Antiviral and Antimicrobial Profiles of Selected Isoquinoline Alkaloids from *Fumaria* and *Corydalis* Species

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In the current study, 33 isoquinoline alkaloids belonging to protopine-, benzylisoquinoline-, benzophenanthridine-, spirobenzylisoquinoline-, phthalideisoquinoline-, aporphine-, protoberberine-, cularine-, and isoquinolone-types as well as 7 derivatives of them obtained from some *Fumaria* and *Corydalis* species growing in Turkey have been evaluated for their *in vitro* antiviral and antimicrobial activities. Both DNA virus *Herpes simplex* (HSV) and RNA virus *Parainfluenza* (PI-3) were employed for antiviral assessment of the compounds using Madine-Darby bovine kidney and Vero cell lines and their maximum non-toxic concentrations (MNTC) and cytopathogenic effects (CPE) were determined using acyclovir and oseltamivir as the references. Antibacterial and antifungal activities of the alkaloids were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* by the microdilution method and compared to ampicilline, ofloxacine, and ketocanazole as the references. The alkaloids did not present any notable antibacterial effect, while they had significant antifungal activity at 8 μg/ml concentration. On the other hand, the alkaloids were found to have selective inhibition against the PI-3 virus ranging between 0.5 and 64 μg/ml as minimum and maximum CPE inhibitory concentrations, whereas they were completely inactive towards HSV.

Key words: Isoquinoline Alkaloids, Antiviral Activity, Antimicrobial Activity

Introduction

Most of the world's cultures have centuries of tradition in the use of crude plant materials to control infectious diseases. Many studies have been conducted on antiviral and antimicrobial activities of such materials, although relatively few studies have been done on pure chemicals. The subject of biological evaluation of compounds from plant species is highly relevant to the identification of lead candidates for drugs.

Alkaloids are bioactive secondary metabolites widely found in nature. A number of isoquinolines, a large and well-known class of alkaloids, was isolated previously in a project which had been initiated to search for alkaloids of the Fumariaceae plant species growing in Turkey (Blasko *et al.*, 1981, 1982; Şener *et al.*, 1983; Şener, 1984, 1985, 1986, 1988, 1989; Şener and Temizer, 1990; Küçükboyaci *et al.*, 1998). This family is represented by 2 genera in the flora of Turkey, namely *Fumaria* L. and *Corydalis* Medik., which are known to produce isoquinoline alkaloids derived from phenylalanine and tyrosine (Davis and Cullen, 1984).

Our focus in the present work was to investigate the antiviral activity of 33 isoquinoline alkaloids and 7 derivatives of them, which are classified as protopine-type [protopine (**1**) and β-allocryptopine (**2**)], benzylisoquinoline-type [(+)-reticuline (**3**) and (+)-norjuziphine (**4**)], benzophenanthridine-type [sanguinarine (**5**), norsanguinarine (**6**), and chelidimerine (**7**)], spirobenzylisoquinoline-type [fumarophycine (**8**), (−)-fumarophycine acetate (**9**), (−)-corpaine (**10**), (±)-sibiricine (**11**), sibiricine acetate (**12**), (±)-dihydrosibiricine (**13**), (+)-fumariline (**14**), (−)-dihydrofumariline (**15**), (+)-parfumine (**16**), parfumine acetate (**17**), and (−)-dihydroparfumine diacetate (**18**)], phthalideisoquinoline-type [α-hydrastine (**19**), (+)-bicuculline (**20**), (−)-bicuculline (**21**), and (−)-adlumidine

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(**22**)], aporphine-type [(+)-bulbocapnine (**23**) and (+)-isoboldine (**24**)], protoberberine-type [berberine (**25**), (−)-stylopine (**26**), (−)-canadine (**27**), (−)-sinactine (**28**), (−)-ophiocarpine (**29**), ophiocarpine-*N*-oxide (**30**), corydalmine (**31**), palmatine (**32**), (±)-corydalidzine (**33**), dehydrocorydaline (**34**), and dehydrocavidine (**35**)], cularine-type [(+)-cularicine (**36**), oxocularine (**37**), oxosarcocapnine (**38**), and oxosarcocapnidine (**39**)], and isoquinolone-type [corydaldine (**40**)], against *Herpes simplex virus* (HSV) and *Parainfluenza-3 virus* (PI-3) using Madine-Darby bovine kidney (MDBK) and Vero cell lines. Moreover, the alkaloids were also tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Bacillus subtilis*, as well as the fungus *Candida albicans* by the microdilution method for their antibacterial and antifungal activities.

