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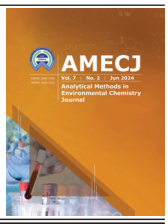

Analysis and preparation of phenolic and alkaloid compounds from Moroccan Berberis vulgaris organic extract: Molecular docking and optimization by UHPLC/DAD/ESI-MS

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ABSTRACT

Among the natural products, phenolic and alkaloid compounds have the most important constituents and common plant-derived secondary metabolites, and thus, analysis deserves particular attention for their therapeutic proprieties. In this study, the phenolic and alkaloid compounds were analyzed using ultra-high-performance liquid chromatography coupled with diode array detector-tandem electrospray ionization mass spectrometry (UHPLC/DAD/ESI-MS) for three extracts of Moroccan *Berberis vulgaris L*. Then we optimized and validated results for four phenolic compounds. To evaluate the biological activity of this species we chose the antimicrobial activity. For the antimicrobial activity of these extracts, we used molecular docking analysis of the compounds identified with DNA gyrase. The method validation results showed a good linear (R^2>0.99) with the coefficient correlation and the significance of regression tests confirmed a linear relationship between x and y. Two concentrations of phenolic and alkaloid compounds (31.25 mg L^{-1} and 500 mg L^{-1}) were determined by the UHPLC/DAD/ESI-MS and used in repeatability tests, the relative standard deviation of 10 repetitions varied to 0.35-2.33 with CV% 1.1-2%. In addition, the analytical method is sensitive, accurate, and precise (CV<5%). The analytical method successfully set for robust quality evaluation and standardization of phenolic and alkaloid compounds from *B. vulgaris* organic extract.

1. Introduction

Plants such as vegetables, fruit, and spices/medicinal herbs play an important role in degenerative diseases. However, it is not yet clear which constituent is responsible for this. Many medicinal plants contain chemical compounds such as flavonoids and alkaloids exhibiting antimicrobial properties. In Moroccan medicine, *Berberis vulgaris* belongs to the family of Berberidaceae locally named “Aghriss” and is used for her antipyretic,

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hepatoprotective, and anti-inflammatory properties [1]. In Iranian traditional medicine, it is used for an enlarged spleen, eye sores, asthma, and skin pigmentation (reference). A decoction of barberry and Emblic myrobalan combined with honey is prescribed to treat acrid urine or painful micturition from bilious [2]. *B. vulgaris* is mainly used as a food cooked with rice and *B. integerrima*, also referred to as black barberry and wild barberry, is used mainly for juice extraction in food industries and as medication [2]. The therapeutic properties of *B. Vulgaris* are probably due to the presence of secondary metabolites like phenolic compounds and alkaloids. To explore these natural sources, several phytochemical and pharmacological studies of Berberis species have been conducted. The presence of many families of natural compounds has been described in the different parts of these plants; alkaloids [3,4, 5], flavonoids [5, 6], fatty acid, and terpenoids [2]. Several studies have been conducted on the biological activities of Berberis species including; anticancer [7] and antioxidant activities [3]. The family of Berberidaceae is known for its strong biological activities. Recently work showed that Ethanol and ethyl acetate extracts of Berberis vulgaris have a higher cytotoxic effect against human breast adenocarcinoma cell line (MCF-7) using the MTT method which indicated their important anticancer capacity[3]. Some alkaloids, including oxyaconthine, berberine, berbamine, brolicin, and columbamine, are present in all of the organs of *B. vulgaris*. Compared to other *B. vulgaris* organs, the root bark contains more alkaloids. One of the plant's most significant alkaloids, berberine, may be useful in preventing coronary artery disease and perhaps lowering triglyceride and total cholesterol levels [2]. Various research showed that polyphenols and alkaloids are extensively used in the pharmaceutical, cosmetic, and food industries due to their various biological activities. Many chromatographical methods like UHPLC/DAD/ESI-MS have been used for the analysis of these compounds. Also, the phenolic and alkaloid compounds, organic compounds such as toluene, benzene, xylene, styrene, and dyes were determined based on nano adsorbents such as bismuth oxide-fullerene nanoparticles, nano-carbon structure, aminopropyl trimethoxysilane-phenanthrene carbaldehyde on graphene oxide, ionic liquids, heterogeneous graphene/graphene oxide, and hydroxyethyl methylimidazolium tetrafluoroborate immobilized on MWCNTs by gas chromatography-mass spectrometry (GC-MS) of gas chromatography flame ionization detection (GC-FID)[8-16].

The present study aims to characterize and validate the phenolic and alkaloid compounds from *B. vulgaris* native to Morocco extracts by the UHPLC/DAD/ESI-MS method. Therefore, this paper describes for the first time the molecular docking analysis and antibacterial activity of Morrocan *B. vulgaris* root extracts.

2. Material and Methods

2.1. Plant material and preparation of extracts

B. vulgaris root samples were collected from Ourika (30 km from Marrakech City, Morocco) in February 2022. The roots were dried at room temperature. Aqueous extracts were prepared by maceration of 10 g of dried roots in 300 mL sterile distilled water for 24 h and following filtration of particulate matter. 10 g of roots was extracted with acetonitrile 80% and methanol 80% separately for 48h.The extracts were evaporated to dryness in a vacuum by a rotary evaporator.

2.2. Chemicals and Reagents

Acetonitrile (HPLC grade ≥ 99.9 % from Honeywell Riedel-de Haen, Germany, CAS Number: 75-05-8) is used as solvent B and 0.1 % formic acid (98 % for LC-MS, Merck Germany, CAS Number: 64-18-6) aqueous solution (ultra-pure water from Pure Lab) is used as solvent A. The phenolic compounds (Gallic acid: CAS Number: 149-91-7, Caffeic acid: CAS Number: 331-39-5, P-coumaric acid: CAS Number: 501-98-4, and Rutin: CAS Number: 207671-50-9) were purchased from Merck (Germany).

