

SHORT COMMUNICATION

# Antifungal Activity of *Mahonia aquifolium* Extract and its Major Protoberberine Alkaloids

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The crude extract of *Mahonia aquifolium* (Berberidaceae) stem bark and its components berberine, palmatine and jatrorrhizine were screened for their inhibitory activity against a variety of dermatophytes and two *Candida* species of human origin using the *in vitro* dilution agar plate method. Jatrorrhizine was found to be the most effective against all fungal species tested (MIC ranges from 62.5 to 125 µg/mL), while the crude extract, berberine, and palmatine exhibited only marginal activity (MIC 500 to ≥1000 µg/mL). Dermatophytes were more susceptible to jatrorrhizine than yeasts, and *Scopulariopsis brevicaulis* appeared the least sensitive species to all the compounds tested. The effects of the alkaloids were compared with those of fluconazole and bifonazole for which the MIC ranges were 12.5 to >100 µg/mL. Our results suggest that jatrorrhizine may serve as a leading compound for further studies to develop new antifungal agents with highly potent antifungal activity and low host toxicity. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: *Mahonia aquifolium*; protoberberine alkaloids; antifungal activity; dermatophytes; yeasts.

## INTRODUCTION

Human mycoses, which may be life-threatening in immuno-compromised patients, are not always successfully treated due to ineffectiveness or toxicity of the available drugs (Feresin *et al.*, 2001). The most important antifungal drugs include camphoterin B, with a polyene structure, and the more recently discovered azole derivatives (Goldstein *et al.*, 2000; Lee *et al.*, 2001).

In addition, the emergence of fungal strains resistant to existing antifungal therapeutics is becoming a further problem. Thus, there are still continuing efforts to develop new, more effective and safe antifungal agents.

Medicinal plants may represent a valuable, untapped source of novel antifungal drugs, especially protoberberine alkaloids, which are readily extractable from Chinese and Korean medicinal plants, and have been shown to possess diverse biochemical and pharmacological actions while being nontoxic to man even at high dosages. Their antifungal activity has been demonstrated against some *Candida* species (Park *et al.*, 1999; Sarma *et al.*, 1999).

Previously, we have reported the isolation of four protoberberine, six bisbenzylisoquinoline alkaloids and some polysaccharide components from the stem bark of *M. aquifolium* (Košťálová *et al.*, 1987; 2001). The main alkaloid constituents of *M. aquifolium* are

berberine and jatrorrhizine with minor amounts of palmatine and columbamine. Although berberine is generally considered the physiologically dominant alkaloid (Iwasa *et al.*, 1998), other components of the *Mahonia* extract may also be important.

In the present communication, we report on the antifungal activity of *Mahonia aquifolium* alcoholic extract and its major protoberberine alkaloids, berberine, jatrorrhizine and palmatine, against dermatophytes and some yeast-like fungi.

## MATERIALS AND METHODS

**Plant material.** The plant material (stem bark of *M. aquifolium*) used in this study was collected in October 1999 in the Arboretum Tesárske Mlyňany, Slovakia. Voucher specimens are deposited at the Herbarium of the Faculty of Pharmacy, Comenius University in Bratislava (No. Ma-108/9).

**Extraction and isolation of alkaloids.** *Mahonia aquifolium* crude extract was prepared according to our previous study (Košťálová *et al.*, 1987). Briefly, 300 g of the *Mahonia* stem bark were extracted with 62% ethanol (1:10 w/v, 5 days, room temperature). The extract was filtered at room temperature and concentrated on a rotary evaporator under vacuum at 40 °C. The dried extract was used for the bioassay. The alkaloid content of the total extract was 0.46%, expressed as berberine. Preparation and subsequent fractionation of the parent extract has been described (Košťálová *et al.*, 1987).

Pure alkaloids (obtained as iodides), berberine (m.p. 265 °C), jatrorrhizine (m.p. 212 °C) and palmatine (m.p.

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**Table 1.** Test fungi used (all of human origin)

No.	Species	Strain No.	Isolated from
1	<i>Epidermophyton floccosum</i>	5905	Inguina
2	<i>Trichophyton rubrum</i>	6029	Toe nail
3	<i>T. interdigitale</i>	6254	Interdigital spaces
4	<i>T. violaceum</i>	Col <sup>a</sup>	Neck
5	<i>T. mentagrophytes</i> v. <i>granulosum</i>	4089	Chest
6	<i>T. equinum</i>	Col <sup>a</sup>	Wrist
7	<i>Microsporum canis</i>	5248	Knee
8	<i>M. gypseum</i>	5497	Glutea
9	<i>Scopulariopsis brevicaulis</i>	6450	Toe nail
10	<i>Candida albicans</i>	6737	Axilla
11	<i>C. tropicalis</i>	6618	Tongue

<sup>a</sup> Strain from a laboratory collection, but also of human origin.