Materials and Methods

Tested alkaloids

The extraction, isolation, and purification procedures were beforehand described in our previous papers (Blasko *et al.*, 1981, 1982; Şener *et al.*, 1983; Şener, 1984, 1985, 1986, 1988; Küçükboyaci *et al.*, 1998). The isoquinoline alkaloids (**1**–**40**) tested were obtained from the respective species among fourteen *Fumaria* [*F. asepala* Boiss., *F. bastardii*, *F. capreolata* L., *F. cilicica* Hausskn., *F. densiflora* DC, *F. gaillardotii* Boiss., *F. judaica* Boiss., *F. kralikii* Jordan, *F. macrocarpa* Parlatore, *F. microcarpa* Boiss., *F. officinalis* L., *F. parviflora* Lam., *F. petteri* Reichb. ssp. *thuretii* (Boiss.) Pugsley, *F. vaillantii*] and six *Corydalis* species [*C. caucasica* DC, *C. rutifolia* (Sibth and Sm.) DC ssp. *erdelii* (Zucc.) Cullen and Davis, *C. rutifolia* (Sibth and Sm.) DC ssp. *kurdica* Cullen and Davis, *C. solida* (L.) Swartz ssp. *brachyloba* (Boiss.) Cullen and Davis, *C. solida* (L.) Swartz ssp. *solida*, and *C. solida* ssp. *Tauricola*] growing in Turkey.

Microorganisms

Standard strains of bacteria, namely *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 7002), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (RSKK 574), and *Acinetobacter baumannii* (RSKK 02026; Culture Collection of Refik Saydam Central Hygiene Institute, Ankara, Turkey), for determination of antibacterial activity and standard strain of the yeast-like fungus *Candida albicans* (ATCC 10231) for evaluation of the antifungal activity were employed. In order to determine the antiviral activity, *Herpes simplex virus* (HSV) and *Parainfluenza*-3 *virus* (PI-3) obtained from the Department of Virology, Faculty of Veterinary, Ankara University (Turkey) were employed.

Antibacterial and antifungal activities

The isoquinoline alkaloids tested (**1**–**40**) were dissolved in ethanol/hexane (1:1, v/v) by using 1% Tween 80 solution at a final concentration of 1024 μg/ml, sterilized by filtration using a 0.22 μm Millipore filter (MA, USA), and used as the stock solutions. Standard antibacterial powders of ampicilline (AMP; Fako Pharmaceutical Company, Istanbul, Turkey) and ofloxacine (OFX; Hoechst Marion Roussel, Istanbul, Turkey) along with standard antifungal powders of ketoconazole (KET; Bilim Pharmaceutical Company, Istanbul, Turkey) were obtained from the respective manufacturers and dissolved in phosphate buffer solution (AMP, pH 8.0, 0.1 M), dimethylsulphoxide (DMSO) (KET), and water (OFX). The stock solutions of the agents were prepared in medium according to the NCCLS recommendations (NCCLS, 1996).

The microdilution method was employed for antibacterial and antifungal activity tests. Media were placed into each well of the microplate. Compound solutions at 1024 μg/ml were added into the first raw of the microplates and 2-fold dilutions of the compounds (512–0.25 μg/ml) were made by dispensing the solutions to the remaining wells. 10 μl of culture suspensions were inoculated into all the wells. The sealed microplates were incubated at 35 °C for 24 h and 48 h in a humid chamber. The lowest concentrations of the compounds that completely inhibit macroscopic growth and minimum inhibitory concentrations (MICs) were determined (NCCLS, 2002; Özçelik *et al.*, 2005).

Mueller-Hinton Broth (Difco) and Mueller-Hinton Agar (Oxoid) were applied for growing and diluting the bacteria. As for growing and diluting of the fungus, Sabouraud liquid medium (Oxoid)

and Sabouraud dextrose agar (SDA) (Oxoid) were applied. The medium RPMI-1640 with L-glutamine was buffered to pH 7 with 3-(*N*-morpholino)-propanesulfonic acid (MOPS). Prior to the tests, strains of bacteria and fungus were cultured on media and passaged at least twice to ensure purity and viability at 35 °C for 24 to 48 h. Culture suspensions were prepared according to NCCLS M27-A (Özçelik *et al.*, 2004). The bacterial suspensions used for inoculation were prepared at 10^5 cfu/ml by diluting fresh cultures at McFarland 0.5 density (10^8 cfu/ml). The fungus suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of 2.5×10^3 cfu/ml (NCCLS, 1996).

