

Antibacterial and ciprofloxacin -potentiation activities of *Berberis vulgaris* L. root extracts against some gram-negative pathogenic bacteria

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ABSTRACT

Background and aims: Some medicinal plants particularly those with antimicrobial properties contain compounds that potentiate the activity of antimicrobials against pathogenic bacteria. This study was planned to search the antibacterial activities of the hydro alcoholic and chloroform extracts of *Berberis vulgaris* L. root and their synergistic effects with ciprofloxacin against some gram-negative pathogenic bacteria.

Methods: After grinding, powder of *Berberis vulgaris* L. roots were extracted with ethanol 85% and chloroform by maceration method. 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 - Naphthylamide (PA β N) as a positive control and efflux pumps inhibitor (EPI).

Results: MIC determination indicated that the hydro alcoholic and chloroform extracts from *Berberis vulgaris* L. root were able to inhibit the growth of all the studied bacteria within a concentration range of 25000 to 75000 and 1562 to 6250 μ g/mL, respectively. Synergistic effects were noted between the extracts from *Berberis vulgaris* L. root extracts and ciprofloxacin on all tested bacteria.

Conclusion: *Berberis vulgaris* L. root extracts act as an antibacterial agent and potentiate ciprofloxacin effects on examined pathogenic bacteria. The present investigation brings about primary information's for the possible use of these extracts in association with fluoroquinolones.

Keywords: Antibacterial activities, *Berberis vulgaris* L., Gram-negative bacteria.

Original article

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

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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 (PAβN) is a compound that has become known as a chemical efflux pump inhibitors (EPIs)⁴. PAβN selectively inhibit 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 is 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 L. is a medicinal herb that traditionally is used for its medicinal properties and is well known in Iran and most countries in the world. The different parts of this plant including root, leaf, bark and fruit have been used widely as folk medicine for the treatment and prevention of various diseases including cardiovascular, gastrointestinal, respiratory, skin, renal and infectious diseases.⁷ Previous studies have also been carried out on chemical composition of the *B. vulgaris* that showed the most important constituents of this plant are isoquinoline alkaloids such as Berberine.⁸

Some reports indicated that *Berberis* species contain compounds that can enhance antibacterial actions of some antimicrobial agents.⁹

This study was planned to search the antibacterial activities of the hydroalcoholic and chloroform extracts of *Berberis vulgaris* L. root and their synergistic effects with ciprofloxacin as a fluoroquinolone representative against some gram-negative pathogenic bacteria.

METHODS

Whole trees of *Berberis vulgaris* L. collected by local people in Khorasan province and transferred to department of botany of Shahrekord University for final confirmation. Dried roots were prepared in microbiology laboratory of veterinary college of the university. After grinding it was used by maceration method for extract preparation. Briefly, ethanol 85% and chloroform added to powdered plant roots in conical flasks and allowed to stand at room temperature for a period of 2 and 3 days for chloroform and ethanol solvents, respectively. Daily filtration and refreshing of the solvent were followed for the ethanol extraction, but for chloroform solvent one stage filtration process were used. The collected filtrates were evaporated by incubating in an incubator at 34°C, in the case of ethanol and in biolaminar safety hood in the case of chloroform solvents.¹⁰ All extracts were kept at 4°C for further investigations.

The studied microorganisms included the reference strains of *Pseudomonas aeruginosa* ATCC 9027 and *Acinetobacter baumannii*, NCTC 13305 kindly delivered by Dr B. Zamanzad and Dr A. Gholipour (Department of Microbiology, Shahrekord Medical school), *E Coli* ATCC 25922 and *Salmonella enteritidis* RTCC 2465 kindly delivered by Dr H. Motamedi (Department of Microbiology, College of Basic Sciences, Shahid Chamran University), were kept in Lauria Bertani broth (LB broth) at 4°C and sub cultured on appropriate agar plates 24 h 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-naphthylamide (PAβN) (Sigma-Aldrich) as microbial growth indicator and efflux pumps inhibitor (EPI) were used, respectively.

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.¹¹ 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 to obtain an inoculum of 5×10^5 CFU/well. The first well of each 96 well micro plate rows 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 considered as positive and negative controls.

The inoculated micro plates were aerobically incubated in shaking for about 18 h at 37 °C. The lowest concentration that inhibits visible growth after incubation was defined as MIC. For verifying synergistic

activity of ciprofloxacin with our extracts, activity of ciprofloxacin with extract associations was compared with that of ciprofloxacin plus PAβN (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).^{12,13} All assays were performed in duplicate.