2.3. UHPLC-DAD-ESI/MS Analysis

The examined extracts were analyzed using UHPLC-

DAD-ESI/MS. Chromatographic separation was carried out using a triple quadrupole mass spectrometer with heated-electrospray (H-ESI) (TSQ Endura, Thermo Fisher Scientific, CA, USA) and the Dionex Ultimate 3000 system (CA, USA). The suggested technique was carried out using a Kinetex C18 reversed-phase column (250 x 4.6 mm, 2.6 m particles, Thermo Fisher Scientific). The mobile phases have acetonitrile as solvent B and formic acid as solvent A in concentrations of 0.1% each. Following are the multiple-step gradients used: With a flow rate of 1 mL min^{-1} and an oven temperature of 25°C, the time intervals are as follows: 0–2 min, 2% B; 2–20 min, 2-25% B; 20–25 min, 25–35% B; 25–28 min, 35–95% B; 28–30 min, 95–95% with a final plateau of 2 min, 2% B. The range of the UV-Vis spectra that were recorded in the range 220–800 nm and chromatograms were also acquired to 350 nm. The capillary temperature was 295◦C. The MS/MS experiments were performed on some selected precursor ions with energy of fragmentation is 50V in negative ion mode with 2500V and the analyses were performed in full scan mode in the range of 100–1000 m/z[17].

2.4. Antibacterial test

In this study, two types of bacteria were tested: Escherichia coli (ATCC 25922), a Gram-negative bacteria, and Staphylococcus aureus (ATCC 25923), a Gram-positive bacteria. The Pasteur Institute (Casablanca, Morocco) provided the bacterial strains. They were kept alive by recurring subcultures and stored at +4 °C before usage.

2.4.1.Disc diffusion method

The agar disc diffusion method was carried out according to the directions provided by Smaili et al. [18]. In a nutshell, Mueller-Hinton agar was updated with 3.10^8 CFU mL^{-1} of bacteria before sterile cellulose discs of 6 mm diameter were placed on it. These discs were impregnated with 10µL of the extracts at a concentration of 500 $\mu g.mL^{-1}$ dissolved in DMSO. A disc containing the common antibiotic tetracycline served as the positive control, and a disc containing DMSO served as the negative control. 24 hours after being incubated at 37 °C. By measuring the inhibitory diameter around the discs in comparison to the common antibiotic, it was possible to quantify how susceptible the harmful bacteria were to the examined chemicals. All experiments were done in triplicate and the results were expressed in mean value ± standard deviation.

2.4.2.Determination of minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration assay was performed using 96-well microdilution plates as described by Anthony et al. [19]. Two-fold serial dilutions of extract products were made in Mueller-Hinton ((3.9-1000 μgmL^{-1}) at a volume of 100 µL /well in 96-well microtiter plates. Each well was then inoculated with 10 µL of the bacterial culture (adjusting the turbidity to reach an optical density of 0.5 McFarland standards). Optical density readings were taken at 24-hour intervals. Experiments were conducted in triplicates each. Positive controls (samples in nutrient broth with no bacterial inoculation) and negative controls (nutrient broth inoculated with inoculum but no samples were included). Turbidity was monitored by measuring absorbance/ optical density at 600 nm (OD600) of the UV-Vis spectrophotometer and corrected by subtracting the background absorbance of the positive control.

2.5. Molecular docking analysis

In silico computational docking studies were performed using AutoDock. The X-ray crystallographic structure of DNA Gyrase B was downloaded from the RCSB Protein Data Bank (RCSB PDB) ID: 4URO. The protein was prepared separately by removing water and co-crystalized ligand bounds with the protein to make receptors free of any ligands before docking. Then, Polar hydrogen and Gastieger charges were added using the MGL Tools (Molecular Graphics Laboratory), and proteins saved in PDBQT format (Protein Data Bank, Partial Charge (Q), and Atom Type (T)). Ligands' structures were created separately using

ChemDraw Ultra 12.0, energy was minimized in Chem3D, and torsional bonds of ligands were set flexibly and saved in PDBQT format. Next, the receptor was kept rigid, and the grid covering all the amino acid residues present inside the active site of proteins was built (grid box size of 30Å x 40Å x 32Å with a spacing of 0.375 Å between the grid points and centered at -0.97 (x), 0.152 (y) and -12.373 (z)). The best conformers were searched by the Lamarckian genetic algorithm (LGA), the population size was set to 150 and the maximum number of energy evaluations was set to 25000000. Finally, the results were analyzed and visualized by Discovery Studio. In molecular docking results, the tetrahydroberberine, berberines, and reticuline as major identified compounds in *B. vulgaris* roots show comparable residual interactions (GLU58, ARG84, PRO87, and ARG144) and docking score of novobiocin.

2.6. Statistical analysis

Statistical analysis was carried out of one-way analysis of variance (ANOVA) test using a statistical package program (SPSS version 23.0) and the significance of the difference between means was followed by the Tukey test, using $p < 0.05$ as the level of significance. Data were expressed with Mean ± Standard error of three parallel measurements.

3. Results and Discussion

Several research reported the UHPLC methods for the characterization of phenolic compounds and alkaloids, in medicinal plants, use organic solvents for their extraction like methanol and acetonitrile[20, 21]. However, most people use the infusion method (water) for the extraction of many secondary metabolites [21]. For this reason, in the present study, we proposed a UHPLC/DAD/ESI-MS method which can be used for aqueous extracts and methanolic, acetonitrile extracts and applied for the analysis of *B. vulgaris* growing in Morocco, to identify and quantify the alkaloids and phenolic compounds in the same method analysis.

3.1. UHPLC-DAD-ESI/MS Analysis

Methanol 80%, acetonitrile 80%, and aqueous extracts of *B. vulgaris* roots were analyzed by UHPLC/DAD/ESI-MS. The chromatographic profiles obtained are given in Figure 1. Under the same chromatographic conditions carried out, 6 peaks were detected in the various extracts of *B. vulgaris* roots (Table 1). The identification of these peaks was based on their UV characteristics and by comparing their mass spectra $(M-H)^-$ with those reported in the literature (Table 1 and Fig. 1) For the convenience of explanation, the 6 identified compounds have been classified into two classes of secondary metabolite namely: alkaloids, and phenolic compounds (Table 1). The profile chromatographic of the aqueous extract showed the predominance of protoberberine alkaloids (m/z 335.2087) with fragment ion m/z 320 (M-H- 15Da) and 305 (M-H- 30Da) loss of two methyl (CH3), followed by glycoside flavones Rutin (m/z 609.1085), the typical ions fragments (m/z 301) corresponding to quercetin was observed in MS^2 mode. We identified tentatively tetrahydroberberine (m/z 339.1064) with an ion fragment (m/z 335.108) corresponding to berberine. The UHPLC-DAD-ESI/MS chromatogram of the acetonitrile 80% showed the presence of two subgroups of phenolic compounds notably, phenolic acid (gallic acid; m/z 169.1052) and flavonol glycosylated (rutin; m/z 609.1085), In addition the acetonitrile 80% extract rich on benzylisoquinoline compounds notably reticuline (m/z 328.4039) with ion fragment m/z 192.021. Likewise, berberine and tetrahydro berberine were identified in acetonitrile and methanol 80% extracts. As can be seen in Figure 1, the reticuline was found only in methanol and acetonitrile extracts. Also, we identified tentatively the Guaijaverin and arabinose quercetin (m/z 433.1059) in three *B. vulgaris* extract.

