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Chemical profiling and *in vitro* antioxidant, antibacterial and anticholinesterase activities of an endemic North African species *Bellevallia mauritanica* Pomel

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Abstract: The aim of this study was to evaluate antioxidant, antibacterial, and anticholinesterase activities of different extracts of an uninvestigated endemic species *Bellevallia mauritanica* Pomel, and identification of its phenolic and flavonoid content by a comprehensive and validated LC-MS/MS method using 37 phytochemical standards. Moreover, the phytochemical profiling of the studied extracts was accomplished by LC-MS-IT-TOF technique. Among all the tested extracts, ethyl acetate (EtOAc) of BRCE (bulbs-roots crude extract) was the most active one. It exhibited a medium antibacterial activity, good inhibition of acetylcholinesterase and a good antioxidant activity in ABTS^{•+} (2,2'-azinobis-(3-

ethylbenzothiazoline-6-sulfonate), DPPH[•] (1,1'-diphenyl-2-picrylhydrazyl radical), and superoxide DMSO (dimethyl sulfoxide) alkaline scavenging assays. LC-MS/MS analysis revealed that EtOAc extract had caffeic acid (1240.7 µg/g extract) and chloroform extract had vanillin (2048.5 µg/g extract) in considerable amounts. They also contained good amounts of certain phytochemicals such as protocatechuic acid (274.3 and 145.2 µg/g extract), p-coumaric acid (382.8 and 310.9 µg/g extract), ferulic acid (361.2 and 969.4 µg/g extract) and kaempferol (146.7 and 150.6 µg/g extract) respectively. LC-MS-IT-TOF screening revealed the presence of 91 major phytochemicals in the three extracts derived from bulb-roots crude extract.

Keywords: *Bellevia mauritanica* Pomel; antioxidant; acetylcholinesterase; antibacterial; LC-MS/MS; LC-MS-IT-TOF

Introduction

People in developing countries have been, so far, referring to traditional practices by using plant extracts to cure themselves [1]. Medicinal plants represent the main source of active compounds as they contain hundreds of molecules as a mixture that should be screened to find the desired phytoconstituents for drug discovery [2, 3].

The fact that synthetic antioxidants used in several pharmaceutical products [4], are considered harmful to the human health [5], justifies efforts for replacing them with natural antioxidant molecules. The abundant reactive species of oxygen (ROS) in our environment, can cause various pathologies [6]. Since synthetic anti-Alzheimer drugs have adverse effects such as hepatotoxicity [7], scientists are eager to search for new natural compounds with potent anti-Alzheimer properties.

In addition, the fact that the genetic, metabolic and physiological diversity of microbial species is constantly changing, they pose a great threat to the health of populations around the world. Antibiotic resistance is constantly evolving and requires the search of new antimicrobial agents that can be an alternative to these antibiotics. Plant-based natural products offer a promise in this sense [8].

Bellevia mauritanica Pomel, endemic to North Africa, extends from Libya to Morocco via Tunisia and Algeria [9] and belongs to the *Bellevia* genus whose species are distributed on three different phytogeographical regions: Irano-Turanian, Mediterranean and Saharo-Sindian. Being represented by 4 sections with 45 species, the genus belongs to the Asparagaceae family and the Scilloideae (*sensu* APG III) subfamily [10].

Members of the *Bellevia* genus are pharmacologically important due to their homoisoflavonoid content which is quite bioactive [11]. These biomarkers were isolated from organic bulb extracts of *B. romana* L., *B. eigii* Feinbrun and *B. flexuosa* Boiss. [12, 13, 14] and were tested for their cytotoxic and

antibacterial activities. Another type of secondary metabolites, norterpeneoids were isolated from methanol bulb extract of *B. romana* L. [15]. No ethnobotanical information was found about *B. mauritanica* according to the literature. However, three species were reported to be used in culinary preparation in Turkey; *B. speciosa* Woronow ex Grossh, *B. paradoxa* (Fisch. & C.A.Mey.) Boiss., and *B. sarmatica* (Pall. ex Georgi) Woronow [16, 17]. Therefore, the present study aims to screen the antioxidant, anti-cholinesterase and antibacterial activities of an uninvestigated endemic plant (*B. mauritanica* Pomel) as well as its phytochemical contents by a previously validated LC-MS/MS method [18]. Besides, phytochemical contents of the studied extracts of the species were screened by liquid chromatography ion trap/time of flight mass spectrometry (LC-ESI-IT-TOF-MS).

