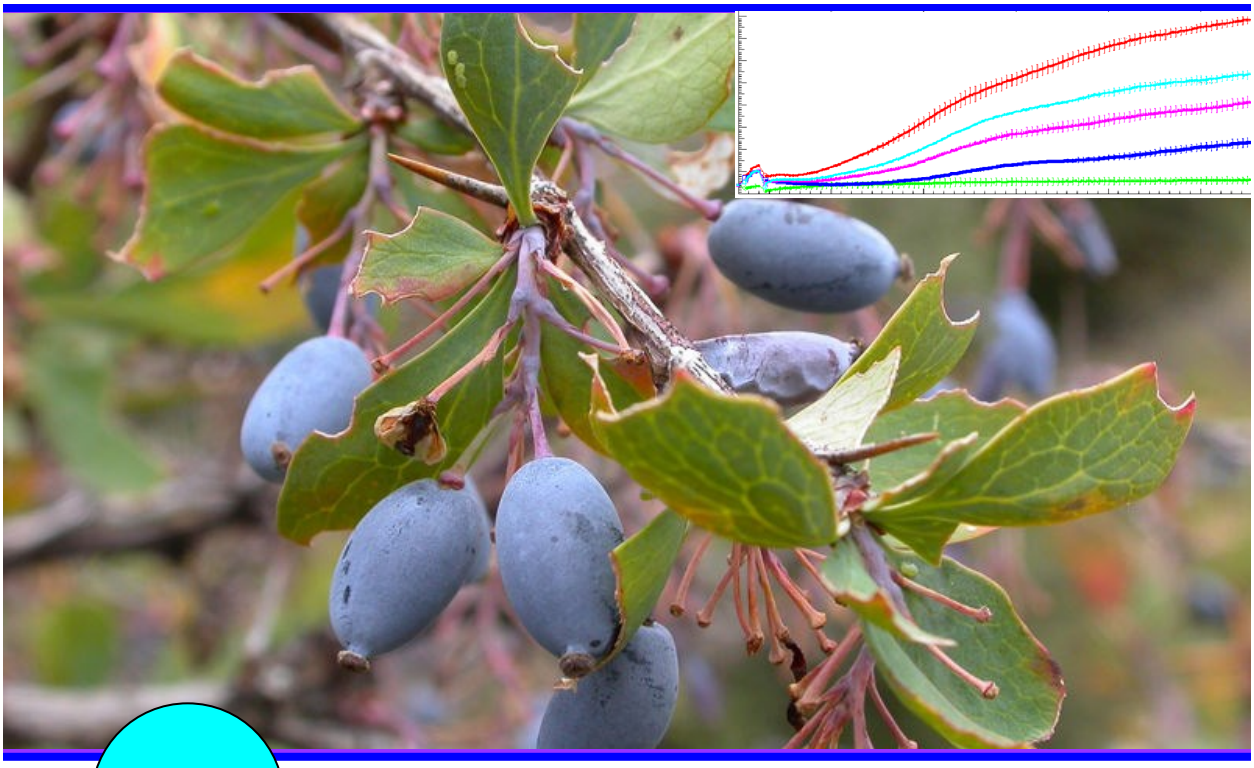

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Isolation of phytoconstituents and evaluation of biological potentials of *Berberis hispanica* from Algeria

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Abstract

The aim of this study was to isolate the phytoconstituents and evaluate the biological activities of *Berberis hispanica*. Three phenolic compounds (tamarixetin, caffeic acid and rutin) were isolated from *B. hispanica.* The structures of the pure compounds were elucidated by spectroscopic and mass-spectrometric analyses, including 1D-, 2D-NMR, and HPLC-TOF/MS. In addition, antimicrobial, anti-oxidant and anti-proliferative effects of the extracts, some fractions and isolated compounds were evaluated. The extracts of *B. hispanica* were evaluated against six bacterial strains and exhibited the highest activity against *Klebsiella pneumonia* (15 mm at 100 mg/mL). Fraction T36 (IC_{50}<5 µg/mL) from the *n*-butanol extract displayed higher radical scavenging activity than butylated hydroxytoluene. The isolated compounds were evaluated for their anti-proliferative effects against human cervical adenocarcinoma (HeLa) cell line by real time cell analyzer assay and tamarixetin exhibited the remarkable effect (IC_{50}<50 µg/mL) on HeLa cells. This study supports the documented medicinal effects of *B. hispanica.*

Introduction

Plant is considered as nature's chemical factory for providing the chemical compounds, and as a source of novel therapeutic compounds (Arora et al., 2003). Several studies have been reported on the antibacterial and anti-oxidant activities of the plant extracts, which may be due to the presence of the natural phenolic compounds (Silva et al., 2010; Prakash et al., 2009). Phenolic compounds play important roles in plants and disease resistance (Servili and Montedoro, 2002; Silva et al., 2006).

