

SHORT COMMUNICATION

Antimicrobial Activity of *Mahonia aquifolium* Crude Extract and its Major Isolated Alkaloids

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The crude extract of *Mahonia aquifolium* (Pursh) Nutt. stem bark and its two main protoberberine alkaloids, berberine and jatrorrhizine, were tested for their *in vitro* antimicrobial activity. Twenty strains of coagulase-negative staphylococci and 20 strains of *Propionibacterium acnes* isolated from skin lesions of patients with a severe form of acne, and 20 strains of *Candida* sp. isolated from chronic vulvovaginal candidosises were tested for their susceptibility to crude extract and two isolated alkaloids. The minimum inhibitory concentrations obtained in this study illustrate the varying degrees of antibacterial and antifungal activity of the tested agents. The results indicate a rational basis for the traditional use of *Mahonia aquifolium* for localized skin and mucosal infection therapy, as well as for the possible development of a preparation for supportive therapy of the diseases mentioned above. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: *Mahonia aquifolium*; protoberberine alkaloids; antimicrobial activity.

INTRODUCTION

The genus *Mahonia* comprises several species, of which *Mahonia aquifolium* (Pursh) Nutt. has long been used in traditional medicine for various skin conditions, and berberine, the main alkaloid of this bush, is used in Asian medicine as a drug due to its antimicrobial activity (Kim *et al.*, 2002). Berberine seems to be active against a number of pathogenic and potentially pathogenic microorganisms, including bacteria, fungi, protozoa and viruses, as indicated by several *in vitro* studies (Kaneda *et al.*, 1998; Lampert and Shaffner, 1995). As to the molecular basis of the biological activity of isoquinoline alkaloids, berberine has been reported to be able to interact with DNA by intercalation with AT base pair preferences (Iwasa *et al.*, 2001). This binding affinity may cause inhibition of replicational and/or transcriptional activity of the target cells. Moreover, the crude extract of plants containing berberine and other protoberberines was found to display differential inhibitory effects on both sterol and cell wall chitin biosynthesis in *Candida albicans* (Park *et al.*, 1999). In previous studies of the stem bark of *M. aquifolium* the inhibitory effect of the crude extract and the isolated protoberberine alkaloids – berberine, palmatine and jatrorrhizine – on a variety of dermatophytes and two *Candida* species of human origin have been detected (Vollková *et al.*, 2003).

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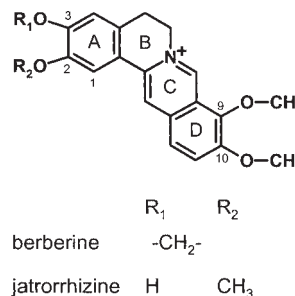


Figure 1. Chemical structure of the protoberberines studied.

The present communication reports on the *in vitro* study of the antimicrobial activity of berberine and jatrorrhizine (Fig. 1) and a crude extract from the stem bark of *Mahonia aquifolium* against 20 strains of coagulase-negative staphylococci (14 strains of *S. epidermidis*, three strains of *S. hominis*, one strain of *S. warneri*, *S. lentus* and *S. hyicus*), 20 strains of *Propionibacterium acnes*, and 20 strains of *Candida* sp. (17 strains of *C. albicans*, two strains of *C. glabrata* and one strain of *C. tropicalis*).

MATERIAL AND METHODS

Plant material. The commercially available air dried and powdered stem bark of *M. aquifolium* (Pursh) Nutt. was obtained from the Arboretum Tesárske Mlyňany, Slovakia. A voucher specimen (no. Ma-112) is kept at the herbarium of the Department of Pharmacognosy and Botany of the Comenius University, Bratislava.

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Isolation of alkaloids. Preparation of the *M. aquifolium* crude extract and isolation of the protoberberine alkaloids was performed using the same procedure as described earlier (Volleková *et al.*, 2003). The chemical structure of the alkaloids tested is shown in Fig. 1. Copies of the original spectral data from alkaloids are obtainable from the author for correspondence.

The tested alkaloids and the dried crude extract of *M. aquifolium* were dissolved in sterile distilled water. The total alkaloid content of the crude extract was measured at 425 nm by spectrophotometry and the results were expressed as the berberine content. The individual concentrations of the tested compounds were 1, 5, 25, 50, 100, 250 and 500 µg/mL for the bacteria, and 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL for the yeasts.

Antimicrobial activity assay. The effect of *Mahonia aquifolium* crude extract and isolated protoberberine alkaloids on bacteria was tested by the broth microdilution test according to the NCCLS M7-A5 (2000) and M11-A-3 (1993) documents. Bacterial inocula were prepared from an overnight culture grown on blood agar in the case of staphylococci, and from a 48 h culture grown anaerobically on VL-agar (Imuna, Slovakia) in the case of propionibacteria. The test was performed in U-shaped microtiter plates. 10 µL volumes of bacterial suspension in physiological saline were added to the tested agents in 100 µL of Mueller-Hinton broth (Oxoid) for staphylococci or Wilkins-Chalgren broth (Oxoid) for propionibacteria. The final concentrations of staphylococci and propionibacteria in the wells were 5×10^5 /mL and 1×10^6 /mL, respectively. Microtiter plates with staphylococci were incubated at 35 °C for 18 h aerobically, and plates with propionibacteria at 35 °C for 48 h anaerobically.

