RESEARCH ARTICLE

Antileishmanial Effect of Berberine and extracts of *Berberis vulgaris* on the Growth of Leishmanial Species

Husain F. Hassan, Zina K. Najim*

Department of Biology, College of Science University of Kirkuk, Iraq

Received: 18th March, 2020; Revised: 24th April, 2020; Accepted: 28th May, 2020; Available Online: 25th June, 2020

ABSTRACT

The present investigation was revealed that the Berberine (0.01, 0.1, 1 mg/cm^3), aqueous extracts and alcoholic extract (1,10,100 mg/cm^3) of *Berberis vulgaris* roots were inhibitory on the growth of *Leishmania tropica*, *L. donovani,* and *L. infantum* promastigotes. High-pressure liquid chromatography (HPLC) analysis of the plant extracts proved to contain Berberine as an active compound. Berberine and *B. vulgaris* root extracts were caused a significant decrease in total nucleic acid contents of three leishmanial species, thus, considering to be an excellent agents for antileishmanial chemotherapy.

Keywords: Antileishmanial effect, Berberine, Berberis vulgaris extracts.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.2.8

How to cite this article: Hassan HF, Najim ZK. Antileishmanial effect of Berberine and extracts of *Berberis vulgaris* on the Growth of Leishmanial Species. International Journal of Pharmaceutical Quality Assurance. 2020;11(2):237-241.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Leishmaniasis is a vector born disease caused by hemoflagellate protozoan parasite of the genus Leishmania.[1] The clinical manifestation ranges from simple cutaneous lesions to life-threatening visceral forms.[2] According to the World Health Organization (WHO), the disease is endemic in 88 countries, with approximately 350 million people at risk.[3]

Pentavalent antimonials are the drug of choice for the treatment of Leishmaniasis,[4] but frequently failed to eradicate the parasite due to toxicity, adverse effects, and lower efficacy.[5] It is generally accepted that there is a great need to develop new chemotherapy drugs for the treatment of the disease.[6] Medicinal plants of several regions of the world have shown promise in use for the treatment of the disease.[7] The present study was undertaken to use the effect of extract of *B. vulgaris* on the *in vitro* growth of leishmanial species.

MATERIALS AND METHODS

Parasite Growth

The promastigote forms of *Leishmania Tropica*, *L. donovani*, and *L. infantum* were grown at 26°C in PY medium, as described by H. Hassan, *et al*.[8]

Collection of Plants

The aerial parts of the *Berberis vulgaris* were collected from Kirkuk city, Iraq. The plant parts (leaves, roots, and fruits) were cleaned with water to remove dirt and placed in a class with diluted chlorine (1%) for 1-minute, then were dried at room temperature, and then kept in free of moisture conditions in paper envelops.[9] A stock solution (1 gm/mL) from each aqueous and alcoholic extract of leaves, roots, and fruits was used to prepare the final concentration (1, 10, 100 mg /mL) in the growth medium. The solution of extracts and Berebrine were sterilized by filtration through 0.45 µm membrane filter.

Estimation of Total Protein and Nucleic Acid

The determination of total protein was carried out using Lowery method,[10] whereas the method of K. W. Giles, *et al.,*[11] were used for estimation of total nucleic acid.

Alkaloids Extraction

Extraction was carried out according to the methodology described by N Cabezas, *et al.,*[12] with some modifications. Oven-dried and powdered leaves (100 grams), stems (300 grams) and roots (300 grams) of *B. vulgaris* were sequentially extracted (24, 48, and 72 hours) with methanol at room temperature. Methanolic extracts were evaporated in vacuum at 40°C, and the residue was reconstituted with 200 mL 10% HCl for 1-hour under agitation (orbital shaker, MS-NOR, Taiwan), and allowed to stand for 12 hours at 10°C, and then filtered. The filtrate was washed with CHCl3 (5 × 100 mL). The aqueous phase was adjusted to pH 10 with NH_4OH and extracted with $CHCl_3$ (5 × 100 mL). The solvent was evaporated for obtaining the extract containing alkaloids.