The genus *Berberis* (Berberidaceae) includes about 500 species distributed largely in Europe, Northeastern region of the United States, Asia and Africa (Harish, 2012; Mokhber-Dezfuli et al., 2014). Many studies have been reported on the medicinal properties of this genus. *I*t has been employed in cholecystitis, cholelithiasis, jaundice, dysentery, leishmaniasis, malaria, gall stones, hypertension, ischemic heart disease, cardiac arrhythmias and cardiomyopathies (Mokhber-Dezfuli et al., 2014). Different types of chemical constituents were found in the *B. jaeschkeana* and *B. thunbergii* species such as alkaloids, glycosides, flavonoids, steroids, saponins reducing sugars and terpenoids (Hussain et al., 2016; Alamzeb et al., 2013).

The plant *B. hispanica* is a deciduous thorny shrub. The infusion of the stem bark of this plant has been used in

traditional medicine to treat the gastro-intestinal infections, inflammation, liver and biliary disorders (El-Youbi et al., 2012a; Bellakhdar, 1997). But, a few studies have been reported on its biological activities and phytochemical analysis (El-Youbi et al., 2012b).

Therefore, this study was undertaken in order to investigate the biological activities of the extracts from *B. hispanica* by various methods, along with to isolate and identificate of its secondary metabolites, and to evaluate the antiproliferative effect of the isolated phenolic compounds on the growth of HeLa cell lines by xCELLigence method.

Materials and Methods

General experimental procedure

NMR measurements were performed on a Bruker Avence III spectrometer in DMSO-d_6, $CDCl_3$ or CD_3OD (^{1}H-NMR 600 MHz; ^{13}C-NMR 150 MHz). Chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard. HPLC-TOF/MS spectrum was recorded in the negative ion mode on an Agilent 6210 spectrometer. ESI-MS analyses were performed in the positive ion mode within the m/z range 100–2000, using a LTQ XL Linear Ion Trap 2D instrument (Thermo Scientific, USA) equipped with Xcalibur® software (Thermo Scientific, USA). Column chromatography was carried out on silica gel (Merck, 60–230 mesh) in glass columns in open atmosphere pressure. For thin-layer chromatography, silica gel F254 (Merck) precoated plates were used. Compounds were detected under UV (254 nm) and sprayed with 5% ceric sulfate H_2SO_4 reagent, followed by heating at 105°C for 1–2 min.

Collection and identification of plant material

The aerial parts of *B. hispanica* was collected from the Cherea Mountain in July 2009 and was identified by Prof. Rebbas Khelaf, Department of Botany, Faculty of Science, M'Sila University, Algeria.

Extraction of plant material

The aerial parts of *B. hispanica* (963 g) were macerated at room temperature with ethanol–water (7:3, v/v) for three days to obtain the crude extract (87 g). The crude extract of ethanol–water (7:3, v/v) was dissolved in the distilled water (1 L), and first extracted with petroleum ether to yielded (4.3 g), after that the aqueous was extracted with chloroform to obtain (2.3 g), then with ethyl acetate (6.1 g). Finally with *n*-butanol to yielded (30.6 g). The ethyl acetate extract (5 g) was subjected on a silica gel column eluted with acetone–chloroform (1:3, v/v) to obtain 56 fractions (S), the precipitate from fraction S8 was purified by TLC eluted with acetone–chloroform (1:4, v/v) to afford compound **1** (tamarixetin). Fraction S24 was precipitated and purified by the TLC eluted with acetone-chloroform (2:4, v/v) to afford the compound **2** (caffeic acid). The *n*-butanol extract (25 g) was then loaded on a silica gel column. The column was eluted with hexane-dichloromethane (100:0-0:100), and dichloromethane-ethyl acetate (100:0-0:100), after that by ethyl acetate-methanol (100:0-0:100) solvent systems, which yielded 69 fractions (T), the fractions T45, T46 and T47 were combined (10 g), then loaded to a silica gel column, and eluted with chloroform-methanol (100:0-0:100) solvent systems, which yielded 25 fractions (X). Fraction X18 was purified by the TLC eluted with ethyl acetate–methanol –water (7.5:1:1, v/v/v) to afford compound **3** (rutin).