225 °C), were isolated from the *M. aquifolium* crude extract according to the method of Košťálová *et al.* (1987) and identified by spectral analysis.

**Control.** Fluconazole and bifonazole, purchased from Sigma Chemical Co. (St. Louis, MO, USA), were used as comparative antifungal drugs and screened under the same experimental conditions.

**Antifungal activity assay.** Antifungal activities of the alkaloid compounds and crude extract of *M. aquifolium* were tested on selected dermatophytes and yeast strains isolated from superficial human lesions. A total of 11 isolates (five *Trichophyton* spp., two *Microsporum* spp., *Epidermophyton floccosum*, *Scopulariopsis brevicaulis* and two *Candida* spp.) identified by conventional procedures were used as test organisms (Table 1).

The minimal inhibitory concentrations (MICs) of the tested compounds were determined by the agar dilution plate method as described previously (Volleková *et al.*, 2001).

For the bioassay, a precisely weighed amount of the concentrated crude extract of *M. aquifolium* and protoberberine alkaloids (crystalline form of berberine, palmatine, and jatrorrhizine) were dissolved in 1:5 diluted dimethylsulphoxide (DMSO) or in sterile distilled water. These stock solutions were diluted with sterile distilled water to give serial two-fold dilutions (working concentrations). They were added to Sabouraud 2% dextrose agar (Pasteur Dg, pH 7.0 adjusted before sterilisation) to reach final concentrations in plates (20 ml of agar in 90-mm Petri dish): 1% (only crude extract) or 0.1% to 0.0031% of extract and each alkaloid compound (i.e. 10 000 or 1000, 500, 250, 125, 62.5 and 31.2 µg/mL). To eliminate the influence of peptones in Sabouraud agar, Yeast Nitrogen Base (BBL) agar (fortified with 1% dextrose, 0.15% of L-asparagine, in phosphate buffer pH 7.0) was also used for testing. Agar plates with fluconazole or bifonazole at concentrations 100 to 3.12 µg/mL and control plates without alkaloids or antifungals were also prepared. The final concentration of DMSO in the assay did not exceed 0.2%.

5 µl of the suspension of each active growing test fungus (density of conidia and hyphae ca 0.5 McFarland was done in sterile distilled water) was inoculated on the surface of testing and control agar plates and incubated for 15 days at 25 ± 1 °C. The growth was verified daily. Two days after the fungi started growth on con-

trol plates the minimal inhibitory concentration (MIC) was determined as the lowest tested concentration of alkaloids or antimycotics that inhibited visible growth of the test fungi. The tests were performed in triplicate.

## RESULTS AND DISCUSSION

The most common superficial mycotic infections of the skin, nails or hair are known to be caused by dermatophytes or yeasts. In the present study, eight species of *Trichophyton*, *Microsporum* and *Epidermophyton*, two *Candida* and one strain of *Scopulariopsis brevicaulis*, all of human origin, were used as test fungi for determination of antifungal properties of the *Mahonia* protoberberine alkaloids. Two azoles were used as a control to verify the test conditions.

The results obtained from the agar plate dilution technique indicated that each alkaloid isolated from *M. aquifolium* exhibited more or less pronounced antifungal potency, affecting both dermatophytes and yeast-like fungi. As the test fungi started to grow on day 2 (yeasts) or 3 to 5 (dermatophytes), the growth after the inoculation was recorded daily. The results and minimal inhibitory concentrations given in Table 2 are those recorded after 6 days of incubation. The value of the MICs did not change from the 6th until the 15th day of incubation when the tests were terminated.

As shown in Table 2, among the alkaloids tested, jatrorrhizine represented the most potent antifungal candidate with MICs ranging from 62.5 to 125 µg/mL for *Trichophyton* and *Microsporum* or from 250 to 500 µg/mL for the two *Candida* spp. However, jatrorrhizine had no activity at 1000 µg/mL against the strain *S. brevicaulis* (Table 2). It should be noticed that this potentially active alkaloid was found in considerably high amounts in the crude extract of *M. aquifolium*.

The crude extract of *M. aquifolium* exhibited weaker activity against all test dermatophytes or yeasts (MICs ≥ 1000 µg/mL), and in particular against *S. brevicaulis* or *M. gypseum*, even at 1% concentration (i.e. 10 000 µg/mL). Interestingly, two protoberberines, berberine and palmatine, showed antifungal activity nearly in the same concentration range as observed for the crude extract of *M. aquifolium*. An exception was *C. tropicalis*: this strain exhibited a good sensitivity to berberine (growth inhibition at 62.5 µg/mL). These results confirm those



larger variety of protoberberine structures are however needed to shed more light into the structure-activity relationships and to acquire an exhaustive spectrum of antifungal activity.

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