J HerbMed Pharmacol. 2016; 5(3): 112-115.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Antibacterial activity and ciprofloxacin-potentiation property of *Berberis vulgaris asperma* stem extracts on pathogenic bacteria

Azizollah Ebrahimi[1*], Mohadaseh Chavoushpour[2], Mohamad Reza Mahzoonieh[1], Sharareh Lotfalian[1]

[1]Department of Pathobiology, School of Veterinary Science, Shahrekord University, Shahrekord, Iran.
[2]Msc Student of Bacteriology, Department of Pathobiology, Veterinary College, Shahrekord University, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 4 March 2016
Accepted: 2 June 2016

Keywords:
Antibacterial activities
Berberis vulgaris asperma
Gram-negative bacteria

ABSTRACT

Introduction: This study was planned to search the antibacterial activities of the ethanol and Chloroform extracts of *Berberis vulgaris asperma* stem and their synergistic effects with ciprofloxacin against some gram-negative pathogenic bacteria.
Methods: Broth micro-dilution method was used for determination of minimum inhibitory concentrations (MICs) of the extracts alone or in association with ciprofloxacin and Phenylalanine-Arginine β-Naphtylamide (PAβN) as a positive control and efflux pumps inhibitor (EPI).
Results: MIC determination indicated that the extracts from *Berberis vulgaris asperma* stem were able to inhibit the growth of all the studied bacteria within a concentration range of 3900 to 37500 µg/mL. Synergistic effects were noted between the extracts from *Berberis vulgaris asperma* stem extracts and ciprofloxacin on all tested bacteria except Acinetobacter bummani.
Conclusion: *Berberis vulgaris asperma* stem extracts act as an antibacterial agent and potentiate ciprofloxacin effects on examined pathogenic bacteria.

Implication for health policy/practice/research/medical education:
Various extracts from *B. vulgaris asperma* stem were able to act as antibacterial agents and increase the susceptibility of pathogenic bacteria to ciprofloxacin. The present data may bring about primary information for the possible use of these extracts in association with fluoroquinolones to combat at least some gram negative pathogens.
Please cite this paper as: Ebrahimi A, Chavoushpour M, Mahzoonieh MR, Lotfalian S. Antibacterial activity and ciprofloxacin-potentiation property of Berberis vulgaris asperma stem extracts on pathogenic bacteria. J HerbMed Pharmacol. 2016;5(3):112-115.

Introduction

Multidrug resistant (MDR) gram-negative bacteria have resistance to many antimicrobial compounds by multiple mechanisms including reduced outer membrane permeability, and active efflux mechanisms by efflux pumps. Efflux pumps in pathogenic bacteria and their roles in excreting entered antimicrobial agents to outer vicinity of the cell is one of several mechanisms that lead to bacterial drug resistances (1).
Reports show this mechanism is involved in fluoroquinolone resistance in gram negative pathogenic bacteria, affecting hospitalized patients (2). The result of over expression of these pumps in pathogenic bacteria is the emergence of pathogenic strains that are clinically resistance to many antimicrobial agents such as ciprofloxacin (3).
Phenylalanyl-arginyl-beta-naphthylamide (PAßN) is a compound that has become known as a chemical efflux pump inhibitor (EPI) (4). PAßN selectively inhibits the efflux activities of a broad range of efflux pumps such as MexAB-OprM, MexEF-OprN, MexCD-OprJ, and MexXY-OprM in *P. aeruginosa* and AcrAB-TolC in some species of the Enterobacteriaceae (5).
It has been reported that the extracts from some medicinal plants contain compounds that inhibit efflux pumps of bacteria and can be considered as EPIs (6). *Berberis vulgaris asperma* is a medicinal herb that traditionally is used for its medicinal properties. Reports indicated that Berberis species contain compounds that could enhance antibacterial actions of some antimicrobial agents and also have many beneficial effects such as anti-bacterial properties (7).
This study was planned to search the antibacterial activities of the ethanol and Chloroform extracts of *Berberis vulgaris asperma* stem and their synergistic effects with ciprofloxacin as a fluoroquinolone representative against some gram-negative pathogenic bacteria.