Chemicals used in the biological tests

Anhydrous sodium carbonate, Folin-Ciocalteu's phenol reagent, methanol were purchased from the Merck (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 2,6-di-*tert*-butyl-4-methylphenol (BHT), dimethyl sulfoxide (DMSO), trypsin-EDTA, fetal bovine serum (FBS), penicillin/streptomycin and Dulbecco's modified Eagle's medium-high glucose (DMEM-HG) were purchased from Sigma-Aldrich GmbH (Germany). All other chemicals were analytical grade and obtained from either Sigma or Merck.

Determination of anti-oxidant activity

Radical scavenging activity was determined according to the method described elsewhere (Blois, 1958). The solution of DPPH in methanol (0.004%) was prepared fresh daily and 1 mL of this solution in methanol was mixed with 1 mL of sample solution of varying concentrations. Each mixture was kept in the dark for 30 min at room temperature and the absorbance was measured at 517 nm against a blank on a UV visible light spectrophotometer (Rayleigh, UV-2601). BHT was used as a positive control. Scavenging of DPPH radical was calculated according to the formula:

$$\text{Radical scavenging \%} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Determination of total phenolic content

Total phenolic contents of the samples were analysed according to the Folin–Ciocalteu method (Singleton and Rossi, 1965). Briefly, 0.1 mL of sample solution, 0.2 mL of 50% Folin-Ciocalteu's reagent and 1 mL of 2% Na_2CO_3 was mixed in a tube. Then, the mixture was incubated at room temperature for 45 min. The absorbance of each mixture was measured at 760 nm. The same procedure was also applied to the standard solutions of gallic acid. Total phenolic contents were expressed as µg gallic acid equivalents per mg of the samples.

Determination of total flavonoids content

The total flavonoid content was determined by the method of aluminum trichloride using quercetin as a reference (Singleton et al., 1965). 1 mL of ethyl acetate and *n*-butanol extracts of aerial parts (1 mg/mL) was

mixed with 1 mL of 2% methanolic aluminum trichloride solution. The absorbance at 420 nm was read after 1 hour. All determinations were realized in couple. The absorption of quercetin standard solutions (15.6 to 1000 µg/mL) was measured in the same conditions. The results are expressed as equivalent quercetin µQE/mg of extract.

Determination of antibacterial activity

The following strains of bacteria were used as test microorganisms: *Acinetobacter oxy*, *Escherichia coli*, *Enterococcus faecalis*, *Proteus mirabilis*, *Klebsiella pneumoniae* ATTC 700603 and *Pseudomonas aeruginosa* ATTC 27853. These microorganisms were obtained from the Microbiology Laboratory, Faculty of Medicine, Badji Mokhtar University.

The dried extract was dissolved in sterile dimethyl sulfoxide (DMSO). Disc diffusion method was used to investigate the antimicrobial activity of the extracts (Toudert et al., 2009; Nascimento et al., 2000). The agar gel (MHA) was treated with the appropriate microorganism suspension (each microorganism was inoculated at a concentration of 10^6 colony forming units per mL), and the antimicrobial activities of the extracts that penetrate into the agar by diffusion was measured. The assays were based on the use of sterile discs filter paper (6 mm diameter) impregnated with 20 µL of the extract solution to be examined and allowed to dry at room temperature. A sterile disc impregnated with DMSO was used as a negative control. After incubation for 24 hours at 37°C, all plates were observed for zone of growth inhibition and the diameter of these zones was measured in millimeters. All experiments were performed in triplicates.

Determination of antiproliferative activity

Antiproliferative effects of the samples (rutin, tamarixetin, fraction T45-47 and *n*-butanol extract) were investigated on HeLa cell lines by xCELLigence real-time cell analyzer–single plate (RTCA-SP) instrument (Roche Applied Science, Switzerland) (Oke-Altuntas et al., 2016). Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 2%penicillin-streptomycin was used as the cell culture medium. First, 50 µL of the medium was added to each of a 96-well E-plate and kept in the hood for 15 min and then in an incubator for 15 min to allow the electrodes to equilibrate with the culture medium. The E-plate was inserted in the RTCA station of the incubator and a background measurement was taken, 100 µL HeLa cell suspensions were then added to the wells to obtain a 2.5 x 10^4 cell/well concentration in each well except three. 100 µL of medium was added to these three wells instead of the cell suspension. After 30 min, the E-plate was inserted in the RTCA station and a second set of measurements was initiated for 80 min. After this step, solutions of the samples that were prepared with DMSO (final concentration of DMSO was less than 1% in each of the wells) and medium were added to the wells to obtain final concentrations in each well, respectively. Upon completion, the final volume of the wells was 200 µL including the medium. After this, the main measurement period of 48 hours was initiated in 10 min intervals in triplicate.