Based on the literature the most important compound in Berberis species is Berberine, many research revealed that this compound can be effective in preventing coronary artery disease and possibly reducing the levels of total cholesterol

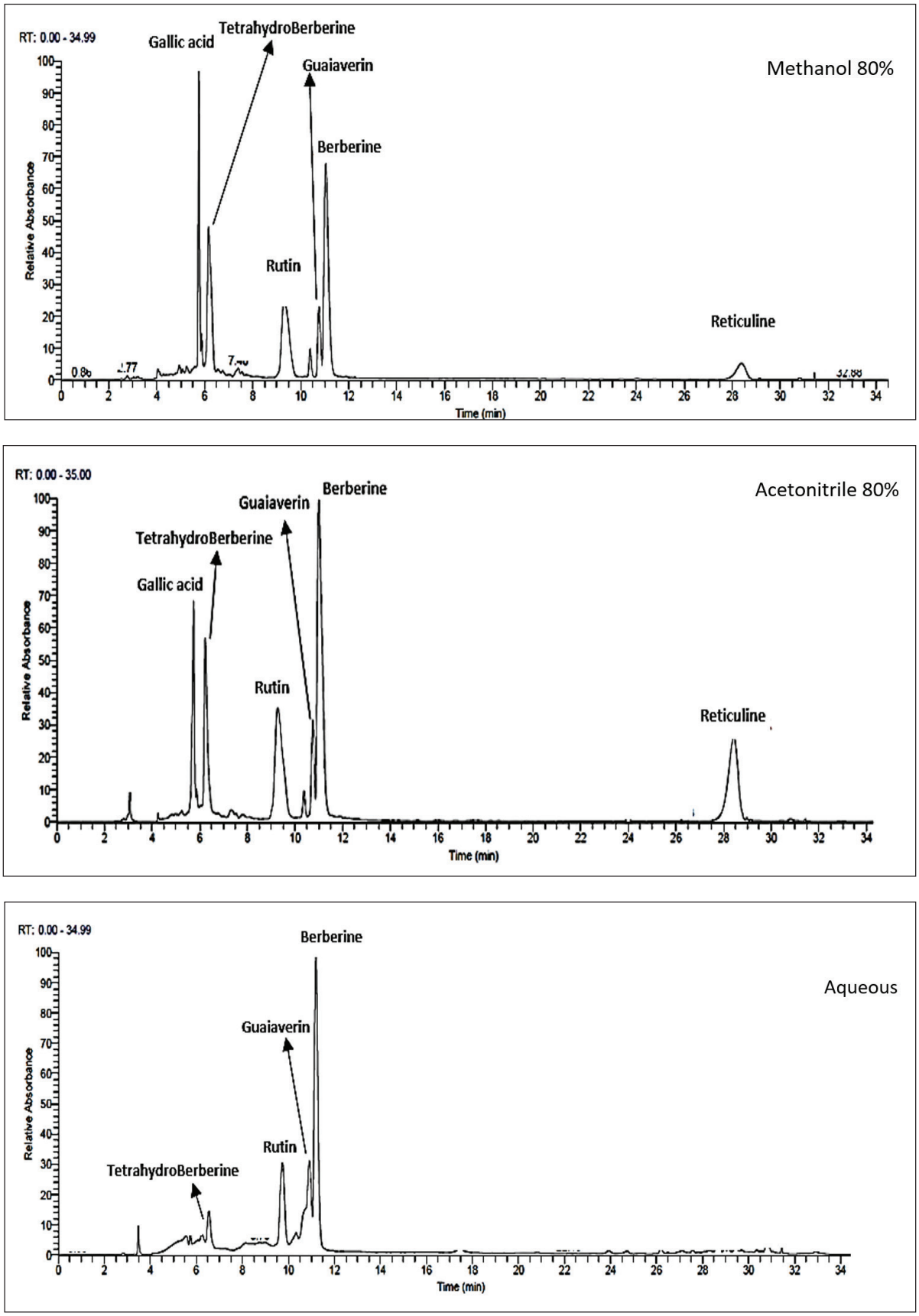

Fig. 1. Profil chromatographic Berberis vulgaris extracts at 350 nm

Table 1. Tentatively identification of phenolic compounds and alkaloids from *B. vulgaris* extracts

RT	Λ_{max} (nm)	m/z experimental	m/z calculated	Mass error (mDA)	MS/MS fragments	Proposed compound	Molecular formula	Reference
5.74	210/272	169.1052	169.0169	-0.088	125/ 107/ 97/ 79/ 69	Gallic acid	$C_7H_6O_5$	This work
6.55	225/258/360	3391064	339.1228	-0.016	335/308/110	Tetrahydroberberine	$C_{20}H_{21}NO_4$	[22]
9.45	255/355	609.1085	609.2959	-0.187	300 /169	Rutin	$C_{27}H_{30}O_{16}$	[5]
10.9	227/274/355	433.1059	433.0776	-0.028	300	Guaijaverin	$C_{20}H_{18}O_{11}$	[5]
11.2	225/257/350	335.2087	335.1230	-0.085	308/231/110	Berberine	$C_{20}H_{18}NO_4$+	[22]
28.88	235/286/350	328.4039	328.3125	-0.091	207/192	Reticuline	$C_{19}H_{23}NO_4$	[22]

and triglyceride. In addition, berberine has been reported protective effects on the central nervous system and helps to treat diseases of this system [2]. Reticuline is one of the alkaloids that possesses potent central nervous system depressing effects and a higher anti-inflammatory effect. When we compared our results with the literature, we found it similar. A few studies reported the presence of reticuline in berberis species, but only the authors [22] have been identified benzylisoquinoline alkaloids (reticuline) in berberis microphylla. Another work, [5] reported the presence of Rutin, berberine, Guaijaverin, citric acid, and palmatine in *Berberis iliensis* extract. Concerning alkaloids, in the first report we detected the presence of reticuline in *B. vulgaris*. As can be seen in our results, rutin and guaijaverin are the derivative of quercetin while, rutin is quercetin-3-O-rutinoside and guaijaverin is quercetin 3-O-arabinose. Besides alkaloids, three phenolic compounds (Guaijaverin, gallic acid, and rutin) were identified for the first time in Moroccan *B. vulgaris* roots.