Alkaloid Standards

Berberine (purity, > 90%) was purchased from Sigma Aldrich (St. Louis, USA), and all solvent used for extraction were analytical grade. Calafatine was obtained from Laboratorio

*Author for Correspondence: zikhn87@gmail.com

de Productos Naturales, Universidad de Magallanes (Punta Arenas, Chile). HPLC grade acetonitrile, methanol-water, and formic acid were purchased from Merck (Darmstadt, Germany).

HPLC Electrospray Ionization-Mass Spectrometer (ESI-MS)/MS

The chromatographic separation was carried out using an RP-C18 BioSuite column (2.1 × 150 mm, 3 µm), injecting 10 µL at 0.2 mL/min and 35ºC. 0.01 g of standards and sample extracts were dissolved in 10 mL of methanol and submitted to LC-MS/MS. The chromatographic separation was performed using a linear gradient solvent system consisting of 0.1% formic acid (A) and acetonitrile (B). The linear gradient was composed of 0–3 minutes 10% B, 3 to 35 minutes 10 to 70% B, 35 to 40 minutes 70% B, 40 to 50 minutes 70 to 10% B, then again, under the initial conditions (10% B) for 10 minutes. Each standard was injected with electrospray ionization (ESI) source into the mass spectrometer (LC-MS-MS Shimadzu Prominence coupled at mass spectrometer Applied Biosystems/ MDS Sciex3200 Qtrap, Massachusetts, USA). The ion source temperature was set to 400ºC, and the capillary voltage was 5.5 kV. For alkaloids determination, data were collected as positive-ion spectra by means of enhanced mass scan (EMS) over a m/z 100 to 1000 Da range at 1000 Da/s and enhanced product ion (EPI) over a m/z 50-1,000 Da range at 4,000 Da/s. The CUR gas was 20 psi, GS1 30 psi, and GS2 60 psi. In addition, the content of alkaloids was performed, calculating the relative amounts of the individual alkaloids present in the plant extracts. The ion intensities were extracted at the m/z values of the molecular (M+) or pseudo-molecular (M+H)+ ions of the corresponding detected compounds. The relative ion peak area of each compound from the sample was compared to the relative ion peak area of the total alkaloids

RESULT AND DISCUSSION

As indicated in Tables 1 to 3 Berberine, aqueous and alcoholic extract of *B. vulgaris* were inhibitory *in vitro* for the promastigote of all three species of Leishmania. On the other hand, the aqueous and alcoholic extract of *B. vulgaris* leaves and fruits were relatively inactive against three species of Leishmania. Berberine exhibited antileishmanial activity and at 1mM reduced the number of *L. tropica, L. donovani,* and *L. infantum* promastigotes by 4 days by 73%, 63%, and 57%, respectively.

Aqueous extract of *B. vulgaris* roots was almost equally effective against leishmanial promastigotes and at 100mg/ ml reduced the number of, *L. tropica, L. donovani* and *L. infantum* promastigotes by 79%, 85% and 81% respectively. The alcoholic extract of *B. vulgaris* roots was also a potent inhibitor and caused at 100 mg/mL 90, 92, and 89% growth inhibition of *L. tropica, L. donovani, and L. infantum,* respectively.

Table 1: Effect of Berberine, aqueous and alcoholic extract of *B. vulgaris* root on the growth of *L.tropica* promastigotes after 96 hours

	Concentrations	*& Growth*	*Inhibition %*	*IC_{50}*
Berberine	0.01 mM	85	15	
	0.1 mM	62	38	0.5 mM
	1mM	27	73	
Aqueous extract	1 mg/mL	71	29	60 mg/mL
		45	55	
		21	79	
	1 mg/mL			
Alcoholic extract	100 mg/mL	62	29	
		51	55	
		10	79	
	1 mg/mL		38	60 mg/mL
	10 mg/mL		49	
			90	
	100 mg/mL			

Table 2: Effect of Berberine, aqueous and alcoholic extract of *B. vulgaris* root on growth of *L.donovani* promastigotes after 96 hours