Statistical analysis

The results were expressed as means ± standard deviations (SD). Statistical analyses were performed using the SPSS 11.5 (USA). Differences among means were done by analysis of variance (ANOVA). Pearson's correlation analysis was used for comparisons of total phenolic contents and the anti-oxidant activity of the fractions.

Results

Identification of isolated compounds

All the compounds shown in Figure 1 were isolated for the first time from this plant, and the spectral data led to identification of two flavonoids and phenolic acid:

Tamarixetin (**1**): yellow powder. ^{1}H-NMR (DMSO-d_6, 600 MHz): 3.82 (3H, s, 4'-OCH_3), 6.17 (1H, *d, J* = 1.76 Hz, H-6), 6.45 (1H, *d, J* = 1.76 Hz, H-8), 6.92 (1H, *d, J* = 8.80 Hz, H-5'), 7.67 (1H, *dd, J* = 8.80-2.35 Hz, H-6'), 7.73 (1H, *d, J* = 2.35 Hz, H-2'), 9.40 (1H, s, 3'-OH), 9.72 (1H, s, 3-OH), 10.76 (1H, s, 7-OH), 12.45 (1H, s, 5-OH); ^{13}C-NMR (DMSO-d_6, 150 MHz): 56.26 (4'-OCH3), 94.09 (C-8), 98.69 (C-6), 103.36 (C-10), 112.25 (C-2'), 116.06 (C-5'), 122.39 (C-1'), 122.24 (C-6'), 132.00 (C-3), 146.96 (C-2), 147.80 (C-3'), 149.25 (C-4'), 156.65(C-9), 161.24 (C-5), 164.52 (C-7), 175.05 (C-4). Molecular formula $C_{16}H_{12}O_7$. (Saewan et al., 2011).

Caffeic acid (**2**): yellow powder. ^{1}H-NMR (DMSO-d_6, 600 MHz): 6.16 (1H, d, *J*= 15.85 Hz, H-8), 6.74 (1H, d, *J*= 8.22 Hz, H-5), 6. 94 (1H, dd, *J*= 8.22- 2.35 Hz, H-6), 7.00 (1H, d, *J*= 2.35 Hz, H-2), 7. 40 (1H, d, *J*= 15.85 Hz, H-7), 9.10-9.50 (3H, s, 3, 4, 9-OH); ^{13}C-NMR (DMSO-d_6, 150 MHz): 115.07 (C-2), 115. 57 (C-8), 116. 18 (C-5), 121.55 (C -6), 126.14 (C-1), 144.98 (C-7), 145.99 (C-3), 148.56 (C-4), 168.28 (C-9). Molecular formula $C_9H_8O_4$ (Bhatt, 2011).

Rutin (**3**): yellow powder. HPLC-TOF/MS [M-H]$^-$ at *m/z* 609, ^{1}H-NMR (DMSO-d_6, 600 MHz): 0.98 (3H, d, *J*= 6.46 Hz, H-6'''), 3.08 (1H, *m*, H-4'''), 3.25 (1H, *m*, H-5'''), 3.30 (1H, *dd*, *J* = 9.39- 2.93 Hz, H-3'''), 3.40 (1H, *dd*, *J* = 2.93-1.76 Hz, H-2'''), 4.37 (1H, *d, J* = 1.76 Hz, H-1'''), 3.03- 3.28 (3H, *m*, H-2'', H-3'', H-4'', H-5''), 3.18, 3.27 (1H, *m*, H_a-6''), 3.68 (1H, d, *J* = 10.56 Hz, H_b-6''), 5.29 (1H, *d, J* = 7.63 Hz, H-1''), 6.13 (1H, *d, J* = 1.76 Hz, H-6), 6.32 (1H, *d, J* = 1.76 Hz, H-8), 6.80 (1H, *d, J* = 8.22 Hz, H-5'), 7.49 (1H, *d, J* = 1.76 Hz, H-2'), 7.52 (1H, *dd, J* = 8.22-1.76 Hz, H-6'); ^{13}C-NMR (DMSO-d_6, 150 MHz): 18.18 (C-6'''), 67.39 (C-6''), 68.69 (C-5'''), 70.73 (C-4''), 70.79 (C-2'''), 70.99 (C-3''''),

