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RESEARCH ARTICLE

Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids

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Abstract

Objective: Some natural products consisting of the alkaloids yohimbine and vincamine (indole-type), scopolamine and atropine (tropane-type), colchicine (tropolone-type), allantoin (imidazolidine-type), trigonelline (pyridine-type) as well as octopamine, synephrine, and capsaicin (exocyclic amine-type); the flavonoid derivatives quercetin, apigenin, genistein, naringin, silymarin, and silibinin; and the phenolic acids namely gallic acid, caffeic acid, chlorogenic acid, and quinic acid, were tested for their *in vitro* antiviral, antibacterial, and antifungal activities and cytotoxicity.

Materials and methods: Antiviral activity of the compounds was tested against DNA virus herpes simplex type 1 and RNA virus parainfluenza (type-3). Cytotoxicity of the compounds was determined using Madin-Darby bovine kidney and Vero cell lines, and their cytopathogenic effects were expressed as maximum non-toxic concentration. Antibacterial activity was assayed against following bacteria and their isolated strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*, although they were screened by microdilution method against two fungi: *Candida albicans* and *Candida parapsilosis*.

Results: Atropine and gallic acid showed potent antiviral effect at the therapeutic range of 0.8–0.05 μg ml^{-1}, whilst all of the compounds exerted robust antibacterial effect.

Conclusion: Antiviral and antimicrobial effects of the compounds tested herein may constitute a preliminary step for further relevant studies to identify the mechanism of action.

Keywords: Alkaloids, antimicrobial activity, antiviral activity, flavonoids, herpes simplex, parainfluenza, phenolic acids

Introduction

Innovation of antimicrobials has long paved the way for human health. However, future effectiveness of antibiotics is somewhat doubtful, because microorganisms are developing resistance in an unavoidable manner to these antimicrobial agents. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a critical problem on the rise in hospitals worldwide (Monnet, 1998). Herpes simplex virus (HSV, types 1 and 2) is pathogenic to humans and is also a risk factor for human immunodeficiency virus (HIV) infection (Whitley et al., 1998; Khan et al., 2005). A frequent occurrence of resistance to anti-herpes drugs has been another growing dilemma. Therefore, discovery of novel antimicrobial agents are always in demand to overcome microbial resistance.

Consequently, we have examined the antiviral activity of a number of commercially available natural compounds, which are namely the alkaloids yohimbine and vincamine (indole-type), scopolamine and atropine (tropane-type), colchicine (tropolone-type), allantoin (imidazolidine-type), trigonelline (pyridine-type) as well as octopamine, synephrine, and capsaicin (exocyclic amine-type); the flavonoid derivatives quercetin, apigenin, genistein, naringin, silymarin, and silibinin; and the phenolic acids namely gallic acid, caffeic acid, chlorogenic acid, and quinic acid for their antiviral

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activity against DNA virus herpes simplex type 1 (HSV-1) and RNA virus parainfluenza type-3 (PI-3). Antibacterial activity of these compounds was evaluated by microdilution using the following strains of bacteria and their isolated strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *S. aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*. The compounds were screened by microdilution method against two fungi *Candida albicans* and *Candida parapsilosis*, although their cytotoxicity was determined using Madin-Darby bovine kidney (MDBK) and Vero cell lines, and their cytopathogenic effects (CPEs) were expressed as maximum non-toxic concentration (MNTC). Although the above-mentioned natural compounds tested are of synthetic origins in this study, they are also well-known secondary metabolites occurring naturally in plants such as vincamine in *Vinca minor* L. (Apocynaceae), atropine in *Atropa belladonna* L. (Solanaceae), colchicine in *Colchicum autumnale* L. (Liliaceae), trigonelline in *Trigonella foenum-graecum* L. (Fabaceae), synephrine and naringin in *Citrus* L. sp. (Rutaceae), capsaicin in *Capsicum annuum* L. (Solanaceae), silibinin and silymarin in *Silybum marianum* L. (Asteraceae), and genistein in *Soja hispida* L. (syn. *Glycine max* L.) (Fabaceae). Also, the phenolic acids such as gallic, chlorogenic, caffeic, and quinic acids are quite abundant in many plant species.