	Concentrations	*& Growth*	*Inhibition %*	*IC_{50}*
Berberine	0.01 mM	92	8	
	0.1 mM	74	26	0.5 mM
	1mM	37	63	
Aqueous extract	1 mg/mL	72	28	60 mg/mL
	10 mg/mL	56	44	
		15	85	
	1 mg/mL			
Alcoholic extract	100 mg/mL	85	15	
		69	31	60 mg/mL
		8	92	
	1 mg/mL			
	10 mg/mL			100 mg/mL
	100 mg/mL			

Table 3: Effect of Berberine, aqueous and alcoholic extract of *B. vulgaris* root on the growth of *L.infantum* promastigotes after 96 hours

	Concentrations	*& Growth*	*Inhibition %*	*IC_{50}*
Berberine	0.01mM	93	7	
	0.1 mM	79	21	0.75 mM
	1 mM	43	57	
Aqueous extract	1 mg/mL	64	36	
	10 mg/mL	51	49	60 mg/mL
	100 mg/mL	19	81	
Alcoholic extract	1 mg/mL	69	31	
	10 mg/mL	56	44	67mg/mL
	100 mg/mL	11	89	

Table 4: Effect of Berberine, aqueous and alcoholic extract of B.vulgaris roots upon L.tropica nucleic acid content

Treatment	*µg DNA/10^8 cells*	*Inhibition %*	*µg RNA/10^8 cells*	*Inhibition %*
Control	12 ± 1	–	3	–
Berberine	7.3 ± 1	39	60 ±	35
Aqueous	7.5 ± 2	37	39 ± 1	37
extract			37.8 ± 2	
alcoholic	7.2 ± 1	40		36
extract			38.4 ± 3	

Table 5: Effect of Berberine, aqueous and alcoholic extract of *B. vulgaris* roots upon *L. donovani* nucleic acid content

Treatment	*µg DNA/108 cells*	*Inhibition %*	*µg DNA/108 cells*	*Inhibition%*
Control	1	–	58 ± 3	–
Berberine	18 ±	41	2	36
Aqueous extract	0.610 ± 1 10.2 ± 2	43	37.2 35.9 ± 2	38
alcoholic extract	9.9 ± 1	45	348 ± 4	40

Table 6: Effect of Berberine aqueous and alcoholic extract of *B. vulgaris* roots upon *L. infantum* nucleic acid content

Treatment	*µg DNA/10^8 cells*	*Inhibition%*	*µg RNA/10^8 cells*	*Inhibition%*
Control	1	–	4	–
Berberine	± 28	42	88 ±	36
Aqueous extract	2 ± 16.2 3 ± 15.1	46	56.3 ± 2 54.5 ± 3	38
Alcoholic extract	2 ± 14.8	47	51.9 ± 1	41