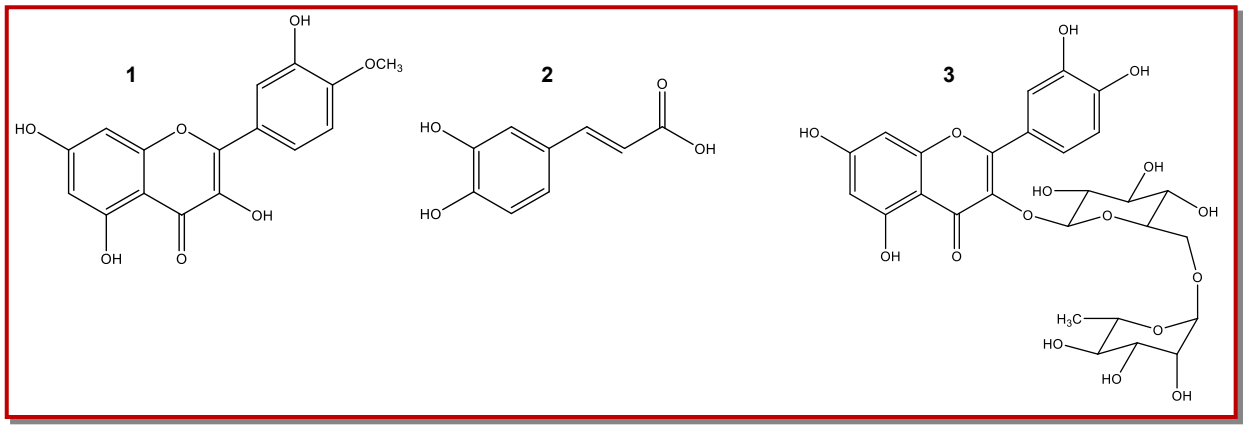

Figure 1: The structures of the isolated compounds

Table I

Radical scavenging activity and total phenolic/ flavonoids contents of the extracts

Extract	DPPH scavenging IC_{50} (µg/mL)	Total phenolic content (µg/mg)	Total flavonoids content (µg/mg)
Ethyl acetate	79.8	384.9 ± 1.5	115.6 ± 1.8
n-Butanol	136.2	500.8 ± 2.3	126.0 ± 2.0
Ascorbic acid	62.3	NS	NS

72.35 (C-4'''), 74.05 (C-2''), 76.29 (C-3'''), 76.96 (C-5''), 94.32 (C-8), 99.69 (C-6), 101.23 (C-1'''), 101.97 (C-1''), 103.54 (C-10), 115.80 (C-5'), 116.61 (C-2'), 121.23 (C-1'), 121.98 (C-6'), 133.58 (C-3), 145.51 (C-3'), 149.52 (C-4'), 156.79 (C-2), 157.00 (C-9), 161.49 (C-5), 166.50 (C-7), 177.44 (C-4). Molecular formula is $C_{27}H_{30}O_{16}$. Spectral data were compared with published literature (Sintayehu et al., 2016).

Anti-oxidant activity

Ethyl acetate extract (IC_{50}= 79.8 µg/mL) showed higher radical scavenging effect than the *n*-butanol extract (IC_{50}= 136.2 µg/mL) (Table I). The highest free radical scavenging was observed for fraction T36 (IC_{50}<5 µg/mL) and this fraction exhibited higher DPPH scavenging activity than the synthetic anti-oxidant BHT (IC_{50}= 23.1 ± 0.2 µg/mL) (Figure 2).

On the other hand, total phenolic and flavonoid contents of the extracts were also determined. *n*-Butanol extract contained higher phenolic and flavonoid contents than ethyl acetate extract (Table I). Among the fractions, the highest total phenolic content was found in fraction T36 (442.5 µg) isolated from the *n*-butanol extract. The contents of total phenolic compounds in the tested fractions ranged between 14.3 to 442.5 µg/mg (Table II). A significant correlation (R= 0.945, p<0.01) was observed between the total phenolic content and DPPH scavenging activity of the fractions indicating

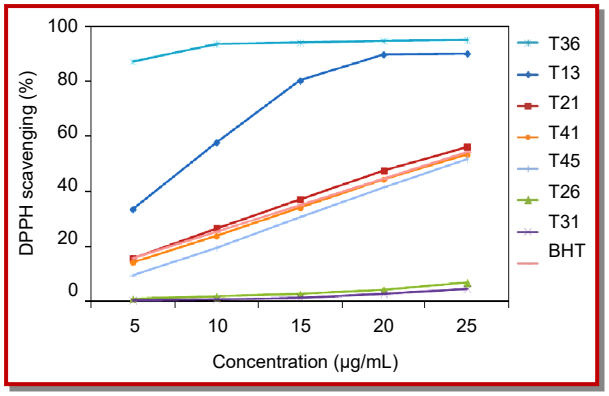

Figure 2: DPPH scavenging activity of the fractions from *n*-buanol extract at tested concentrations

phenolic compounds were primarily responsible for this activity.