Materials and methods

Tested compounds

The alkaloids used in this study, namely yohimbine (Y3125, Sigma, St. Louis, MO), vincamine (V2127, Sigma), scopolamine (S0929, Sigma), atropine (A0132, Sigma), colchicine (C9754, Sigma), allantoin (A7878, Sigma), trigonelline (5509, Sigma), octopamine (O0250, Sigma), synephrine (S0752, Sigma), and capsaicin (V9130, Sigma); the flavonoid derivatives quercetin (Serva, 34120), genistein (G6776, Sigma), apigenin (13700, Serva, Germany), naringin (4161h, Koch-Light Laboratories, Germany), silibinin (S0417, Sigma), and silymarin (S0292, Sigma); the phenolic acids namely chlorogenic acid (C3878, Sigma), caffeic acid (822029, Schuchardt, Germany), gallic acid (G7384, Sigma), and quinic acid (ASB-D0017175-001, ChromaDex, Irvine, CA) were purchased from the respective manufacturers.

Antiviral activity

Test viruses

To determine the antiviral activity of the samples, HSV-1 as a representative of DNA viruses and PI-3 as a representative of RNA viruses were used. The test viruses were obtained from Faculty of Veterinary Medicine, Department of Virology, Ankara University, Turkey.

Cell line and growth conditions

The Vero cell line (African green monkey kidney) and MDBK cell line used in this study were obtained from the Department of Virology, Ankara University, Turkey. The cells were grown in Eagle's minimal essential medium (EMEM) (Seromed, Biochrom, Berlin, Germany), enriched with 10% fetal calf serum (Biochrom), 100 mg ml^{-1} of streptomycin and 100 IU ml^{-1} of penicillin in a humidified atmosphere of 5% carbon dioxide (CO_2) at 37°C. The cells were harvested using trypsin solution (Gibco, Paisley, UK).

Determination of antiviral activity

EMEM was placed into each of the 96 wells of the microplates (Greiner®; Essen, Germany). Stock solutions of the samples were added into the first row of each microplate and two-fold dilutions of the compounds (512–0.012 μg ml^{-1}) were made by dispensing the solutions to the remaining wells. Two-fold dilution of each material was obtained according to Log_2 on the microplates. Acyclovir (Biofarma, Istanbul, Turkey) and oseltamivir (Roche, basel, Switzerland) were used as the references. Strains of HSV-1 and PI-3 titers were calculated as tissue culture infecting dose and inoculated into all of the wells. The sealed microplates were incubated in 5% CO_2 at 37°C for 2 h to detect the possible antiviral activities of the samples. After incubation, 50 μl of the cell suspension of 300,000 cells ml^{-1}, which were prepared in EMEM together with 5% fetal bovine serum were put into each well and the plates were incubated in 5% CO_2 at 37°C for 48 h. After the end of this period, the cells were evaluated using cell culture microscope by comparison with treated–untreated control cultures and with acyclovir and oseltamivir. Consequently, maximum CPE concentrations as the indicator of antiviral activities of the extracts were determined (Özçelik et al., 2006).

Cytotoxicity

The MNTCs of each compound were determined by the method described previously by Özçelik et al. (2006) based on cellular morphologic alteration. Several concentrations of each test compound were placed in contact with confluent cell monolayers and incubated in 5% CO_2 at 37°C for 48 h. After the incubation period, drug concentrations that are not toxic to viable cells were evaluated as non-toxic and also compared with non-threatening cells for confirmation. The rows that caused damage in all cells were evaluated as toxic in this concentration. In addition, maximum drug concentrations that did not affect the cells were evaluated as non-toxic concentrations. MNTCs were determined by comparing treated and controlling untreated cultures.