RESULTS

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

Table 1: Minimum inhibitory concentrations (MICs), (µg/mL) of ciprofloxacin (cip), and Phenylalanine arginine β-aphthylamide (PAβN) in the absence and presence of *Berberis vulgaris* L. root extracts against some gram negative bacteria*

Combination	Cip.	Eth.E.+ cip.	Eth.E.	Ch.E.+ cip.	Ch.E.	PAβN	PAβN+ cip.
Bacteria							
<i>Ps. aeruginosa</i>	1	0.2	3125	0.2	2340	3.75	0.125
<i>S. enteritidis</i>	0.008	0.001	75000	0.002	3125	5	0.05
<i>E- coli</i>	0.007	0.003	25000	0.0002	6250	6.25	0.002
<i>A. baumannii</i>	0.26	0.05	75000	0.8	1562	6.25	R

*Synergistic combinations appeared as bold numbers; *Eth.E., Ch.E. + cip. and R Stand for hydroalcoholic extracts, chloroform extract, ciprofloxacin and resistant respectively.

The MICs of hydroalcoholic and chloroform extracts and drug combinations against examined bacteria are appeared in Table 1. The chloroform extract of the extracts investigated generally showed a greater activity against examined bacteria (MIC, 1562 to 6250 µg/mL). By MIC

determination, chloroform extract of *Berberis vulgaris* L. root showed synergistic activity with ciprofloxacin against all of the examined bacteria other than *A. bummani* (Table 1). This synergy showed by hydro alcoholic extract against all tested bacteria.

In *A. bummani*, chloroform extract did not show synergy with ciprofloxacin but PA β N also did not reduce the MIC of ciprofloxacin (Table 1).

DISCUSSION

Pathogenic microorganisms have different ways to deal with antimicrobial agents such as antibiotics and indiscriminate uses of these compounds have led to the development of drug resistance.

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* L. root hydroalcoholic and chloroform extracts for antibacterial activity and possible synergy with ciprofloxacin.

It is reported that examined bacterial strains tested with a combination of *Berberis vulgaris* L. extracts, ciprofloxacin and PA β N all contains multidrug resistance efflux pumps.^{3,14}

It appears that extracts of *B. vulgaris* L. root prevent the growth of all examined bacterial strains in 1562 to 75000 μ g/mL concentration range (Table 1). The lowest MIC value (1562 μ g/mL) was obtained with the chloroform extract of *B. vulgaris* L. root against *A. baumannii*. Reports show other plant extracts contain compounds that potentiate antibiotic activity or have antibacterial activity.^{15,16} Also, it is reported that essential oil from a Corsican plant, *Helichrysum italicum*, reduced the MIC of chloramphenicol against *Enterobacter aerogenes*, *A. baumannii* and *P. aeruginosa*.¹⁶

In terms of antibiotic potentiation with ciprofloxacin, the chloroform extract of *B. vulgaris* L. root showed the best activity against *E. coli*, *Ps. aeruginosa* and *S. enteritidis*, respectively. The best

synergistic activity of hydroalcoholic extract was recorded against *S. enteritidis*, *Ps. aeruginosa* and *A. baumannii*. These observations may imply that *B. vulgaris* L. root 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, many reports indicate activity of other plant extracts that synergised with this agent.^{1,17}

In *A. baumannii*, PA β N and association of chloroform extract had no effect on MIC of ciprofloxacin. Since the presence of AcrB efflux pumps confers resistance to the cells against PA β N.¹⁸ This may imply that our examined strain of *A. baumannii* over expressed AcrB efflux pumps and chloroform extracts from *B. vulgaris* L. root may also behave such 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.¹⁹

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* L. with other antibiotics were noted on other bacteria also indicate that extracts of this herb can act as efflux pump inhibitor.²⁰

Phytochemical screening of barberry's roots have also revealed the presence of alkaloids, flavonoids, saponin, phenolic contents, terpenoids and cardiac glycosides. Some of them have antimicrobial effects beside other benefits.⁷

However, a detailed study on active constituents, phytochemical and toxicological properties of *B. vulgaris* L. root is suggested for evaluating its safety.

Taking together, from our results we can suggest that using the extracts of *B. vulgaris* L.

root in associations with fluoroquinolones might be helpful in treating infections caused by examined pathogenic bacteria.

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

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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