Antimicrobial activity

The results of the antibacterial activity of the crude *n*-butanol and ethyl acetate extracts of *B. hispanica* are

Table II

Radical scavenging activity and total phenolics contents of the fractions*

Fractions	DPPH scavenging IC_{50} (µg/mL)	Total phenolic content µg GAE/mg extract
T21	23.8 ± 0.2^{b}	178.1 ± 0.9^{d}
T41	22.8 ± 0.5^{b}	214.5 ± 0.9^{b}
T45- 47	24.1 ± 0.5^{b}	169.5 ± 0.5^{e}
T36	<5	442.5 ± 0.6^{a}
T26	98.5 ± 0.5^{d}	44.5 ± 0.8^{f}
T31	>100	14.3 ± 0.7^{g}
BHT	23.1 ± 0.1^{b}	ND
EDTA	ND	ND

*Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscripts are significantly (p<0.05) different, ND: Not determined

Table III

Antimicrobial activity of the *B. hispanica* extracts*

Microorganism	*n*-Butanol extract (mg/mL)						Ethyl acetate extract (mg/mL)					
	3.1	6.3	12.5	25	50	100	3.1	6.3	12.5	25	50	100
Acinetobacter. oxy	6.2 (0.2)	8.1 (0.2)	13.8 (0.2)	14.1 (0.1)	14.3 (0.3)	15.2 (0.2)	5.8 (0.2)	6.0 (0.1)	6.3 (0.1)	9.8 (0.3)	10.2 (0.2)	15.0 (0.2)
E. coli	7.2 (0.3)	8.0 (0.3)	12.9 (0.2)	13.2 (0.3)	14.3 (0.3)	14.9 (0.1)	6.8 (0.1)	6.0 (0.1)	6.1 (0.2)	6.2 (0.2)	7.1 (0.1)	12.2 (0.2)
Enterococcus faecalis	5.8 (0.2)	6.1 (0.2)	10.9 (0.1)	13.4 (0.3)	14.0 (0.2)	14.1 (0.2)	5.9 (0.1)	5.9 (0.1)	6.3 (0.3)	11.9 (0.1)	12.1 (0.2)	15.0 (0.2)
Proteus mirabilis	5.7 (0.3)	5.8 (0.2)	5.9 (0.2)	6.2 (0.2)	6.3 (0.3)	6.4 (0.3)	5.9 (0.1)	5.9 (0.1)	6.1 (0.1)	6.2 (0.2)	6.3 (0.3)	10.0 (0.1)
Klebsiella pneumoniae ATTC700603	5.8 (0.1)	6.1 (0.3)	7.2 (0.2)	8.2 (0.2)	14.0 (0.2)	15.4 (0.4)	5.8 (0.2)	5.9 (0.1)	6.2 (0.2)	8.1 (1.7)	10.1 (0.1)	15.0 (0.1)
Pseudomonas aeruginosa ATTC 27853	5.8 (0.2)	6.3 (0.3)	6.9 (0.1)	7.2 (0.2)	13.0 (0.1)	15.2 (0.2)	5.8 (0.2)	6.0 (0.1)	6.0 (0.1)	6.1 (0.2)	10.1 (0.2)	14.0 (0.1)

*The results were given as zone diameter (mm)

presented in Table III. The aerial parts of the extracts generally showed increase activity with increasing of the concentration against Gram positive bacteria and Gram negative bacteria. Both of the crude extracts exhibited the highest activity against *Klebsiella pneumoniae* (15 mm at 100 mg/mL).

