Original Article

In Vitro Anti-*Helicobacter pylori* Activity of Berberine and Barberry Extracts: A Preliminary Report

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Abstract

The berberine accumulation in *Berberis vulgaris* and antibacterial activity of plant extracts against *Helicobacter pylori* were examined. The antibacterial activities of berberine, the main alkaloid of *B. vulgaris*, and standard antimicrobials metronidazole and tetracycline were also determined. Berberine content was investigated using high-performance liquid chromatography. The antibacterial activity of plant extracts, berberine and reference antibiotics was tested by the broth microdilution method. The highest amount of berberine was found in the root bark (3.99 g/kg, dry wt). The plant extracts tested exhibited in vitro antibacterial activity against *H. pylori,* but the activities were lower than those of berberine.

Keywords

HPLC, berberine, *Berberis vulgaris*, *Helicobacter pylori*, antibacterial activity

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Helicobacter pylori is the primary etiologic agent responsible for gastroduodenal diseases in humans: chronic active type B gastritis, gastric ulcers, and gastric cancer.[1]

According to traditional medicine, *Berberis vulgaris* L. is used to treat fever, cough, liver disease, depression, hyperlipidemia, and hyperglycemia. The most important alkaloid of this plant is berberine (BBR), which shows antimicrobial activity towards a variety of organisms, including bacteria, fungi, protozoans, and viruses.[2] Chu et al[3] showed the important action of berberine as an enhancer of the action of levofloxacinin the treatment of methicillin-resistant *Staphylococcus aureus* infections.[3] Also of interest is that a simple chemical modification of berberine to its 13-hexyl derivative increases its activity by up to 100-fold against a few bacterial strains.[4] Berberine combined with triple therapy can be an option for increasing *H. pylori* eradication rates and reducing overall the incidence of therapy-related adverse effects.[5]

The aim of this research was to investigate berberine accumulation in *B. vulgaris* and anti-*H. pylori* activity of barberry extracts obtained from wild and cultivated plant populations. The antibacterial activities of berberine and the standard antimicrobials metronidazole and tetracycline were also determined. The results of berberine accumulation in the examined plant organs are shown in Table 1. The yield of crude methanol extract (CME) varied among organs. The highest was obtained from the leaf extract, followed by the bark of branches, and the lowest from the root bark. However, the root bark accumulated the highest amount of berberine. The berberine content of stem bark was higher at the fruit-forming stage than that at the blooming stage; the leaves had the lowest content, as expected.[6] Our results are not in agreement with those suggesting that the maximum yield of berberine can be achieved by harvesting *Berberis* in the summer season, as compared with winter.[7] The anti-*H. pylori* activities of barberry extracts, berberine, and reference antibiotics are presented in Table 2.

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Table 1. The Content of Berberine in Organs of *B. vulagris*.

	Leaf[a]	Bark of branches	Bark of root
Yield of CME (%)	20.0	7.2[a]	-
		9.3[b]	5.0[b]
		4[c]	4.4[c]
BBR (g/kg dry plants)	0.05 ± 0.01	2.0 ± 0.02[a]	-
		2.7 ± 0.01[b]	3.4 ± 0.06[b]
		2.3 ± 0.09[c]	4.0 ± 0.07[c]

[a]Wild plants at blooming stage.
[b]Wild plants at fruit forming stage.
[c]Cultivated plants at fruit forming stage.

The examined extracts possessed antibacterial activities with minimum inhibitory concentration (MIC) values ranging from 512 to 1024 µg/mL and minimum bactericidal concentration (MBC) values from 512 to 4096 µg/mL. Our results correspond with the literature data.[8] It is interesting to note that berberine exhibited higher antibacterial activity than that of the commercial antibiotics. On the other hand, the leaves accumulate significantly lower amounts of berberine in comparison with the bark of branches and root, but the leaf extract exhibited the lowest MIC value. Stermitz et al[9] had reported that flavonolignan and porphyrin, from berberine-containing *Berberis* species, form potent synergistic complexes with sub-inhibitory concentrations of berberine. These synergistic complexes could be a bacterial multidrug resistant pump inhibitor.[9] Further research into this is required.