Materials and methods

Plant material

Bulbs and roots as well as aerial parts of *Bellevia mauritanica* Pomel were collected from Ibn ziad township N36° 21. 807' and E06° 28.661' at 681 m in the city of Constantine, and identified by Dr Gérard de Bélair, University of Badji Mokhtar Annaba, Faculty of Nature and Life Sciences (Voucher specimen No. 107_43).

Preparation of extracts and samples for LC-MS/MS analysis

Air dried and frozen powdered parts (aerial and bulbs-roots) were extracted with ethanol-water (80:20). Then, the crude extract of bulbs-roots was subjected to liquid-liquid extraction with chloroform, ethyl acetate and 1-butanol. Extracts were dissolved in methanol at a concentration of 1 g/L and filtered through microfilter (0.2 µm) prior to the LC-MS/MS and LC-MS-IT-TOF analyses.

Chemicals and apparatus used in bioactivity studies

The bioactivity measurements were performed on a 96-well microplate reader (Perkin Elmer Multimode Plate Reader EnSpire) at the National Center of Biotechnology Research. Gallic acid, Folin-Ciocalteu's Reagent (FCR), aluminum trichloride, 1,1'-diphenyl-2-picrylhydrazyl (DPPH), butylatedhydroxytoluene (BHT), ascorbic acid, α-tocopherol, butylatedhydroxyanisole (BHA), dimethyl sulfoxide (DMSO), diammonium salt (ABTS), nitro blue tetrazolium (NTB), neocuproine 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), butyrylcholinesterase from horse serum (BChE, EC 3.1.1.8, 7.8 U/mg, Sigma), acetylcholinesterase from electric eel (AChE, Type-VI-S, EC 3.1.1.7, 827.84 U/mg, Sigma), acetylthiocholine iodide, S-butyrylthiocholine iodide, galantamine, 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB), anis aldehyde, sulfuric acid, and glacial acetic acid were obtained from Sigma Aldrich (Stern-heim, Germany). Potassium persulfate, copper (II) chloride and sodium carbonate were obtained from Biochem Chemopharma. All other chemicals and solvents were of analytical grade. Antibiotics were obtained from Titan Media, India.

LC-IT-TOF-MS conditions

The qualitative identification of phytochemical constituents was carried out using a Shimadzu LC-MS-IT-TOF instrument. This instrument is a combination of ultra-high performance liquid chromatograph (U-HPLC) and IT-TOF-MS, a high-resolution mass spectrometer. The U-HPLC system was composed of an autosampler (SIL-20AC), a gradient pump, (LC-20AD), a degasser (DGU-20A3), a column oven (CTO-20AC) and a communication bus module (CBM-20A). An Agilent Eclipse XDB model reversed phase chromatography column (150 mm, 4.6 mm, 3.5 μ m) was used for the separation of analytes. The mobile phase flow rate, the column temperature and the injection volume were optimized as 35°C, 0.35 mL/min and 4 μ L. The mobile phase consisted of A (aqueous 5 mM ammonium formate with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). The following gradient elution profile was used: 7.5% B (0–5 min), 7.5–95% B (5–45 min), 95% B (7 min), 7.5% B (13 min). The hybrid high resolution mass spectrometer used (IT-TOF-MS) possessed an electrospray ionization (ESI) source operating in both negative and positive ionization modes. The optimized mass spectrometer (MS) conditions were as follows: positive and negative ionization modes; drying gas (N_2) pressure, 100 kPa; nebulizer gas (N_2) flow rate, 1.5 L/min; heater block temperature, 200°C; CDL (curved desolvation line) temperature, 200°C; electrospray voltage, 4.5 kV; detector voltage, 1.63 kV; mass range, m/z 100–1000 (MS1); IT pressure, 1.8×10^4 Pa; TOF pressure, 1.4×10^4 Pa; ion accumulation time, 30 msec. LC-MS Solution Version 3.4.1 (Shimadzu, Japan) software and Formula Predictor Software were used to analyze the obtained data.