Based on the characterization results of three extracts of B.vulgaris we optimized and validated a UHPLC/DAD/ESI-MS method for four phenolic compounds: gallic acid, p-coumaric acid, caffeic acid, and Rutin

3.2. Statistical methods

3.2.1.Specificity, Linearity, Repeatability and Reproducibility

For specificity, multistandard solutions such as gallic acid, caffeic acid, rutin, and p-coumaric acid were characterized by UHPLC/DAD/ESI-MS [23]. For linearity**,** three series of standard stock solutions at 5 concentration levels were used (31.75, 61.5, 125, 250, and 500 mgL^{-1}). Three repetitions for each level of each series were performed by UHPLC/DAD/ESI-MS. To evaluate the linearity test we used six assays as the test of intercept, the nullity of the slope, the significance of slope, the Cochran test, the significance of the regression, and the coefficient correlation [24]. For repeatability, two concentration levels (31.75 and 500 mg L^{-1}) were measured by the same operator and characterized by UHPLC/DAD/ESI-MS. Ten repetitions were performed for each level on the same day. We calculated the average, standard deviation, and coefficient of variation (CV%) of ten measurement [25]. As reproducibility**,** for 3 days, a series of multi-standard solutions with five concentration levels (31.75, 61.5, 125, 250, and 500 mg L^{-1}) was prepared by the same operator. Three repetitions for each level of each series were performed by UHPLC/DAD/ESI-MS[24].

3.2.2.Statistica Tests

In the intercept test, The following inequality will make it possible to establish the correlation from our right with zero (Equation 1). For the nullity of the slope test, we used Equation 2. For the test of the significance of the slope, we used Equation 3. For the Cochran test, we used Equation 4. The purpose of this test is to verify the homogeneity of the variances constituting

the experimental error and thus detect the presence of suspicious values. For the test of the significance of the regression, F was calculated using the LINEST function in Microsoft Excel, and the acceptance criteria were calculated when the slope was not different from zero if $F_{calculated} \leq F_{(1-\alpha,\ 1,\ k-2)}$. Also, the coefficient correlation is calculated by Equation 5.

$$tc = \left|\frac{b}{Sb}\right| \qquad \text{(Eq. 1)}$$

b: intercept; Sb: Standards deviation of intercept; tc: T calculated

Acceptance criteria:_The line passes through the origin if $t_{calculated} \leq t_{(1-\alpha/2,\ k-2)}$

$$tc = \left|\frac{a}{Sa}\right| \qquad \text{(Eq. 2)}$$

a: slope; Sb: Standards error of slope; tc: T calculated

Acceptance criteria:_The slope is null if $t_{calculated} \leq t_{(1-\alpha/2,\ k-2)}$

** One-tail student test: Equation of regression curve: (y: area; x: concentration mg L^{-1}) / α: risk = 0.05 / k: number of concentration levels

$$F = \frac{Si^2}{Sr^2} \qquad \text{(Eq. 3)}$$

Si^2: variance due to linear regression; Sr^2: residual variance which represents the difference between the theoretical value and the actual value

Acceptance criteria: The slope is not significant if $F_{calculated} \leq F_{(1-\alpha,\ 1,\ Nk-2);}$

One-tail Fischer test: Equation of regression curve: (y: area; x: concentration mg L^{-1})

$$C = \frac{S^2max}{\sum S^2j} \qquad \text{(Eq. 4)}$$

$S^2_{max:}$ highest variance of j groups; S^2j: Variance of j groups

Acceptance criteria:_There is a homogeneity of variances if $C_{calculated} \leq C_{(1-\alpha,\ s,\ nk-1)}$

$$Sr = \sqrt{\left(\frac{1-Coefficient\ of\ determination}{n-2}\right)}$$

$$to = \sqrt{\left(\frac{R}{Sr}\right)}$$

(Eq. 5)

With: n: number of repetitions; R: coefficient of determination

Acceptance criteria:_if $t_{calculated} > t_{(1-\alpha/2,\ k-2)}$ there is a linear relationship between x and y

α: risk = 0.05 / s: Number of series / n: number of repetitions per level in the series/ k: number of concentration levels / N: number of repetitions per level all series combined

3.2.3.Detection and quantification limit

Ten repetitions for the control (acetonitrile) were performed by UHPLC/DAD/ESI-MS and detection and quantification limits (DL/LOD and QL/LOQ) were calculated using Equations 6 and 7 [25].

$$DL = 3 \times h_{max} \times R \qquad \text{(Eq. 6)}$$

$$QL = 10 \times h_{max} \times R \qquad \text{(Eq. 7)}$$

h_{max}: Average of h_{max} of each repetition of the control sample (10 repetitions in total)

Factor R: quantity/signal of peak

3.3. Validation method

Validation of four phenolic compounds, gallic acid, p-coumaric acid, caffeic acid, and rutin was conducted on UHPLC/DAD/ESI-MS analysis. We estimated various tests like specificity, linearity, repeatability, intermediate precision and detection, and quantification limits.