In conclusion, the largest amount of berberine was in the bark of the root, harvested at the fruit-forming stage. The plant extracts exhibited in vitro activity against *H. pylori,* but the activities were lower than those of berberine.

Table 2. Antibacterial Activity of *B. vulagris* Extracts, Berberine, and Reference Antibiotics (µg/mL).

Sample	MIC	MBC
Leaf[a]	512	4096
Bark of branches[a]	1024	4096
Bark of branches[b]	1024	2048
Bark of branches[c]	1024	1024
Bark of root[b]	512	512
Bark of root[c]	512	512
Berberine	256	256
Metronidazole	512	512
Tetracycline	512	512

[a]Wild plants at blooming stage.
[b]Wild plants at fruit forming stage.
[c]Cultivated plants at fruit forming stage.

Experimental

Plant Material and Chemicals

The leaves, root bark, and stem bark of *B. vulgaris* were collected from wild and cultivated healthy specimens at Bela Palanka (wild) and Niš (cultivated), Serbia in 2017. Voucher specimens (accession numbers 17 464 and 17 465) are deposited at the Herbarium of the Department of Botany, Faculty of Biology, University of Belgrade-Herbarium Code BEOU. The plant material was collected (a) at the blooming stage (7th June) and (b) at the fruit-forming stage (27th October). All chemicals, reagents, and standards were of analytical reagent grade and were purchased from the Sigma-Aldrich Chemical Company and Thermo Fisher Scientific, USA.

Extract Preparation

The samples were cleaned, air-dried, powdered, and extracted with methanol (ratio of solvent to plant material 10:1). Extraction was performed 4 times for 15 minutes in an ultrasound bath, followed by maceration for 24 hours at room temperature. The extracts were filtered and evaporated to dryness.

Extraction of Berberine

Berberine was extracted according to the method described by Pradhan et al.[10]

Prepared of Standard Solution

A 10 mg/mL stock solution of berberine was prepared in methanol. A standard series was prepared in concentrations of 400, 800, 1200, 1600, and 2000 µg/mL.

HPLC Conditions

Quantification and qualification of berberine were performed using a high-performance liquid chromatograph Agilent-1200 series with a diode array detector for multiwavelength measurements. The column was thermostatted at 30°C. After injecting 10 µL of the sample, separation was performed on an Agilent-Eclipse XDB C-18 4.6 · 150 mm column. The solvent system for isocratic elution was (A) HCOOH and (B) acetonitrile (70:30), with a flow rate of 1 mL/min and a detection wavelength of 370 nm.

Antibacterial Testing

The barberry extracts, berberine, metronidazole, and tetracycline were tested for their activity against *H. pylori* NCTC 11638. The inocula of the bacterial strain were prepared from overnight broth cultures and the suspensions were adjusted

to 0.5 McFarland standard turbidity (corresponding to 10^8 CFU/mL, depending on genera-consensus standard by the Clinical and Laboratory Standards Institute).[11]

Micro-Well Dilution Assay

A micro-well dilution assay, slightly modified, was used to determine the MIC and MBC in vitro, as previously described.[12] Mueller-Hinton broth was used and supplemented with 10% horse serum. The final volume in each well of microtiter plates was 100 µL. The plates were incubated at 37°C under microaerobic conditions in a moist atmosphere for 3 days. In order to improve the broth method, equal volumes (100 µL) of double strengthened Christensen's urea broth were added into each well after incubation, and plates were additionally incubated in an aerobic atmosphere at 37°C. The plates were examined visually for color change 3 hours after Christensen's urea broth addition. To determine MBC, the broth was taken from each well without visible growth and inoculated in Columbia agar for 3 days at 37°C.

Declaration of Conflicting Interests

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