Antiviral activity and cytotoxicity evaluation

Media (EMEM) were placed into each well of 96-well microplates (Greiner®, Essen, Germany). Stock solutions of the alkaloids were added into the first raw of the microplates and 2-fold dilutions of the compounds (512–0.25 μg/ml), which were prepared according to Log_2 on the microplates, were made by dispensing the solutions to the remaining wells. Acyclovir (Biofarma, Istanbul, Turkey) and oseltamivir (Roche, Istanbul, Turkey) were used as the references. Strains of HSV and PI-3 titers were calculated as $TCID_{50}$ and inoculated into all the wells (Frey and Liess, 1971). The sealed microplates were incubated in 5% CO_2 at 37 °C for 2 h to detect the possible antiviral activities of the samples. Following incubation, 50 μl of the cell suspension of 300.000 cells/ml, which were prepared in EMEM together with 5% fetal bovine serum, were put in each well and the plates were incubated in 5% CO_2 at 37 °C for 48 h. After that, the cells were evaluated using a cell culture microscope, comparing with treated-untreated control cultures and with acyclovir and oseltamivir. Consequently, maximum cytopathogenic effect (CPE) concentrations as the indicator of antiviral activities of the compounds were determined (Özçelik *et al.*, 2005).

The maximum non-toxic concentration (MNTC) of each compound was determined by the method described beforehand based on cellular morphologic alteration (Özçelik *et al.*, 2005). Several concentrations of each sample were placed in contact with confluent cell monolayers and incubated in 5% CO_2 at 37 °C for 48 h. MNTC value for each compound was determined by comparing treated and untreated (control) cultures.

Results and Discussion

The results of the antibacterial and antifungal activities evaluation of the tested alkaloids are presented in Table I. For determining the antibacterial effect, the alkaloids were tested against five Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, and *A. baumannii*) and two Gram-positive bacteria (*S. aureus* and *B. subtilis*) using AMP and OFX as the reference compounds.

All types of alkaloids appeared to be more active against Gram-negative bacteria than Gram-positive ones. Most of the alkaloids including protopine, β-allocryptopine, chelidimerine, fumarophycine, (±)-sibiricine, sibiricine acetate, (±)-dihydrosibiricine, parfumine acetate, α-hydrastine, (+)-bulbocapnine, berberine, (–)-canadine, (–)-ophiocarpine, ophiocarpine-*N*-oxide, corydalmine, oxosarcocapnidine, and corydaldine showed significant inhibition towards *K. pneumoniae* and *A. baumannii*, in particular, better than the rest of the Gram-negative bacteria, at 8 μg/ml concentration as compared to AMP (2 μg/ml). All of the alkaloids, regardless of their structural differences, inhibited *E. coli* and *P. mirabilis* with a MIC of 32 μg/ml, while they inhibited *S. aureus* at 64 μg/ml.

Interestingly, the alkaloids, which were found to inhibit *K. pneumoniae* and *A. baumannii* at 8 μg/ml, had also remarkable activity against *C. albicans* (4 μg/ml) as compared to KET (2 μg/ml), while the notable occurrence of antifungal activity for the rest of the alkaloids was observed at 8 μg/ml concentration.

The tested isoquinolines were observed to possess a selective inhibition against PI-3 as seen in Table II, except for (+)-isoboldine, (–)-stylopine, and (±)-corydalidzine, that were totally ineffective against both viruses. In addition, another alkaloid, berberine, had no antiviral action, but it exhibited lower cytotoxicity than acyclovir (64 μg/ml) and the same as oseltamivir (32 μg/ml). According to the data we obtained, protopine, β-allocryptopine, chelidimerine, fumarophycine, α-hydrastine, (+)-bulbocapnine, (+)-isoboldine, (–)-sinactine, palmatine, dehydrocorydaline, dehydrocavidine, (+)-cularicine, oxocularine, and oxosarcocapnine were completely inactive against HSV, whereas maximum CPE inhibitory concentrations of the rest of the alkaloids were the same as for acyclovir (16 μg/ml). However, the alkaloids were revealed to be less cytotoxic than acyclovir

Table I. Antimicrobial activity of the alkaloids expressed as minimum inhibitory concentrations (MICs) (μg/ml).