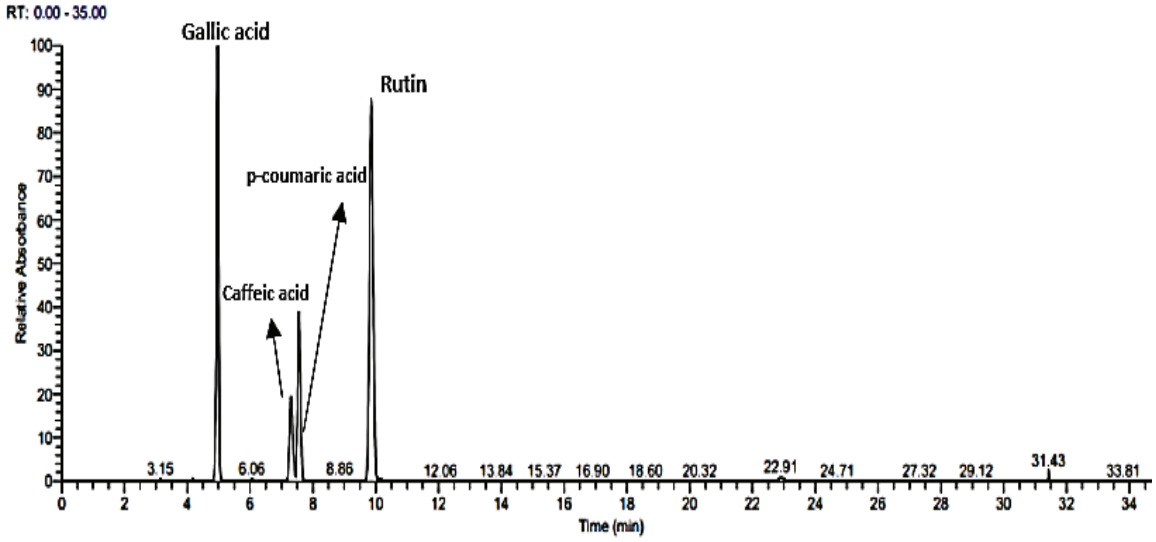

Fig. 2. Profil chromatographic of multi-standards solution at 350nm

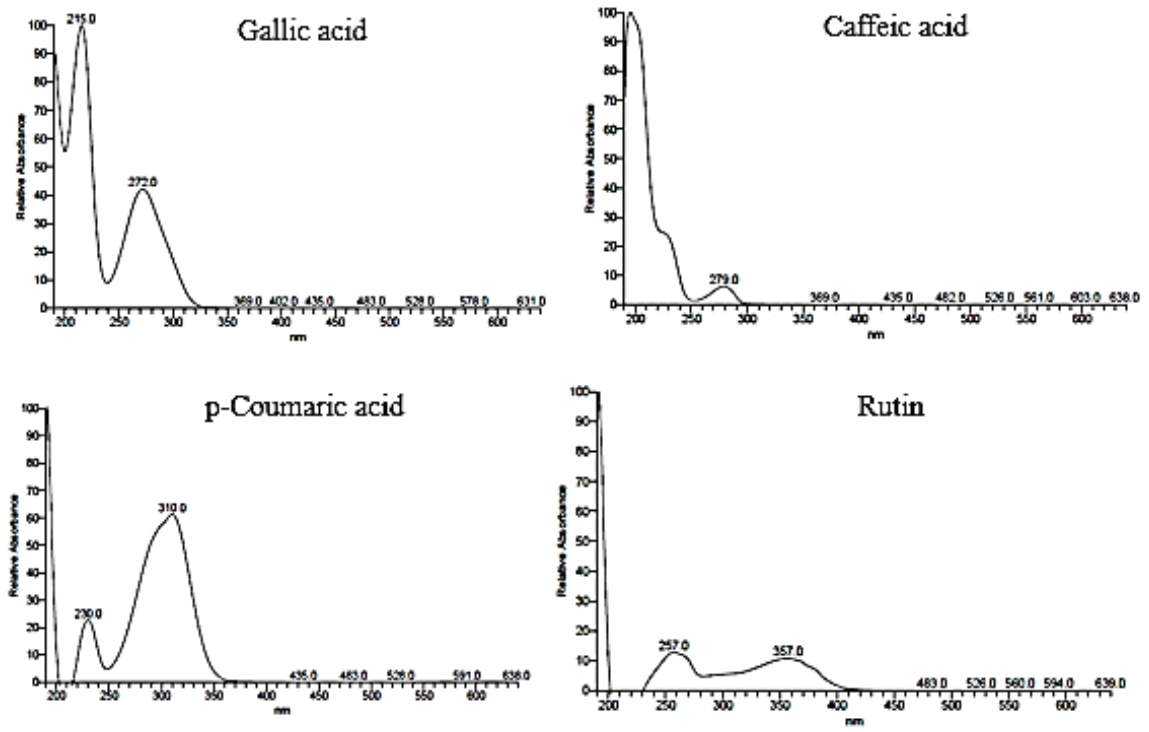

Fig. 3. UV spectrum of four phenolic compounds at 350nm

3.3.1. The specificity

The chromatograms show that the separation of all four phenolic compounds was successfully achieved with good resolution. Additionally, no interfering peaks were observed in Figures 2 and 3.

3.3.2. Linearity tests

Linear regression was plotted between standard concentration and peak area. These plots were found to be linear with a correlation coefficient (R^2) in the range of 0.993 to 0.997. In this study, we

calculated various parameters like the coefficient of determination (R^2), slope, intercept, and standard deviation and we deduced six approaches such us: a test of intercept, the test of the nullity of the slope, a Test of the significance of slope, Test of the significance of regression, Test of Coefficient correlation and Cochran test (Table2).

The regression was found in Equations 8-11.

(gallic acid): y=14708746,13x+155441,9785 (Eq. 8)

(caffeic acid): y=4399929,254x+28863,56444 (Eq. 9)

(p-coumaric acid): y=8097205,429x+55936,00681 (Eq. 10)

(rutin): y=22760604,74x+227220,6614 (Eq. 11)

the linearity method was evaluated by considering retention time precision, R^2 values which are superior to 0.96 for all the compounds tested and can confirm the linearity of the method.