Determination of antibacterial and antifungal activities

Preparation of the test compounds

All of the compounds were dissolved in dimethylsulfoxide to prepare a final concentration of 256 μg ml^{-1}, sterilized by filtration using 0.22 μm Millipore (MA 01730), and used as the stock solutions. Reference antibacterial agents of ampicillin (AMP; Fako) and ofloxacin (OFX; Hoechst Marion Roussel) were obtained from their respective manufacturers and dissolved in phosphate

buffer solution (AMP pH 8.0, 0.1 mol l^{-1}), and in distilled water (OFX). The stock solutions of these agents were prepared in medium according to Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards, NCCLS) recommendations (CLSI/NCCLS, 1996).

Microorganisms and inoculum preparation

Antibacterial activity tests were carried out against standard (American type culture collection, ATCC; Culture collection of Refik Saydam Central Hygiene Institute, RSKK) and isolated strains (clinical isolate obtained from the Faculty of Medicine, Department of Microbiology, Gazi University, Ankara, Turkey) of Gram-negative type *E. coli* ATCC 35218, *P. aeruginosa* ATCC 10145, *P. mirabilis* ATCC 7002, *K. pneumoniae* RSKK 574, *A. baumannii* RSKK 02026, and the strains of Gram-positive type *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, and *B. subtilis* ATCC 6633. *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 were employed for determination of antifungal activity. Mueller Hinton broth (Difco, Lawrence, KS) and Mueller Hinton agar (Oxoid, Cambridge, UK) were applied for growing and diluting of the bacterium suspensions as described beforehand by Özçelik et al. (2005). The synthetic medium RPMI-1640 with L-glutamine was buffered to pH 7 with 3-[*N*-morpholino]-propanesulfonic acid and culture suspensions were prepared. The microorganism suspensions used for inoculation were prepared at 10^5 cfu ml^{-1} (colony forming unit) by diluting fresh cultures at McFarland 0.5 density (10^8 cfu ml^{-1}). Suspensions of bacteria and fungi were added to each well of the diluted samples, density of 10^5 cfu ml^{-1} for fungi and bacteria. The bacterial suspensions used for inoculation were prepared at 10^5 cfu ml^{-1} by diluting fresh cultures at McFarland 0.5 density (10^8 cfu ml^{-1}). The fungus suspensions were prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of 2.5×10^3 cfu ml^{-1} (CLSI/NCCLS, 1996).

Antibacterial and antifungal tests

The microdilution method as described in our previous studies was employed for antibacterial and antifungal activity tests (Özçelik et al., 2005, 2006). Medium was placed into each well of 96-well microplates. Sample solutions at 512 μg ml^{-1} were added to the first row of each microplate and two-fold dilutions of the compounds (256–0.125 μg ml^{-1}) were made by dispensing the solutions to the remaining wells. Culture suspensions of 10 μl were inoculated into all of the wells. The sealed microplates were incubated at 35°C for 24 and 48 h in a humid chamber. The lowest concentration of the compounds that could completely inhibit macroscopic growth was determined and minimum inhibitory concentrations (MICs) were calculated. All tests were performed in triplicate in each run of the experiments.

Results

Results of the antiviral activity and cytotoxicity of the compounds are tabulated in Table 1 in comparison with the references (acyclovir and oseltamivir), although antibacterial and antifungal outcomes of the compounds are listed in Table 2. Accordingly, the alkaloids investigated showed a remarkable inhibitory effect against HSV-1 with CPE varying between 0.05 and 1.6 μg ml^{-1}, although only atropine and octopamine had inhibition against PI-3, having MNTCs between 0.05 and 0.8 μg ml^{-1}. A noteworthy occurrence of anti-HSV-1 activity was observed in all of the flavonoids screened, although apigenin and naringin had the highest inhibition against HSV-1 with the widest therapeutic range (0.4–1.6 μg ml^{-1}). Among the phenolics, only genistein, gallic, chlorogenic, and quinic acids exerted varying degrees of anti-PI-3 effect. In MDBK cells, most of the compounds had better cytotoxicity than that of acyclovir (1.6 μg ml^{-1}).