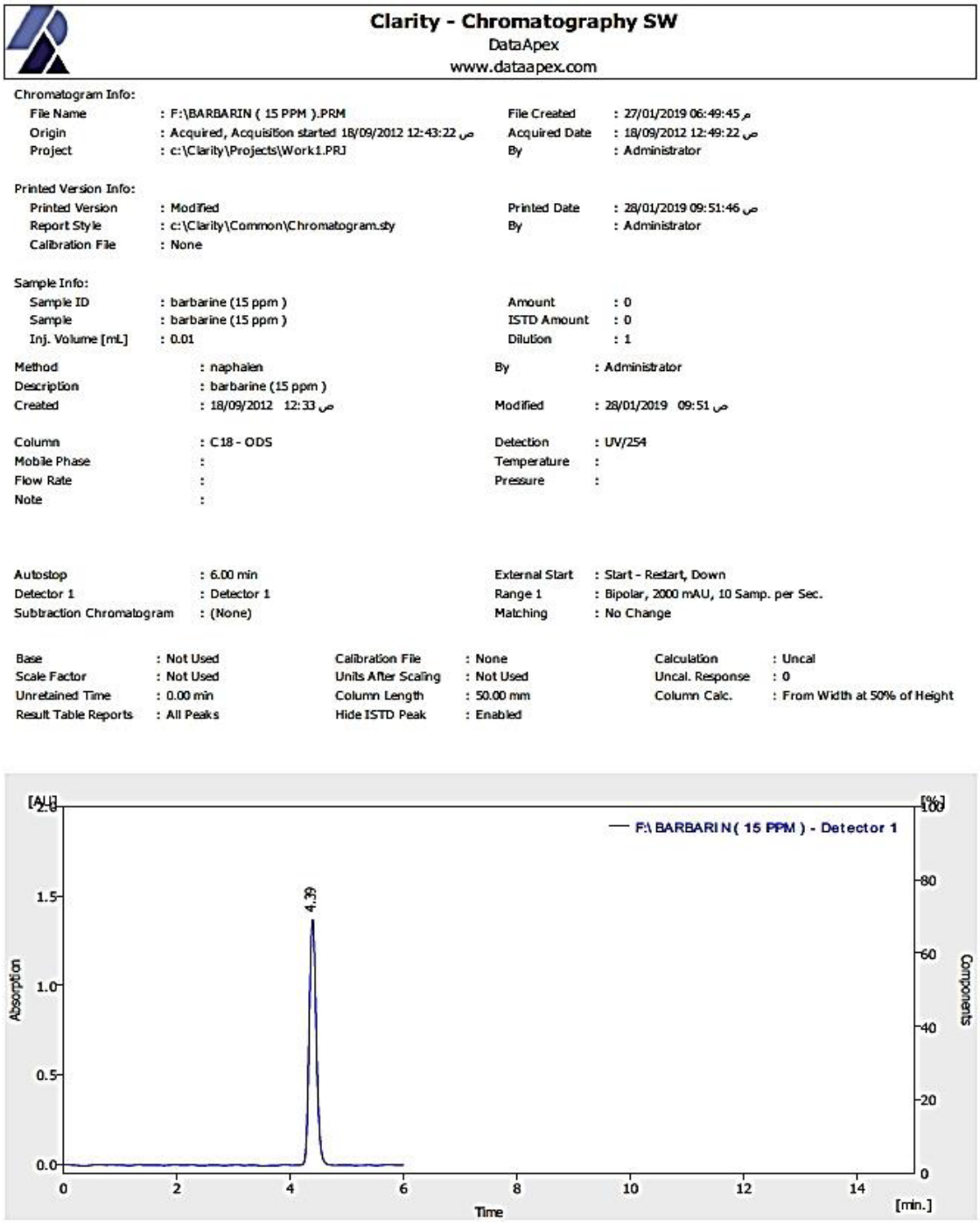

Figure 1: HPLC profile of Beberine

Table 7: Retention of Berberine

	Resit Table (Until- F:Ibarbarin (IS PPM-Detector I)					
	Reten time (min)	*Area (m.AU.s)*	*Height (mAU)*	*Area (%)*	*Compound Name*	*Correction Factor*
1	4.393	2,822.399	562.733	100.0		
	Total	2,822.399	562.733	100.0		

Clarity - Chromatography SW
DataApex
www.dataapex.com

Chromatogram Info:
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Project : c:\Clarity\Projects\Work1.PRJ
File Created : 27/01/2019 06:48:31 م
Acquired Date : 08/03/2016 11:47:38 ص
By : Administrator

Printed Version Info:
Printed Version : Modified
Report Style : c:\Clarity\Common\Chromatogram.sty
Calibration File : None
Printed Date : 28/01/2019 09:54:33 ص
By : Administrator

Sample Info:
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Sample : sample 1
Inj. Volume [mL] : 0
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
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Created : 31/08/2006 04:43 م
By : DataApex Ltd.
Modified : 28/01/2019 09:54 ص

Column : c18
Mobile Phase :
Flow Rate : 1 ml/min
Note :
Detection :
Temperature :
Pressure :

Autostop : 15.00 min
Detector 1 : Channel 1
Subtraction Chromatogram : (None)
External Start : Start - Stop, Up
Range 1 : Bipolar, 10000 mV, 10 Samp. per Sec.
Matching : No Change

Base : Not Used
Scale Factor : Not Used
Unretained Time : 0.00 min
Result Table Reports : All Peaks
Calibration File : None
Units After Scaling : Not Used
Column Length : 50.00 mm
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0
Column Calc. : From Width at 50% of Height