The coefficient correlation and significance of regression tests confirmed that there is a linear relationship between x and y with calculated values

Table 2 .Linearity parameters for selected phenolic compounds using UHPLC/DAD/ESI-MS

Standards		**Gallic acid**	**Caffeic acid**	**p-Coumaric acid**	**Rutin**
Slope a		14708746,13	4399929,254	8097205,429	22760604,74
The standard deviation of the slope a		669530,3449	139784,4542	258921,3805	629993,4294
Intercept b		155441,9785	28863,56444	55936,00681	227220,6614
The standard deviation of intercept b		172787,5609	36074,56344	66820,56183	162584,159
Coefficient of determination R^2		0,993822407	0,996981198	0,996941865	0,99770687
number of degrees of freedom		3	3	3	3
square sum residual (residual variation)		1,95418E+11	8518085198	29225372620	1,7302E+11
Test of intercept	T	0,899613246	0,800108489	0,8371077	1,397557196
	t(0,975, 3)	3,1824			
	Conclusion	The line passes through the origin			
Test of nullity of the slope	T	21,97	31,48	31,27	36,13
	t(0,975, 3)	3,1824			
	Conclusion	The slope is different from zero, there is a linear relationship between x and y			
Test of the significance of slope	F	26011,31	23034,82	23098,18	33126,60298
	F(0,95, 1, 43)	4,08			
	Conclusion	The slope is different from zero, there is a linear relationship between x and y			
Test of the significance of regression	F	482,1	990,77	977,9	1305,26
	F(0,95, 1, 3)	10,13			
	Conclusion	there is a linear relationship between x and y			
Cochran test	C	0,76	0,78	0,83	0,84
	C(0,95;5;2)	0,877			
	Conclusion	The homogeneity of variances is confirmed			
Test of Coefficient correlation	T	21,97	31,48	31,27	36,13
	t(0,975, 3)	3,1824			
	Conclusion	there is a linear relationship between x and y			

of each compound ≤ t critical (3.1824) and > F critic (10.13) respectively. The Cochran test confirmed the homogeneity of variances with C values of gallic acid (0.76), p-coumaric acid (0.78), caffeic acid (0.83), and Rutin (0.84) inferior to C critical (0.877). As can be seen in Table 2**,** the nullity of the slope and significance of the slope test revealed that the slope is different from zero with t values of each standard superior to t critical (3.1824) and F calculated superior to F critical (4.08) according to student and Fischer test respectively. In our study, the six approaches confirm the linearity of the method. Comparatively with the literature[26], mentioned that the method validation of phenolic compounds using UHPLD/DAD/ESI-MS presented a good linearity with a correlation coefficient higher than 0.98 to all phenolic compounds.

3.3.3.Repeatability and Intermediate precision

Repeatability was calculated by intra-day variation from the same sample at two levels (level 1 and 5) *B. vulgaris* extracts and analyzing them ten times, for reproducibility by inter-day variation, analyzing the same sample at five levels in triplicate on three different days. Repeatability and intermediate precision methods were evaluated by calculating the standard of deviation (RSD) and coefficient of variation (CV%). The coefficient variation values ranged from 1% to 2.8% in the case of the samples analyzed on the same day and on different days (Tables 3 and 4).

The CV% during intra-dayis less than 5% for gallic acid (1.39-2.8%), caffeic acid (1.17-1.48%), p-coumaric acid (1.06-1.15%), and rutin(1.19-1.32%) which is acceptable. The intermediate precision of the analysis method was also measured by calculating %CV of inter-day precision of four standards, which were found in the range of 1.15 to 2.8% (Table 4)

Table 3. Repeatability parameters for phenolic compounds using UHPLC/DAD/ESI-MS

Standards	**Gallic acid**		**Caffeic acid**		**p-Coumaric acid**		**Rutin**	
Concentration (mg L^{-1})	31.25	500.00	31.25	500	31.25	500	31.25	500.00
*Average (mg L^{-1})	31.46	507.27	29.54	502.70	33.07	502.80	31.32	502.54
*SD (mgL^{-1})	0.82	0.70	0.42	2.33	0.35	2.49	0.41	0.63
(CV)	2.80	1.39	1.48	1.17	1.06	1.15	1.32	1.19
Repeatability (r)	2.28	1.96	1.18	5.21	0.98	6.92	1.15	1.96

*10 repetitions

Table 4. Intermediate precision parameters for selected phenolic compounds using UHPLC/DAD/ESI-MS

Concentration (mgL^{-1})		**0.03125**	**0.0625**	**0.125**	**0.25**	**0.5**
Gallic acid	CV (%)	2.8	1.61	0.38	1.47	1.36
	Reproducibility	1.98	2.38	1.25	5.95	2.96
Caffeic acid	CV (%)	1.48	1.53	0.28	1.38	1.17
	Reproducibility	1.28	1.54	0.54	5.11	5.21
p-Coumaric acid	CV (%)	1.06	1.39	0.04	1.18	1.15
	Reproducibility	0.98	2.5	0.15	6.99	6.94
Rutin	CV (%)	1.32	1.31	0.21	1.21	1.19
	Reproducibility	1.15	4.45	1.15	2.94	1.96

3.3.4.LOQ and LOD Analysis

LOD and LOQ were determined to define the sensitivity of the developed method. Detection and quantification limits were calculated using signal-to-noise ratios (S/N) of ten replicates. LOD and LOQ with their respective for gallic acid (0.35 and 4.38 mg L^{-1}), caffeic acid (0.32 and 3.57 mg L^{-1}), p-coumaric acid (0.1 and 3.62 mg.L^{-1}), and rutin (0.06 and 2.22 mg L^{-1}) (Table 5). When we compared our results with the literature we found similar results with other method validation, for example[27], reported a lower detection and quantification limit in the method validation of phenolic compounds analysis.

3.4. Antibacterial activity

The antibacterial effect of *Berberis vulgaris* extracts against *E coli and staphylococcus aureus*, are shown in Table 6. The formation of inhibition zones around the wells shows bacterial sensitivity to antibacterial and antibiotics (which are used as positive controls). The positive control used in the well was a tetracycline. These results revealed that the antibacterial activity against *E coli* and *staphylococcus aureus* was increased in acetonitrile 80% extract. However, lower antibacterial activity against E coli and *staphylococcus aureus* was indicated in the inhibition zone diameter equal to 7mm and 9 mm respectively, in methanol 80% extract.