***Corresponding author:** Azizollah Ebrahimi, Department of Pathobiology, School of Veterinary Science, Shahrekord University, P. O. Box: 115, Shahrekord, Iran. Fax: +98-3832324427; Phone: +98-3832324427; Mobile: 09133197980; Email: A_kahrizsangi@yahoo.com

Materials and methods

Dried stems of *Berberis vulgaris asperma* were prepared from Khorasan province local markets and transferred to microbiology laboratory of veterinary college of Shahrekord university. After grinding, the resulting powder was extracted with ethanol 85% and chloroform for 48 hours at 25°C. After filtration the extracts were concentrated under vacuum (8). All extracts were kept at 4°C for further investigations.

Bacterial cultures and materials

The studied microorganisms included the reference strains of *Pseudomonas aeruginosa* ATCC 9027, *Acinetobacter baumannii* NCTC 13305, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* RTCC 2465, were kept in Lauria Bertani broth (LB broth) at 4°C and sub-cultured on appropriate agar plates 24 hours prior to antimicrobial tests. Mueller Hinton broth (MHB) was used for all the antibacterial assays. Ciprofloxacin (CIP) (Sigma-Aldrich) as fluoroquinolone representative and Phenylalanine arginine β-aphthylamide (PAβN) (Sigma-Aldrich) as microbial growth indicator and EPI were used, respectively.

Antimicrobial testing

After a preliminary assay by tube dilution method on examined drugs against four standard bacterial strains, the MICs for drug combinations were determined following the method of double-serial micro dilution, according to guidelines of the Clinical and Laboratory Standards Institute (9). Briefly, the bacterial cultures were incubated aerobically at 37°C for 18-24 hours. The turbidity of the cultures adjusted to 0.5 McFarland (1.5×10^8 CFU/ml) and then diluted in saline solution so that to obtain an inoculum of 5×10^5 CFU/well. The first well of each 96 well micro plate row inoculated with four MIC of drug/drugs followed by double dilution in successive wells to detect any possible antagonistic or synergistic combinations. The two last wells were considered as positive and negative controls.

The inoculated micro plates were aerobically incubated in shaking for about 18 hours at 37°C. The lowest concentration that inhibited visible growth after incubation was defined as MIC. For verifying synergistic activity of ciprofloxacin with the extracts, activity of ciprofloxacin with extract associations was compared with that of ciprofloxacin plus PAßN (30 µg/ml in prepared stock solution) whose antimicrobial activity was also tested.

Interaction of drugs in combinations was calculated as the ratio of $MIC_{Antibiotic}$ in combination/$MIC_{Antibiotic}$ alone and the results were discussed as follows: synergy (<0.5), indifferent (0.5 to 4), or antagonism (>4) (10,11). All assays were performed in duplicate.

Results

Ethanol and chloroform extracts of *Berberis vulgaris asperma* stem were examined to detect synergy with ciprofloxacin. The positive control was PAβN whose antimicrobial activity was also assayed on the examined strains (Table 1).

The MICs of ethanolic and chloroform extracts and drug combinations against examined bacteria are appeared in Table 1. Of the extracts investigated, the chloroform extract generally showed a greater activity against examined bacteria, (MIC, 3900 to 7810 µg/mL.). By MIC determination, chlorophorm extract of *Berberis vulgaris asperma* stem showed synergistic activity with ciprofloxacin against all of the examined bacteria other than *A. bummani (*Table 1*)*. However, for ethanol extract this synergy was recorded only against *E. coli* and *S. enteritidis*. In *A. bummani* there were no synergy with ciprofloxacin, PAβN also did not reduce the MIC of ciprofloxacine (Table 1).

Discussion

There is continued clinical pressure for novel approaches to combat antibiotic-resistances and identifying new antimicrobials for treating resistant bacterial infections. Screening plants for natural products with antibiotic potentiating effects was a successful approach (7). So, in the present work we examined *Berberis vulgaris asperma* stem ethanolic and chloroform extracts for antibacterial activity and possible synergy with ciprofloxacin.