Alkaloids tested	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*K. pneumoniae*	*A. baumannii*	*S. aureus*	*B. subtilis*	*C. albicans*
Protopine	32	64	32	8	8	64	128	4
β-Allocryptopine	32	64	32	8	8	64	128	4
(+)-Reticuline	32	32	32	32	32	64	64	8
(+)-Norjuziphine	32	32	32	32	32	64	64	8
Sanguinarine	32	32	32	32	32	64	64	8
Norsanguinarine	32	32	32	32	32	64	64	8
Chelidimerine	32	64	32	8	8	64	128	4
Fumarophycine	32	64	32	8	8	64	128	4
(−)-Fumarophycine acetate	32	32	32	32	32	64	64	8
(−)-Corpaine	32	32	32	32	32	64	64	8
(±)-Sibiricine	32	64	32	8	8	64	128	4
Sibiricine acetate	32	64	32	8	8	64	128	4
(±)-Dihydrosibiricine	32	64	32	8	8	64	128	4
(+)-Fumariline	32	32	32	32	32	64	64	8
(−)-Dihydrofumariline	32	32	32	32	32	64	64	8
(+)-Parfumine	32	32	32	32	32	64	64	8
Parfumine acetate	32	64	32	8	8	64	128	4
(−)-Dihydroparfumine diacetate	32	32	32	32	32	64	64	8
α-Hydrastine	32	64	32	8	8	64	128	4
(+)-Bicuculline	32	32	32	32	32	64	64	8
(−)-Bicuculline	32	32	32	32	32	64	64	8
(−)-Adlumidine	32	32	32	32	32	64	64	8
(+)-Bulbocapnine	32	64	32	8	8	64	128	4
(+)-Isoboldine	32	32	32	32	32	64	64	8
Berberine	32	64	32	8	8	64	128	4
(−)-Stylopine	32	32	32	32	32	64	64	8
(−)-Canadine	32	64	32	8	8	64	128	4
(−)-Sinactine	32	32	32	32	32	64	64	8
(−)-Ophiocarpine	32	64	32	8	8	64	128	4
Ophiocarpine-*N*-oxide	32	64	32	8	8	64	128	4
Corydalmine	32	64	32	8	8	64	128	4
Palmatine	32	32	32	32	32	64	64	8
(±)-Corydalidzine	32	32	32	32	32	64	64	8
Dehydrocorydaline	32	32	32	32	32	64	64	8
Dehydrocavidine	32	32	32	32	32	64	64	8
(+)-Cularicine	32	32	32	32	32	64	64	8
Oxocularine	32	32	32	32	32	64	64	8
Oxosarcocapnine	32	32	32	32	32	64	64	8
Oxosarcocapnidine	32	64	32	8	8	64	128	4
Corydaldine	32	64	32	8	8	64	128	4
AMP[a]	2	–[d]	2	2	2	<0.12	0.12	–
OFX[b]	0.12	1	<0.12	0.12	0.12	0.5	0.5	–
KET[c]	–	–	–	–	–	–	–	2

[a] AMP, ampicilline. [b] OFX, ofloxacine. [c] KET, ketoconazole. [d] No activity observed.

on MDBK cells, (−)-canadine being the least cytotoxic alkaloid (128 μg/ml). The most active alkaloid with anti-PI-3 effect was shown to be protopine (1–32 μg/ml), followed by fumarophycine (2–32 μg/ml), chelidimerine, (+)-bulbocapnine, and (−)-ophiocarpine (4–32 μg/ml), as well as β-allocryptopine and oxosarcocapnidine (8–32 μg/ml). Besides, the alkaloids tested exhibited lower or the same degree of cytotoxicity as oseltamivir (32 μg/ml) against Vero cells.

Table II. Antiviral activity and cytotoxicity of the alkaloids and references.