For determining the antifungal activity of the tested agents, a modified broth microdilution test was used according to the NCCLS M27-A1 (2000) document. 100 µL aliquots of yeast cell suspension in physiological saline, prepared from 24 h cultures of yeast strains grown aerobically on Sabouraud agar (BBL, USA), were added to the samples of two-fold concentrated tested agents in a 100 µL volume of Sabouraud liquid broth modified-antibiotic medium 13 (BBL, USA) in the U-shaped microtiter plate wells. The final concentration of the yeast cells in each well was $1-5 \times 10^4$ /mL. Microtiter plates were incubated at 35 °C for 48 h aerobically. Samples without microbes (sterility

control) and samples without tested agents (microbial growth control) were run in each test plate. The individual concentrations were tested in parallel samples. The MICs were read visually after cultivation as the lowest concentrations without a visible microbial growth.

RESULTS AND DISCUSSION

Berberine, jatrorrhizine and a crude extract of *M. aquifolium* showed the strongest activity against the 20 clinical isolates of *Propionibacterium acnes* with minimal inhibitory concentration (MIC) values between 5 and 50 µg/mL (Table 1) and are consistent with those reported by other authors (e.g. Lampert and Shaffner, 1995). When assayed against coagulase-negative staphylococci, berberine seems to be more active than jatrorrhizine (MIC values of 17 tested strains were between 25 and 100 µg/mL for berberine, 100 and 250 µg/mL for the crude extract, and between 100 and 500 µg/mL for jatrorrhizine). However, one strain of *S. epidermidis* and two strains of *S. hominis* were not inhibited even by the highest tested concentration of the investigated agents (500 µg/mL). In fact, different strains within the same species might have a different sensitivity to the tested compounds, probably due to variations of the cell envelope constitution and permeability. Another explanation of this fact may be the increased activity of a multidrug resistance pump, such as NorA, for which berberine and palmatine serve as substrates (Hsieh *et al.*, 1998). However, all 20 staphylococcal strains tested in the study were susceptible to ciprofloxacin. This observation makes the second possible explanation highly improbable. The results reported in Table 2 show the antifungal activity of the compounds tested, though again to varying extents in various strains. The only tested strain of *C. tropicalis* (resistant to nystatin, miconazole and econazole) was most strongly inhibited by all the agents tested, with the MIC of berberine and the crude extract equal to 31.3 µg/mL and jatrorrhizine 125 µg/mL. On the other hand, both tested strains of *C. glabrata* were resistant to even the highest concentrations of the investigated agents (MIC > 500 µg/mL). The most active against the 17 strains of *C. albicans* was jatrorrhizine with MICs between 125 and 250 µg/mL. The MICs of berberine were between 125 and 500 µg/mL, and of the crude extract 500 and >500 µg/mL. Interestingly, our previous

Table 1. Antibacterial effect of *Mahonia aquifolium* crude extract and its major isolated alkaloids berberine and jatrorrhizine on the tested bacterial strains

Species	n	Susceptibility to the tested agent		
		<i>Mahonia aquifolium</i> crude extract MIC (µg/mL)	Berberine MIC (µg/mL)	Jatrorrhizine MIC (µg/mL)
<i>S. epidermidis</i>	14	100 – > 500	25 – > 500	100 – > 500
<i>S. hominis</i>	3	250 – > 500	50 – > 500	500 – > 500
<i>S. warneri</i>	1	100	25	250
<i>S. lentus</i>	1	100	50	250
<i>S. hyicus</i>	1	100	25	250
<i>P. acnes</i>	20	25–50	5–25	25–50

n, the number of tested strains; MIC, minimal inhibitory concentration.

Table 2. Antifungal effect of *Mahonia aquifolium* crude extract and its major isolated alkaloids berberine and jatrorrhizine on the tested strains of *Candida*

Species	n	Susceptibility to the tested agent		
		<i>Mahonia aquifolium</i> crude extract MIC (µg/mL)	Berberine MIC (µg/mL)	Jatrorrhizine MIC (µg/mL)
<i>C.albicans</i>	17	500 – > 500	125 – 500	125 – 250
<i>C.glabrata</i>	2	>500	>500	>500
<i>C.tropicalis</i>	1	32.3	31.3	125

n, the number of tested strains; MIC, minimal inhibitory concentration.

studies suggest, too, that jatrorrhizine may be the most active alkaloid tested and may serve as a lead substance for further studies to obtain more effective agents against the fungal species tested so far (Volleková *et al.*, 2003).

In conclusion, the results suggest that the antimicrobial activity of the crude extract and the tested protoberberines of *M. aquifolium in vitro* is considerably inferior to some antimicrobial drugs used in

medical practice. Further studies are required regarding the overall mode of action of *Mahonia aquifolium* main alkaloids, as well as their *in vivo* activity.

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