The examined bacterial strains tested with a combination of *Berberis vulgaris asperma* extracts, ciprofloxacin and PAβN all contained multidrug resistance efflux pumps (3,12). It appears that extracts of *B. vulgaris asperma* stem prevent the growth of all examined bacterial strains in

Table 1. MICs (µg/mL) of CIP, and PAβN in the absence and presence of *Berberis vulgaris asperma* stem extracts against some gram negative Bacteria[a]

Combination PAβN+Cip	CIP	Eth.E.+ CIP	Eth.E.	Ch.E. + CIP	Ch.E.	PAβN
Bacteria						
Ps. aeruginosa	1	0.625	3125	**0.19**	4680	3.75
0.125						
S. enteritidis	0.008	**0.003**	37500	**0.003**	3900	5
0.05						
E. coli	0.007	**0.0015**	25000	**0.0002**	7810	6.25
0.002						
A. baumannii	0.26	0.2	37500	*0.4*	6250	6.25
R						

Abbreviations: Eth.E., ethanolic extracts; Ch. E., chloroform extract; CIP, ciprofloxacin respectively; MIC, Minimum inhibitory concentration; PAβN, phenylalanine arginine β-aphthylamide.

[a] Synergistic combinations appeared as bold numbers.

3900 to 37500 µg/mL concentration range (Table 1). The lowest MIC value (3900 µg/mL.) was obtained with the Chloroform extract of *B. vulgaris asperma* stem against *S. enteritidis*.

Reports show other plant extracts contain compounds that potentiate antibiotic activity or have antibacterial activity (14). Also it has been reported that essential oil from a Corsican plant, *Helichrysum italicum*, reduced the MIC of chloramphenicol against *Enterobacter aerogenes*, *A. baumannii* and *P. aeruginosa* (14).

In terms of antibiotic potentiation with ciprofloxacin the chloroform extract of *B. vulgaris asperma* stem showed the best activity against *S. enteritidis*, *E. coli* and *pseudomonas aeroginosa*. The best synertgistic activities of ethanolic extract were recorded against two former bacteria. These observations may imply that *B. vulgaris asperma* stem extracts contain active compounds that target the bacterial cell and might be powerful substrates of mentioned bacterial efflux pumps. As ciprofloxacin is a substrate of many bacterial efflux pumps (1), many reports indicate activity of other plant extracts that synergised with this agent (15). *A. baumannii,* PAβN and association of extracts had no effect on MIC of ciprofloxacin. Since the presence of AcrB efflux pumps confers resistance to the cells against PA*β*N (16), this may implies that our examined strain of *A. baumannii* over expressed AcrB efflux pumps and the extracts from *B. vulgaris asperma* stem may also behave the same as PA*β*N.

It is also worth to note that PA*β*N cannot reverse drug resistance for all drugs and has, for instance, no effect on efflux pump-mediated resistance to the dye ethidium (17). Berberine is an isoquinoline-type alkaloid with antimicrobial properties isolated from many kinds of Berberis species such as *Berberis aristata*, *Berberis aquifolium* and *Berberis vulgaris.* The synergistic effects of extracts of *B. vulgaris asperma* stem with other antibiotics were noted on other bacteria also indicate that extracts of this herb can act as EPI (18). However, a detailed study on active constituents, phytochemical and toxicological properties of *B. vulgaris asperma* stem is suggested for evaluating its safety.

Taking together, from our results we can suggest that using the extracts of *B. vulgaris asperma* stem in associations with fluoroquinolones might be helpful in treating infections caused by *Pseudomonas aeruginosa*, *E. coli* and *Salmonella enteritidis* strains.

In conclusion extracts from *B. vulgaris asperma* stem were able to increase susceptibility to ciprofloxacin and the present investigation brings about primary information for the possible use of these extracts in association with fluoroquinolones to combat at least some gram negative pathogens.

Acknowledgement

The authors thank to Dr. B. Zamanzad and Dr A. Gholipour (Dept. of microbiology, Shahrekord Medical School), and Dr H. Motamedi (Department of microbiology, College of basic Sciences, Shahid Chamran University) for their kind corporation in preparation of the examined standard strains.