Anti-proliferative activity

The anti-proliferaitve effects of the isolated compounds (rutin and tamarixetin), fraction T45-47 and *n*-butanol extract (rutin isolated from this fraction and extract) were evaluated against HeLa cell line. Real-time cell monitoring of the proliferation of the cells treated with the compounds for 48 hours period (Figure 3). *n*-Butanol extract and fraction T45-47 showed dose- and time-dependent effect. Tamarixetin exhibited significant anti-proliferative effect against HeLa cell line (IC_{50}<50 µg/mL), whereas rutin exhibited low cytotoxic effect (IC_{50} >100 µg/mL). Figure 4 shows the anti-proliferative effect of the compounds on HeLa cells for 24 and 48 hours after the treatment at the concentrations of 10, 50 and 100 µg/mL. Tamarixetin exhibited approximately 80% inhibitory effect at the highest concentration of 100 µg/mL.

Discussion

In this study, three known compounds were isolated from *B. hispanica* for the first time and their structures

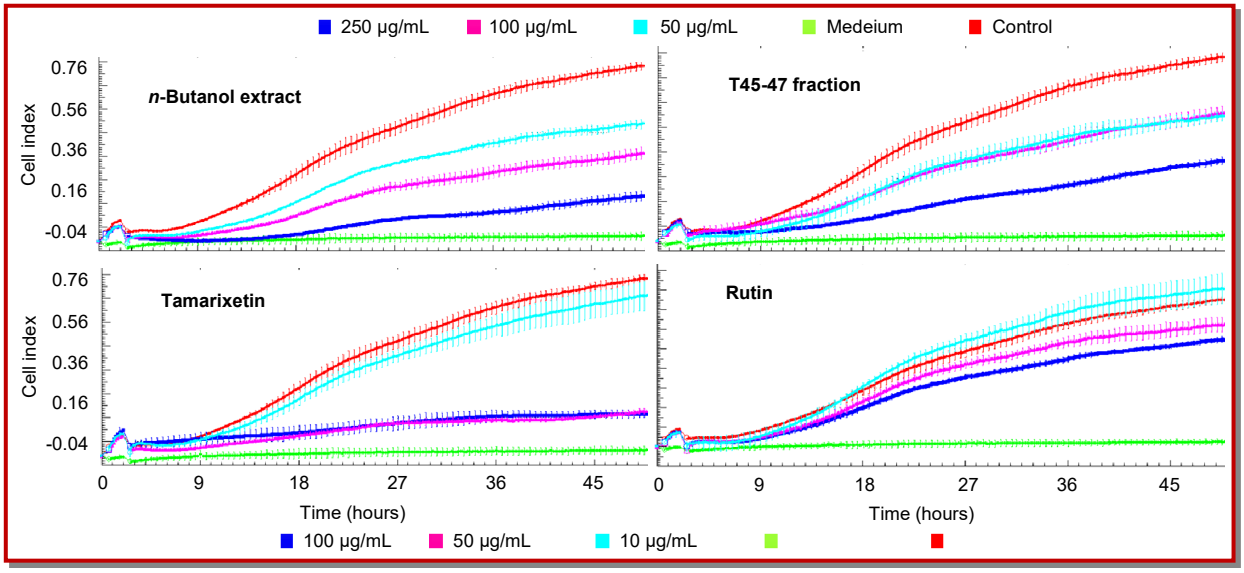

Figure 3: Anti-proliferative effect of the n-butanol extract, fractions T45-47, tamarixetin and rutin

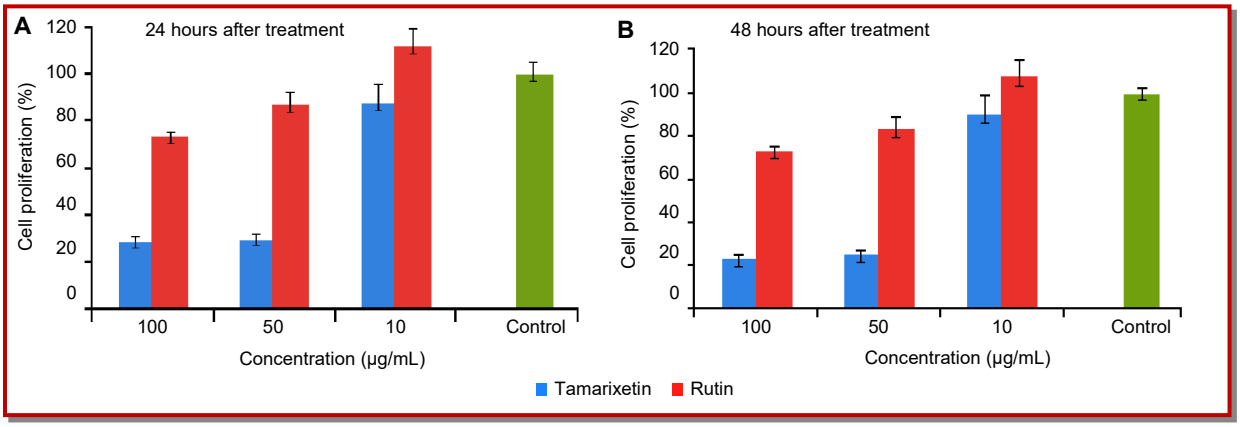

Figure 4: Cell proliferation (%) for 24 and 48 hours after the sample treatment at tested concentrations