The compounds displayed a very high activity towards all of the ATCC and RSKK strains of the tested bacteria and were revealed to be ineffective against MRSA and extended-spectrum beta-lactamases (ESβL+) strains. Among the alkaloids, yohimbine and vincamine emerged as the most effective against the bacteria with MIC values between 2 and 8 μg ml^{-1}. On the other hand, the compounds exhibited better antifungal effect against the opportunistic pathogen *C. albicans* rather than *C. parapsilosis*. The most effective compounds having anti-*Candida* activity were found to be vincamine, trigonelline, and silibinin at 4 μg ml^{-1}.

Discussion

Because microbial resistance has become an increasing problem for humans, an enormous amount of research has focused on discovery or extension of lifespan of novel antimicrobial agents. For the same purpose, there have also been numerous studies on antimicrobial activity of natural products including phenolics and alkaloids (Iwasa et al., 2001; Cushnie & Lamb, 2005; Gul & Hamann, 2005; Ríos & Recio, 2005; Khan et al., 2005; Orhan et al., 2007). In many cases, antimicrobial effects of various plant extracts have been attributed to their flavonoid contents (Tsao et al., 1982; Cafarchia et al., 1999). Flavonoid derivatives have also been reported to possess antiviral activity against a wide range of viruses such as HSV, HIV, Coxsackie B virus, coronavirus, cytomegalovirus, poliomyelitis virus, rhinovirus, rotavirus, poliovirus, sindbis virus, and rabies virus (De Bruyne et al., 1999; Evers et al., 2005; Chávez et al., 2006; Nowakowska, 2007). In a study by Chiang et al. (2002), *Plantago major*, which has been used in the treatment of viral hepatitis in Chinese traditional medicine, showed a strong anti-herpes activity against HSV-1 and antiviral activity of the aqueous extract of this species mainly attributed to its rich phenolic content, caffeic acid, in

Table 1. Antiviral activity and cytotoxicity of the compounds and references.

	MDBK cells			Vero cells		
		CPE inhibitory concentration HSV-1			CPE inhibitory concentration PI-3	
	MNTC (μg ml^{-1})	Maximum	Minimum	MNTC (μg ml^{-1})	Maximum	Minimum
Alkaloids						
Yohimbine	1.6	0.8	0.2	1.6	–	–
Vincamine	1.6	0.8	0.2	1.6	–	–
Scopolamine	3.2	1.6	0.8	0.8	0.4	–
Atropine	3.2	0.8	0.05	1.6	0.8	0.05
Colchicine	3.2	1.6	0.8	0.8	–	–
Allantoin	3.2	1.6	0.4	0.8	0.4	–
Trigonelline	1.6	0.4	0.1	1.6	0.4	–
Octopamine	3.2	1.6	0.05	1.6	0.8	0.05
Synephrine	3.2	1.6	0.8	0.8	–	–
Capsaicin	1.6	0.4	0.05	1.6	0.2	–
Flavonoids						
Quercetin	1.6	0.2	0.1	1.6	–	–
Apigenin	3.2	1.6	0.4	0.8	0.2	–
Genistein	1.6	0.8	0.4	1.6	0.4	0.2
Naringin	3.2	1.6	0.4	0.8	0.2	–
Silymarin	3.2	1.6	0.8	0.8	–	–
Silibinin	1.6	0.4	0.1	1.6	0.4	–
Phenolic acids						
Gallic acid	3.2	0.8	0.05	1.6	0.8	0.05
Caffeic acid	3.2	0.8	0.4	1.6	0.8	–
Chlorogenic acid	3.2	0.8	0.4	3.2	1.6	0.4
Quinic acid	3.2	0.8	0.05	3.2	1.6	0.4
References						
Acyclovir	1.6	1.6	<0.012	–	–	–
Oseltamivir	–	–	–	1.6	1.6	<0.012

MDBK, Madine-Darby bovine kidney; MNTC, maximum non-toxic concentration; CPE, cytopathogenic effect; HSV-1, herpes simplex virus (type-1); PI-3, parainfluenza (type-3), –, no activity observed.