Alkaloid	MDBK cells			Vero vells		
	MNTC [μg/ml]	CPE inhibitory concentration		MNTC [μg/ml]	CPE inhibitory concentration	
		HSV			PI-3	
		Max.	Min.		Max.	Min.
Protopine	64	–	–	32	32	1
β-Allocryptopine	64	–	–	32	32	8
(+)-Reticuline	32	16	–	64	32	16
(+)-Norjuziphine	32	16	–	64	32	16
Sanguinarine	32	16	–	32	32	16
Norsanguinarine	32	16	–	32	32	16
Chelidimerine	64	–	–	32	32	4
Fumarophycine	64	–	–	32	32	2
(–)-Fumarophycine acetate	32	16	–	64	32	16
(–)-Corpaine	32	16	–	64	32	16
(±)-Sibiricine	32	16	–	64	32	16
Sibiricine acetate	32	16	–	32	32	16
(±)-Dihydrosibiricine	32	16	–	64	32	16
(+)-Fumariline	32	16	–	64	32	16
(–)-Dihydrofumariline	32	16	–	64	32	16
(+)-Parfumine	32	16	–	64	32	16
Parfumine acetate	32	16	–	64	32	16
(–)-Dihydroparfumine diacetate	32	16	–	64	32	16
α-Hydrastine	64	–	–	64	32	16
(+)-Bicuculline	32	16	–	64	32	16
(–)-Bicuculline	32	16	–	64	32	16
(–)-Adlumidine	32	16	–	64	32	16
(+)-Bulbocapnine	64	–	–	32	32	4
(+)-Isoboldine	–	–	–	–	–	–
Berberine	64	–	–	32	–	–
(–)-Stylopine	–	–	–	–	–	16
(–)-Canadine	128	–	–	64	32	16
(–)-Sinactine	32	16	–	64	32	16
(–)-Ophiocarpine	64	–	–	32	32	4
Ophiocarpine-*N*-oxide	64	–	–	32	32	16
Corydalmine	64	–	–	64	64	32
Palmatine	32	16	–	32	32	16
(±)-Corydalidzine	–	–	–	–	–	–
Dehydrocorydaline	32	16	–	64	–	16
Dehydrocavidine	32	16	–	64	32	16
(+)-Cularicine	32	16	–	32	32	16
Oxocularine	32	16	–	64	32	16
Oxosarcocapnine	32	16	–	64	32	16
Oxosarcocapnidine	64	–	–	32	32	8
Corydaldine	64	–	–	32	32	16
Acyclovir	16	16	<0.25	–	–	–
Oseltamivir	–	–	–	32	32	<0.25

A number of antimicrobial, antiviral, antitumoral, antimalarial, and cytotoxicity studies have been so far reported on various derivatives of natural or synthetic isoquinoline alkaloids (Capilla *et al.*, 2001; An *et al.*, 2001; Satou *et al.*, 2002; Zhang *et al.*, 2002; Gomez-Monterrey *et al.*, 2003; Morrell *et al.*, 2004; Fischer *et al.*, 2004). In one study (Iwasa *et al.*, 2001), antimicrobial, cytotoxic, and anti-HIV activities of 26 simple isoquinolines and 21 benzylisoquinolines were investigated and it was stated that a quaternary nitrogen atom of isoquinoline- or dyhydroisoquinoline-type may enhance the potency of antimicrobial activity and cytotoxicity, whereas anti-HIV activity was higher with tetrahydroisoquinolines. In Cui *et al.*'s study (2006), 17 simple isoquinolines, 15 of which were of 1-benzylisoquinoline-type and 19 of which were protoberberine derivatives, were screened against Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which is considered to be an indicator of the evaluation for antitumor-promoting activity, in Raji cells and all 1-benzylisoquinolines and 11 of the protoberberines exerted higher inhibitory activity than β-carotene. Regarding structure-activity relationship, it was concluded that the inhibitory activity of 1-benzylisoquinolines increased as the number of hydroxy groups on the aromatic ring increased and, additionally, the size of substituents at C-8 and C-13 as well as the type and position of the oxygenated substituents on A and D rings influenced the virus inhibition. Moreover, derivatives of the isoquinoline skeleton attached to the carboxamide moiety were declared to be the potent and selective inhibitors of human cytomegalovirus (HCMV) (Chan *et al.*, 1999).