Based on the literature, previous studies have also reported the antibacterial activity of *Berberis vulgaris*, Kazemipoor, et al [28] revealed that the ethanol extract of leaves and fruits showed moderate antibacterial activity against *streptococcus sp* with the diameter zone inhibition increase from 7mm to 13mm. Another work[29], reported that ethanolic extracts from Berberis vulgaris have a higher antibacterial capacity against *E coli and Staphylococcus aureus.* The Minimum inhibitory

Table 5. Data used for the calculation of the detection and quantification limits[27]

Standard	Gallic acid	Caffeic acid	p-Coumaric acid	Rutin
Retention time (min)	5.76	7.14	7.68	9.47
Detection limit (mg L^{-1})	0.35	0.32	0.10	0.06
Quantification limit (mg L^{-1})	4.38	3.57	3.62	2.22

Table 6. Inhibition zone (mm) of three *B. vulgaris* extracts and antibiotic against two pathogenic bacteria

Compound	Inhibition zones (mm)	
	***E. coli* (ATCC 25922)**	***S. aureus* (ATCC 25923)**
Methanol 80%	9±0.1	8±0.57
Acetonitrile 80%	15±0.15	13±0.14
Aqueous	11±0.11	10±0.2
Tetracycline	20±0.028	19±0.05
DMSO	------	------

Table 7. Minimum inhibitory concentration (MIC) ppm of samples

Bacteria	Methanol 80%	Acetonitrile 80%	Aqueous	Tetracycline
E coli	250 ± 0.05	62.5± 0.11	250±0.50	50±0.3
SA	500± 0.02	125± 0.07	250±0.06	25±0.08

concentration test of aqueous, methanol 80%, and acetonitrile 80% extracts was determined by E. *coli* and *Staphylococcus aureus*. These results have been reported in Table 7. The acetonitrile 80% extract exhibited a higher activity with MIC values ranging from 62.5 and 125 µg mL^{-1} against *E coli and Staphylococcus aureus(SA),* respectively.

3.5. Molecular docking analysis

The molecular docking of the identified compounds was performed to understand the mechanism of antimicrobial activity and explain the obtained results. Thus, gallic acid, caffeic acid, p-coumaric acid, tetrahydroberberine, berberine, rutin, and reticuline were docked to the active site of DNA gyrase protein (4RUO) and compared to the potential inhibitor novobiocin. Indeed, DNA gyrase plays a crucial role in bacterial survival and is considered a target of antibacterial agents [30]. First, our docking protocol was validated by redocking the cognate ligand (novobiocin) on the crystal structure 4URO. The RMSD (root-mean-square deviation) between expected and actual poses is 0.86 Å (<2 Å). Thus, the selected protocol is suitable for reproducing the native poses. All molecular docking results are summarized in Table 8 and Figures 4-6. The eight products displayed minimum binding energies ranging from −3.63 to −7.36 kcal/mol. For instance, tetrahydroberberine, berberines, and reticuline have comparable estimated free energy of binding compared to novobiocin. As a result, they are the most potent inhibitors of DNA

Table 8. Binding affinities and potential molecular interactions of identified compounds with DNA Gyrase

Product	E (Kcal mol^{-1})	Ki (µM)	Hydrogen Bonds Interactions	Electrostatic Interactions	Hydrophobic Interactions
Gallic acid	-3.63	2190	ARG144, GLY85, GLU58, ASP81	GLU58	ILE86 (×2), GLY85
Caffeic acid	-5.1	183.75	ARG144, GLY85, ASP81 (×2)	GLU58	ILE86
p-coumaric acid	-6.11	248.08	,ARG144, GLY85 ASP81	GLU58	ILE86, ASN54
Tetrahydro-berberine	-7.36	4	ARG144, GLY125	GLU58, ARG84	ILE86 (×3), PRO87 (×2), ARG84 (×2)
Berberine	-7.37	3.9	ARG144, ASP81	GLU58, ARG84	ILE86 (×4), PRO87 (× 3), ARG84 ,(×2), ASN54, SER55, GLY85 ILE175 (×2)
Rutin	-3.97	1230	ARG84, GLY85, GLY125, ASP81 GLU58 (×2), ASP57, ASN54(×3), PRO87	GLU58	ILE86 (×5), PRO87, ASN54, SER55, GLY85 (×2), ILE102
Reticuline	-7.14	5.8	,ARG144 (×2), ASP81 GLU58, ASN54 (×2), ASP57	ARG84	ILE86,PRO87 (×2), ARG84
Guaijaverin	-5.66	70.84	ARG144 (×3), GLU58, ASP81 (×2)	GLU58	ILE86 (×2), PRO87
Novobiocin	-8.57	0.52	ARG144 (×3), GLN91, ASN54, ASP89, SER55	GLU58, ARG84 ,(×2) ARG144, ,ARG200	PRO87 (×4), ARG84 (×2),ILE102 ,(×2) ALA98

E = Estimated Free Energy of Binding Ki = Estimated Inhibition Constant

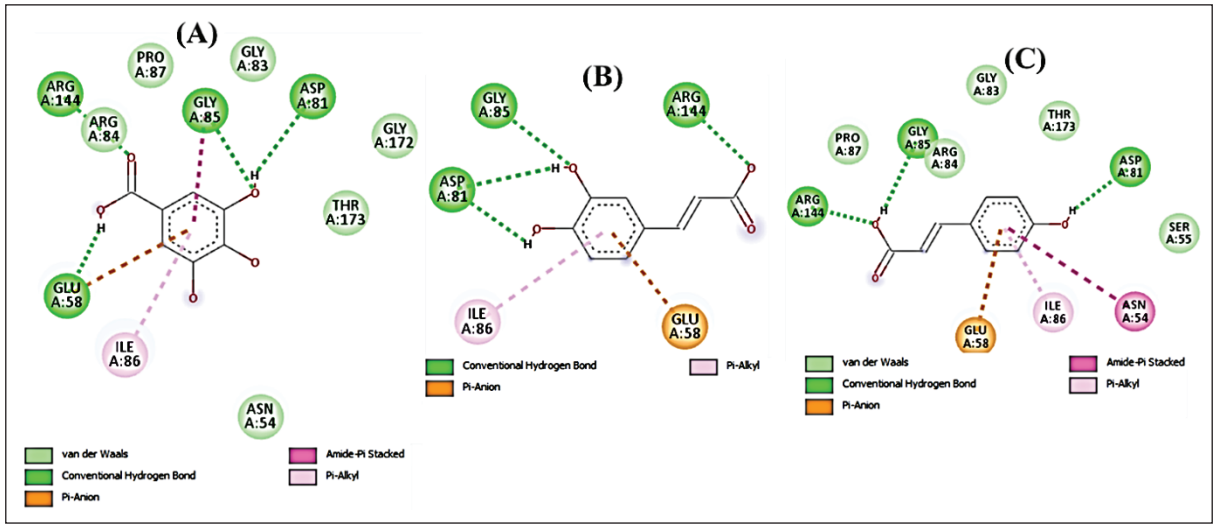

Fig. 4. 2-D interactions of Gallic acid (A), Caffeic acid (B), and p-coumaric acid (C) with DNA Gyrase