particular, which is consistent with our data (Table 1). In some studies (Amoros et al., 1992; Kujumgiev et al., 1999), the major flavonoid derivatives (quercetin, procyanidin, pelargonidol, catechin, hesperidin, and luteolin) identified in propolis (bee glue) were tested for their anti-HSV effect and quercetin, catechin, and hesperitin were found to cause direct inactivation of HSV. The results also verified that flavonols were more active than flavones. Fritz et al. (2007) investigated anti-herpes asset of the methanol extract of *Hypericum connatum* along with its isolated components—amentoflavone, hyperoside, guaijevenine, and luteoforol. Among them, luteoforol (a flavan-4-ol) had the best activity against HSV-1. Relevantly, leachianone G (a prenylated flavonoid) exerted the most potent inhibition against HSV-1 on Vero cells among all the compounds isolated from the root bark of *Morus alba* (Du et al., 2003). We formerly examined antibacterial, antifungal, and antiviral activities of four flavonoid derivatives, namely scandenone (prenylated isoflavone), tiliroside, quercetin-3,7-*O*-α-L-dirhamnoside, and kaempferol-3,7-*O*-α-L-dirhamnoside in the same manner as the current study (Özçelik et al., 2006). None of those compounds was active against HSV-1, although only quercetin-3,7-*O*-α-L-dirhamnoside inhibited strongly PI-3 with therapeutic range of 32–8 μg ml^{-1}. On the other hand, all of them exhibited better cytotoxicity on MDBK and Vero cells than those of acyclovir and oseltamivir. Quercetin was formerly reported to enhance greatly the antiviral effect of tumor necrosis factor that produces a dose-dependent inhibition of vesicular stomatitis virus, encephalomyocarditis virus, and HSV-1 replication in WISH cells (Ohnishi & Bannai, 1993). In our assay, we also found it to be effective in spite of its narrow therapeutic range of 0.2–0.1 μg ml^{-1}. Strong inhibition of HSV-1 and -2 by the aqueous extract of *Pelargonium sidoides*, which mainly contains simple phenolics, coumarins, flavonoids, and catechins (Schnitzler et al., 2008) was reported, although the flavonoid-rich extracts of *Vitex polygama* exhibited a strong inhibition towards acyclovir-resistant HSV-1 (Gonçalves et al., 2001). However, ineffectiveness of its quercetin-containing fraction in this assay was suggested to be due to its low quantity within the fraction. We have not encountered any report on anti-HSV or anti-PI effect of naringin up to date, but it was previously reported to be ineffective against sindbis neurovirulent strain, although naringenin was strongly active (Paredes et al.,

Table 2. Antimicrobial activity of the compounds and references against standard and isolated strains of microorganisms expressed as MIC (μg ml^{-1}).

	E. coli		*P. aeruginosa*		*P. mirabilis*		*K. pneumoniae*		*A. baumannii*		*S. aureus*		*E. faecalis*		*B. subtilis*			
	ATCC 35218	Isolated strain ESβL+	ATCC 10145	Isolated strain	ATCC 7002	Isolated strain ESβL+	RSKK 574	Isolated strain ESβL+	RSKK 02026	Isolated strain	ATCC 25923	Isolated strain MRSA	ATCC 29212	Isolated strain	ATCC 6633	Isolated strain	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019
Alkaloids																		
Yohimbine	4	32	4	32	4	32	4	64	8	64	2	64	2	64	2	64	8	8
Vincamine	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	4	8
Scopolamine	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Atropine	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Colchicine	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Allantoin	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Trigonelline	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	4	8
Octopamine	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Synephrine	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Capsaicin	4	32	4	32	4	32	4	64	8	64	2	64	2	64	2	64	8	8
Flavonoids																		
Quercetin	4	32	4	32	4	32	4	64	8	64	2	64	2	64	2	64	8	8
Apigenin	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Genistein	4	32	4	32	4	32	4	64	8	64	2	64	2	64	2	64	8	8
Naringin	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Silymarin	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Silibinin	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	4	8
Phenolic acids																		
Gallic acid	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Caffeic acid	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Chlorogenic acid	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Quinic acid	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
References																		
AMP	2	>128	–	–	2	>128	2	>128	2	>128	<0.12	>128	0.5	>128	0.12	0.5	–	–
GM	–	–	0.5	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
OFX	0.12	0.5	1	64	<0.12	1	<0.12	0.5	0.12	64	0.25	64	1	32	–	–	–	–
LVX	<0.12	0.5	1	64	<0.12	1	<0.12	1	0.12	64	0.25	128	0.5	32	–	–	–	–
VAN	–	–	–	–	–	–	–	–	–	–	0.5	2	0.12	2	–	–	–	–
KET	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1
FLU	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	4