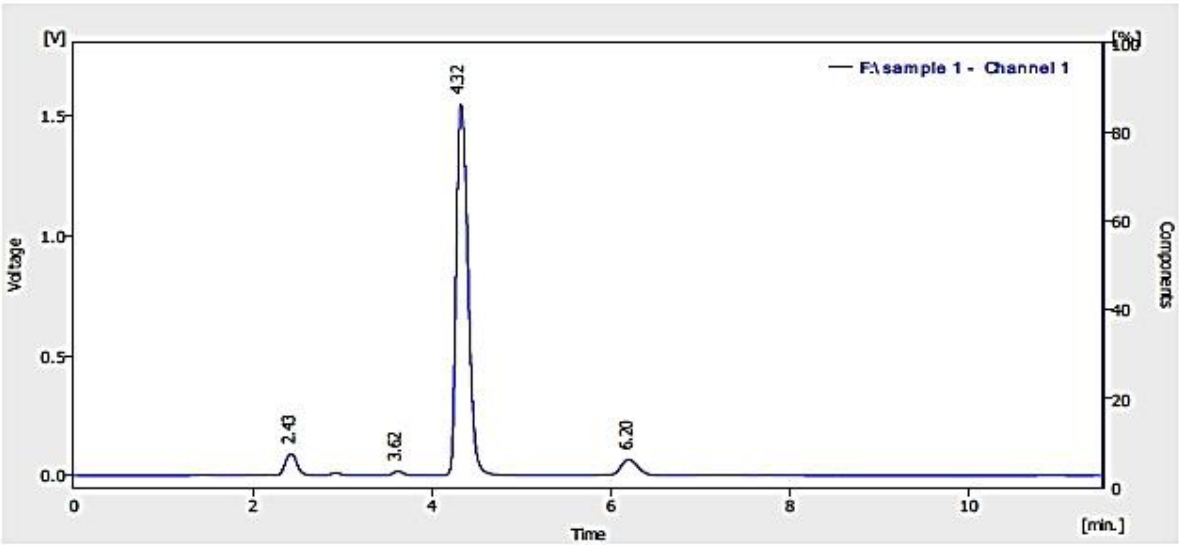

Figure 2: HPLC profile of B.vulgaris root extracts

Table 8: Retention time of Berberis vulgaris roots

Resit Table (Until- F:IBARBARIN (IS PPM) - Detector I)						
	Reten time [min]	*Area [mV.s]*	*Height [mV]*	*Area [%]*	*Compound name*	*Correction factor*
1	2.433	451.818	62.469	3.6		
2	3.617	132.806	16.970	1.1		
3	4.317	11,289.484	1,375.087	90.02		
4	6.200	639.416	56.674	5.1		
	Total	12,513.524	1,511.200	100.0		

- To determine the effect of Berberine, aqueous, and alcoholic extract of *B. vulgaris* roots, we have measured the nucleic acid content and protein synthesis of drug-treatedd promastigotes (Table 4 to 6). Protein content did not reduced by Berberine, aqueous and alcoholic extract of *B. vulgaris* roots, whereas nucleic acid content (RNA and DNA)were decreased by more than 35% by all the three drugs.

As presented in Figures 1 and 2, Tables 7 and 8, the HPLC profile, the standard, and B. vulgaris root extracts have shown the presence of Berberine.

- Therapeutic evaluation for medicinal plants is essential because of the growing interest in alternative therapies and the use of natural products.[13] It is also generally accepted that there is a great need to develop more effective and less toxic drugs for the treatment of Leishmaniasis and the recent efforts in the search for new drugs alternatives has been the demonstration that many medicinal plants were active and safe in cutaneous and visceral Leishmaniasis.[14]
- The data presented here suggest that Berberine and *B. vulgaris* inhibit the growth of leishmanial promastigotes by inhibition of nucleic acid contents by mechanisms related to decrease in the net amount of RNA per cell and its loss of function or may in part due to the breaking the covalently bonds between purine and pyrimidine nucleotide in the DNA. The use of Berberine or *B. vulgaris* extract would be an excellent candidate agent for antileishmanial chemotherapy.

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