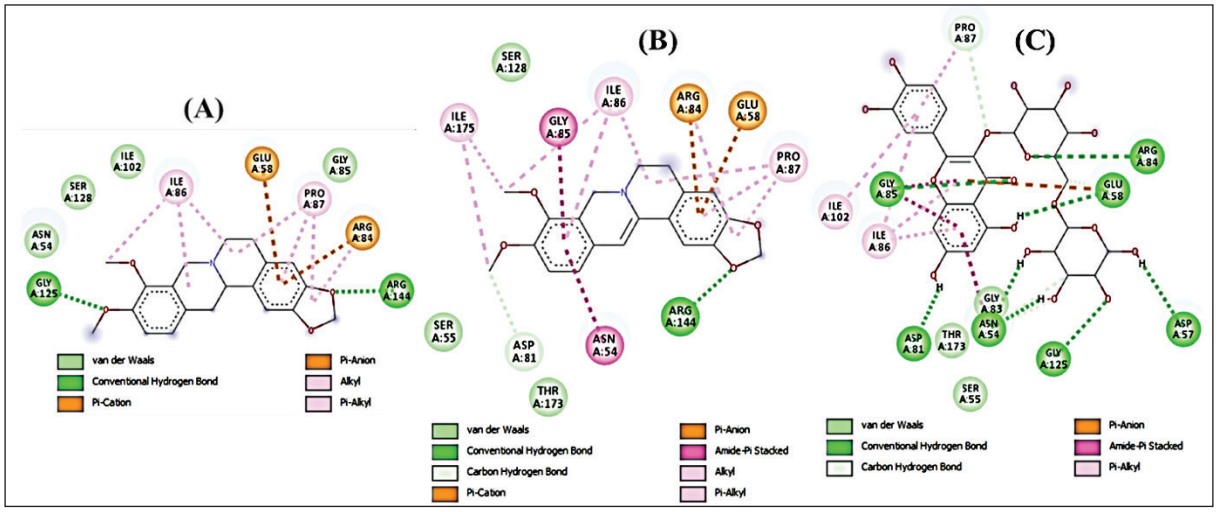

Fig. 5. 2-D interactions of Tetrahydro-berberine (A), Berberine (B), and Rutin (C) with DNA Gyrase

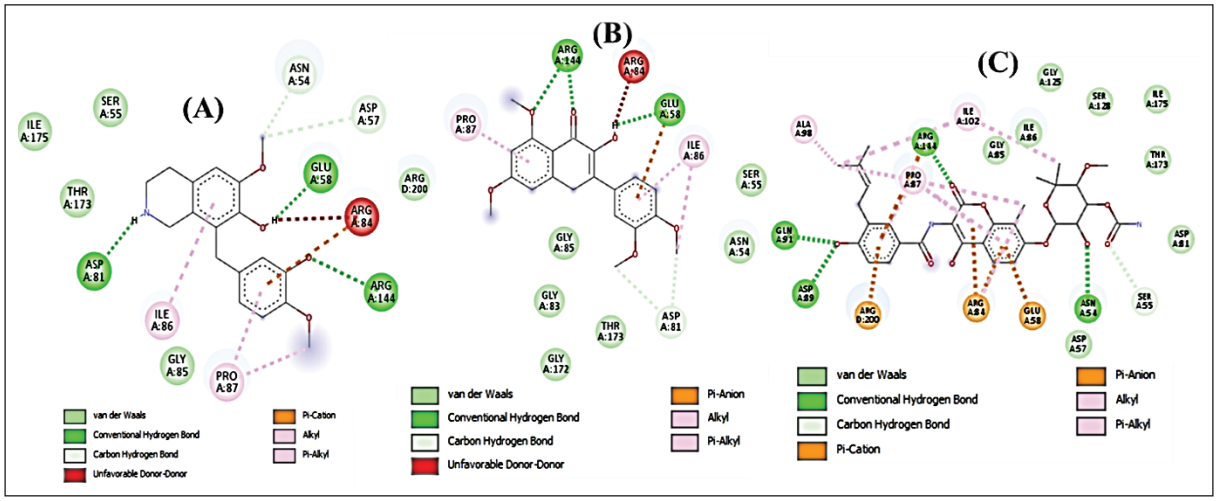

Fig. 6. 2-D interactions of Reticuline (A), Guaijaverin (B), and Novobiocin (C) with DNA Gyrase

gyrase, with estimated inhibition constants of 3.9 - 5.8 µM. From antimicrobial results, we notice that the acetonitrile extract is the most active compared to aqueous and methanolic solutions. This result may be explained by the fact that the potent compounds (tetrahydroberberine, berberines, and reticuline) were abundant in acetonitrile compared to the other extracts.

For the interaction studies, all compounds (except rutin) make hydrogen bonds with the crucial amino acid ARG144 as the reference inhibitor (novobiocin). Also, most products form electrostatic interactions with the amino acid GLU58 (rutin interacts with ARG84). The tetrahydroberberine and berberines make additional residual interaction with ARG84. In comparison to novobiocin, the most active ligands (tetrahydroberberine, berberines, and reticuline) have shown similar residual amino acids binding hydrophobic interactions with PRO87 and ARG84. Overall, in silico molecular docking analysis of the identified compounds matches with antibacterial results. Among analyzed compounds, tetrahydroberberine, berberines, and reticuline show comparable residual interactions and docking scores of novobiocin.

4. Conclusion

In the present study, The UHPLC/DAD/ESI-MS method was also successfully applied to *B. vulgaris* extracts and analysis which identified the phenolic compounds and alkaloid content in the same method for the first time (recovery more than 95%). The antimicrobial activity of the extracts was demonstrated by molecular docking studies of identified compounds to DNA-gyrase, and the mechanism was explained. A validation method showed good linearity, intermediate precision, repeatability and quantification, and detection limits. Our results revealed the successfully applied determination of phenolic and alkaloid compounds in medicinal plants.

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