated compounds (1 mg/ml) were placed into the wells and the plates were incubated at 24–72 h at room temperature. Amphotericin B (1.6 mg/ml) (Sigma) and itraconazol (1.6 mg/ml) (Pfizer) were dissolved in DMSO (Sigma) and served as positive controls. Electrospray mass spectrometry analysis was conducted at the Mass Spectrometry & Proteomics Facility, Ohio State University, Columbus, OH 43210. The spectral data and our analysis compared satisfactorily with those reported in the literature.9,11)

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