On the other hand, berberine was shown to have important cytotoxic effects against divergent cell lines such as HT-29 (colorectal cancer), MCF-7 (breast cancer), Hep-2 (larynx cancer), MKN-45 (gastric cancer), HeLa (uterus carcinoma), ECC (esophageal cancer), T84 (intestinal epithelial cell line), and $SVKO_3$ (ovary carcinoma) as well as inhibitory effects on topomerase-I (Taylor *et al.*, 1999; Iizuka *et al.*, 2000; Orfila *et al.*, 2000; Tsai, 2001; Mazzini *et al.*, 2003; Cordero *et al.*, 2004). In another study (Iwasa *et al.*, 1996), structure-activity relationship of berberine and its derivatives was examined for their antibacterial activity and among the 13-alkyl-substituted and the 13-unsubstituted protoberberinium salts, an increase in antibacterial activity against *Staphylococcus aureus* was observed with the 13-ethyl-9-ethoxy, the 13-ethyl and the 13-methyl derivatives by 8-, 4-, and 2-fold, respectively, over berberine, which suggested that steric effects played an noteworthy role in the antibacterial activity. Additionally, replacement of methoxy groups at C-2 and the C-3 of ring A by a methylenedioxy group caused a boost in activity. In this report, it was stated that the quaternary nitrogen atom such as in protoberberinium salts, an alkyl substituent at C-13, and a methylenedioxy function at C-2 and C-3 are required for enhanced antibacterial activity. In a study by Nakamoto *et al.* (1990), berberine was revealed to have a strong antifungal effect against *C. albicans*, *C. tropicalis*, and *C. glabrata*, respectively, which is in accordance with our data on berberine. In a recent publication, a high occurrence of antibacterial activity of berberine was shown towards *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. fluorescens*, *S. aureus*, *Salmonella typhi*, *Enterococcus* sp., and *Serratia marcescens*, showing better activity than streptomycin at 50 μg/ml by the paper disc diffusion method and, consequently, berberine was concluded to be responsible for the high antibacterial activity of *Coscinium fenestratum* (Nair *et al.*, 2005). However, we herein found by the microdilution method that berberine was only active against *K. pneumoniae* and *A. baumannii*, which might result from the application of two different methods. In another former study, berberine obtained from *Berberis heterophylla* was tested by the agar diffusion method against the ATCC strains of *S. aureus*, *Enterococcus faecalis*, *P. aeruginosa*, *E. coli*, and *C. albicans* at 50, 100, and 200 μg/ml concentrations and the alkaloid was highly active against *S. aureus* at 100 and 200 μg/ml, whereas it did not possess any inhibitory effect against *E. faecalis*, *P. aeruginosa*, and *E. coli* (Freile *et al.*, 2003). This data has been consistent with ours for berberine in case of *E. coli* and *P. aeruginosa*, whereas it was also not active against *S. aureus*, which might be again resulted from the use of two dissimilar methods. In the same work, antifungal activity screening was performed with berberine using the clinical strains of several *Candida* sp. such as *C. albicans*, *C. glabrata*, *C. haemulonii*, *C. lusitaniae*, *C. krusei*, and *C. parapsilosis*. Being the most active against *C. krusei* followed by the rest at decreasing degrees, berberine was expressed as a novel antifungal agent.

In one report, protopine and α-allocryptopine isolated from *Glaucium oxylobum* were tested for

their antifungal activity against *Microsporium canis*, *M. gypseum*, *Tricophyton mentagrophytes*, *Epidermophyton floccosum*, *C. albicans*, *Aspergillus niger*, and *Penicillium* sp. (Morteza-Semnani *et al.*, 2003). Among these fungi, protopine exerted low activity against *M. canis* and *T. mentagrophytes*, while α-allocryptopine had low activity towards *M. gypseum* and good inhibition on *E. floccosum*. In contrary, protopine was found to be inactive against *C. albicans*, whereas this alkaloid had a high inhibition against the same fungus in our study (4 μg/ml). α-Allocryptopine was also inactive against *C. albicans*, whose β-counterpart exhibited a very good antifungal effect against *C. albicans*, which may be reasonably due to α- and β-conformation of the compound. Protopine, isolated from *Chelidonium majus*, was found to be highly toxic in the brine shrimp lethality test with LC_{50} of 49.7 ppm (Sağlam and Arar, 2003). In a previous study, the molluscicidal activity of *Argemone mexicana* seeds was tested against the snail *Lymnaea acuminata*, which led to isolation of protopine and sanguinarine as the active components (Singh and Singh, 1999).

From the structure-activity point of view, a few features about the isoquinoline alkaloids investigated herein can be pointed out. Quaternary nitrogen atom found on some of the isoquinolines such as dehydrocorydaline, dehydrocavidine, berberine, sanguinarine, and palmatine may have an effect on the decrease of antiviral activity. On the other hand, the synergistic interaction among the isoquinoline alkaloids isolated from *F. vaillantii* may be stated to contribute to the higher antiviral activity of this extract. Protopine-type alkaloids seem to display higher antiviral effects than the rest.

In conclusion, to the best of our knowledge, there has been no report on the antiviral, antibacterial, and antifungal activity of the above-mentioned isoquinoline alkaloids, except for protopine, berberine, and sanguinarine. Among them, protopine, fumarophycine, (+)-bulbocapnine, and (+)-ophiocarpine could be considered as new alternatives for the treatment of PI-3.

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