Authors' contributions

AE designed and supervised the study and wrote the manuscript. MC followed the examinations and arranged the data sheet. MM supervised the study. SL did the technical supports.

Conflict of interests

The authors declared that there is no conflict of interest

Ethical considerations

The authors have been entirely regarded ethical issues such as plagiarisms, data assembly, fraud, double publication or submission.

Funding/Support

This study was financially supported by veterinary college of Shahrekord university as a grant for MSc thesis project.

References

1. Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev. 2006;19(2):382-402.
2. Ardebili A, Talebi M, Azimi L, Lari AR. Effect of efflux pump inhibitor carbonyl cyanide 3-chlorophenylhydrazone on the minimum inhibitory concentration of ciprofloxacin in acinetobacter baumannii clinical isolates. Jundishapur J Microbiol. 2014;7(1):e8691. doi: 10.5812/jjm.8691.
3. Blot S, Depuydt P, Vandewoude K, De Bacquer D. Measuring the impact of multidrug resistance in nosocomial infection. Curr Opin Infect Dis. 2007;20(4):391-6.
4. Lamers RP, Cavallari JF, Burrows LL. The efflux inhibitor phenylalanine-arginine beta-naphthylamide (PAβN) permeabilizes the outer membrane of gram-negative bacteria. PLoS One. 2013;8(3):e60666.
5. Dreier J, Ruggerone P. Interaction of antibacterial compounds with RND efflux pumps in Pseudomonas aeruginosa. Front Microbiol. 2015.
6. Stavri M, Piddock LJ, Gibbons S. Bacterial efflux pump inhibitors from natural sources. J Antimicrob Chemother. 2007;59(6):1247-60.
7. Alimirzaee P, Gohari AR, Hajiaghaee R, Mirzaee S, Jamalifar H, Monsef-Esfahani HR, et al. 1-methyl malate from Berberis integerrima fruits enhances the antibacterial activity of ampicillin against Staphylococcus aureus. Phytother Res. 2009;23(6):797-800.
8. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82.
9. Wayne P. Clinical and Laboratory Standarts Institute: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A7. USA: CLSI; 2006.
10. Shahverdi A, Monsef-Esfahani H, Tavasoli F, Zaheri A, Mirjani R. Trans-cinnamaldehyde from cinnamomum zeylanicum stem essential oil reduces the clindamycin resistance of clostridium difficile in vitro. J Food Sci. 2007;72(1):S055-S8.
11. Braga LC, Leite AA, Xavier KG, Takahashi JA, Bemquerer MP, Chartone-Souza E, et al. Synergic interaction between pomegranate extract and antibiotics against Staphylococcus aureus. Can J Microbiol. 2005;51(7):541-7.
12. Hsieh PC, Siegel SA, Rogers B, Davis D, Lewis K. Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. Proc Natl Acad Sci. 1998;95(12):6602-6.
13. Tegos G, Stermitz FR, Lomovskaya O, Lewis K.

Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob Agents Chemother. 2002;46(10):3133-41.
14. Lorenzi V, Muselli A, Bernardini AF, Berti L, Pagès JM, Amaral L, et al. Geraniol restores antibiotic activities against multidrug-resistant isolates from gram-negative species. Antimicrob Agents Chemother. 2009;53(5):2209-11.
15. Piddock LJ, Garvey MI, Rahman MM, Gibbons S. Natural and synthetic compounds such as trimethoprim behave as inhibitors of efflux in Gram-negative bacteria. J Antimicrob. Chemother. 2010;65(6):1215-23.
16. Ohene-Agyei T, Mowla R, Rahman T, Venter H. Phytochemicals increase the antibacterial activity of antibiotics by acting on a drug efflux pump. Microbiology Open. 2014;3(6):885-96.
17. Lomovskaya O1, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, et al. Identification and characterization of inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa: novel agents for combination therapy. Antimicrob Agents Chemother. 2001;45(1):105-16.
18. Wojtyczka RD, Dziedzic A, Kępa M, Kubina R, Kabała-Dzik A, Mularz T, et al. Berberine enhances the antibacterial activity of selected antibiotics against coagulase-negative Staphylococcus strains in vitro. Molecules. 2014;19(5):6583-96.