were elucidated as tamarixetin (**1**), caffeic acid (**2**) and rutin (**3**). In addition, anti-oxidant, antimicrobial and anti-proliferative activities of the samples from *B. hispanica* were evaluated. The extracts and fractions showed remerkable radical scavenging activity, especially fraction T36 exhibited higher radical scavenging effect than synthetic anti-oxidant BHT. To the best of our knowledge, there is only one study on *B. hispanica* about its anti-oxidant activity in the literature. Similar to our study, in the study of El-Youbi et al. (2012b), the aqueous extract of *B. hispanica* exhibited higher radical scavenging effect than BHT.

Berberis species is known to contain a variety of phenolic compounds which exhibit antibacterial potency (Birdsall and Kelly, 1997; Meenakshi et al., 2007). Both of the extracts have shown occurrence of phenolic and flavonoids content and that antibacterial actions may be due to their compounds content or could be belongs to the isolated compounds (tamarixetin and caffeic acide) from ethyl acetate extract of *B. hispanica,* which had been reported to inhibit the growth of different and same bacterial strains in this study (Sultanova et al., 2001; Alves et al., 2013), along with the presence of rutin compound in the *n*-butanol extract could be concluded that the antibacterial activity of this extract belongs to rutin (Basile et al., 2000). The result supports previously reported studies on antimicrobial activity of ethanol and water extracts from the root bark of *B. hispanica* against *M. smegmatis*, *M. aurum* and *S. aureus* activity (Amina et al., 2014; Ibtissem et al., 2017). The antibacterial results and occurrence of flavonoids and phenolic acid in crude extracts of *B. hispanica* are interest to discover new active plant compounds, and lead us to isolate more compounds for evaluate the antimicrobial activity of these extracts.

In this study, tested compounds inhibited the proliferation of HeLa cells in a dose- and time-dependent manner. Polyphenolic compounds from natural sources are currently of interest due to their possible anti-cancer activities (Greenwell et al., 2014). However, anti-proliferative effects of tamarixetin on human tumor cells have a few assessed. In the present study, we examined the effects of tamarixetin on the growth of human cervical cell line and found that it displays significant anti-proliferative effect. Similar to our study, Nicolini et al. (2013) investigated the effects of tamarixetin on viability of some human tumor cell lines and found that it was cytotoxic against leukemia cells and in particular P-glycoprotein-overexpressing K562/ADR cells. They indicated that tamarixetin inhibited proliferation in a concentration- and time-dependent manner, induced apoptosis and blocked cell cycle progression at G2-M phase. Delgado et al. (2014) reported that quercetin and its methylated metabolites including tamarixetin had significant anti-proliferative activity against breast (MCF-7), colon (Caco-2) and pancreas (BxPC-3) cancer cell lines.

On the other hand, in this study, rutin isolated from *B. hispanica* exhibited time and dose-dependent activity on HeLa cells. Some studies have been reported that rutin exhibited a dose- and time-dependent effect on U-937 and HT-60 (Srinivasan et al., 2016) and glistoma (Santos et al., 2011) cell lines. Rutin has also shown cytotoxic effects on several human cancer cells (Alonso-castro et al., 2011; ben-Sghaer et al., 2016). However, Kuntz et al. (1999) found that the flavonoids with glycosylated hydroxyl group (rutin, naringin) were not effective on human colon cancer cell lines. The remarkable anti-proliferative activity by *B. hispanica* extract and fraction against human cervical cell line in our study might be due to the presence of anti-cancerous compounds, need to be isolated and identified for more investigation.

Conclusion

Tamarixetin, rutin and caffeic acid were isolated from *B. hispanica.* Tamarixetin exhibited a powerful cytotoxic effect on HeLa cells. Moreover, the crude extracts of *B. hispanica* exhibited remarkable antibacterial and anti-oxidant activities. This study supports the documented medicinal effects of *B. hispanica* and opens up the possibilities of pharmaceutical applications.

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Conflict of Interest

Authors declare no conflict of interest

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