AMP, ampicillin; GM, gentamicin; OFX, ofloxacin; LVX, levofloxacin; VAN, vancomycin; KET, ketoconazole; FLU, fluconazole; –, not tested.

2003). In a study by Chiang et al. (2005), the extracts of *Ocimum basilicum* and its purified compounds including apigenin were tested against a number of viruses (HSV, adenovirus, and hepatitis B virus) and apigenin displayed a broad range of antiviral activity, which is in accordance with our data. Genistein, a soy isoflavone, was previously reported to inhibit bovine HSV-1 on MDBK cells as found by us herein (Akula et al., 2002). Silymarin and silibinin, the hepatoprotective principles of *S. marianum* (milk thistle), are known to be the potent antivirals against hepatitis virus, in particular (Saller et al., 2001; Mayer et al., 2005; Pradhan & Girish, 2006; Ferenci et al., 2008). However, we did not find any article on their anti-herpes effects in the literature.

In our previous study (Orhan et al., 2007), we screened a number of isoquinoline alkaloids for their antiviral, antibacterial, and antifungal activities using the same methods as herein and found out that they were quite active against PI-3, although their anti-herpes (HSV-1) effect was negligible. The most active anti-PI-3 alkaloids seemed to be protopine (32–1 μg ml^{-1}), followed by fumarophycine (32–2 μg ml^{-1}), chelidimerine, ophiocarpine, and (+)-bulbocapnine (32–4 μg ml^{-1}). Some antiviral compositions containing yohimbine as the active constituent have been patented, which is again in agreement with our data (Leone, 2002). Atropine, which displayed a high activity against both HSV-1 and PI-3 in our assays, was previously tested for its antiviral effect against HIV-1 and reported to be strong inhibitor of this virion as in good consistence with our data (Yamazaki & Tagaya, 1980; Alarcón et al., 1984). Although capsaicin was stated not to have a direct anti-herpes effect, *cis*-capsaicin (civamide) exhibited remarkable inhibition against genital HSV (Bourne et al., 1999).

In this study, we have screened antimicrobial activity of several alkaloids, flavonoid derivatives, and simple phenolic acids, and most of them showed remarkable antiviral activity against HSV-1, although they were less active on PI-3. Doubtless phenolic compounds and alkaloids constitute unique templates associated with desired bioactivities. However, it is also apparent that antimicrobial activity depends on specific substitution patterns in chemical structures of the tested compounds. Relevant literature and our own data point to the fact that natural compounds are the most attractive sources in the search for exploring new antimicrobial agents. Among the tested compounds, atropine, gallic acid, and quinic acid had a potent anti-herpes activity, whilst atropine, octopamine, and gallic acid exerted strong anti-influenza effect at the therapeutic range of 0.8–0.05 μg ml^{-1}. All of the compounds have also possessed sturdy antibacterial effect against ATCC and RSKK strains of the tested bacteria and antifungal properties. To the best of our knowledge, our study describes the anti-HSV (type-1) and anti-PI (type-3) activity of some of the compounds screened such as naringin, silymarin, silibinin, scopolamine, vincamine, colchicine, allantoin, octopamine, synephrine, quinic acid as well as anti-PI (type-3) activity of gallic, caffeic, and chlorogenic acids for the first time.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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