LC-MS/MS (chromatography Tandem Mass Spectrometry) Instrument and Chromatographic Conditions

For this analysis, the extracts were dissolved in methanol at a concentration of 1 g/L into 2 mL vials and filtered with a microfilter (0.2 μ m) for LC-MS/MS analysis. The phytochemical quantification of the extracts was performed by a previously developed and validated LC-MS/MS method.^[21] The LC-MS/MS system consisted of a Shimadzu U-HPLC combined with a Shimadzu LCMS triple quadrupole mass spectrometer (8040 model). The U-HPLC comprised a gradient pump (LC-30 AD model), a degasser (DGU-20A3R model), a column oven (CTO-10ASvp model) and an autosampler (SIL-30AC model). The chromatographic separation was performed on a RPC18 Inertsil (ODS-4 model) reversed phase column (100 mm $\times$ 2,1 mm, 2 μ m). The column temperature was kept at 35°C throughout the analysis. The mobile phases consisted of A (water; 10 mM ammonium formate, 0.1% formic acid) and B (acetonitrile; 0.1% formic acid). The applied gradient profile was optimized as 5–20% B (0–10 min), 20% B (10–22 min), 20–50% B (22–36 min), 95% B (36–40 min) and 5% B (40–50 min). The flowrate of the mobile phase and the injection volume were optimized as 0.25 mL/min and 4 μ L, respectively. The optimum ESI parameters for the mass spectrometer were as follows: 350°C interface temperature, 250°C DL temperature, 400°C heat

block temperature and 3 L/min and 15 L/min were nebulizer and drying gas (N₂) flowrates, respectively [18].

Determination of phenolic content

Total phenolic content

The total phenolic contents of the studied extracts were determined using FCR (Folin-Ciocalteu) according to the method of Vernon et al. [19]. The calculation of the content was expressed as gallic acid equivalent (GAEs) using the equation below:

$$Y=0.007 [\text{GAEs}] + 0.116 \quad r^2= 0.994$$

Total flavonoids content

The total flavonoids contents of the studied extracts were assessed using aluminum nitrate method [20]. The calculation of the content was expressed as quercetin equivalent (QEs) using the equation below:

$$Y= 0.009 [\text{QEs}] + 0.063 \quad r^2=0.997$$

Antioxidant activity assessment

DPPH[•] free radical-scavenging activity assay

The free radical-scavenging activity was determined spectrophotometrically by the DPPH[•] assay according to Blois Blois [21] with slight modifications. In this method, ethanol was used as negative control and α -tocopherol and BHA were used as positive control. The results were expressed as IC₅₀ values and were graphically calculated from the DPPH[•] scavenging effect percentage against the sample concentration.

$$(\text{DPPH}^{\bullet}) \% \text{ inhibition} = 100 - (A \text{ sample} / A \text{ control} \times 100)$$

ABTS^{•+} radical decolorization assay

ABTS^{•+}-scavenging activity measurements were carried out using the method of Re et al. [22] with slight modifications. Water was used as negative control and BHA and BHT were used as positive control standards. The results were given as the IC₅₀, that was graphically calculated from the ABTS^{•+} scavenging effect percentage against the sample concentration.

$$(\text{ABTS}^{\bullet+}) \% \text{ inhibition} = 100 - (A \text{ sample} / A \text{ control} \times 100)$$

Superoxide DMSO alkaline scavenging assay

The superoxide anion radical-scavenging activity of extracts was determined according to Kunchandy and Rao [23] with slight modifications. IC₅₀ values were used to express the results and they were graphically calculated from the activity percentage against the sample concentration.

$$(\text{superoxyde DMSO alkaline}) \% \text{ inhibition} = 100 - (A \text{ sample} / A \text{ control} \times 100)$$

Cupric reducing capacity assay (CUPRAC)

The CUPRAC antioxidant activity was assessed according to the study conducted by Apak et al. [24] with slight modifications. The results were given as $A_{0.50}$ which corresponds to the concentration producing 0.500 absorbance. It was calculated graphically from the cupric reducing antioxidant capacity absorbance, against the sample concentration. BHT and BHA were used as standard antioxidants to compare the activity.

Evaluation of anticholinesterase activity

The measurement of butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities, were assessed by the spectrophotometric method, developed by Ellman et al. [25] Galantamine was used as reference cholinesterase inhibitory standard and the results were given as the 50% inhibition concentration (IC_{50}). IC_{50} values were calculated from the graph of the enzyme-inhibition percentage, against the sample concentration.

$$(AChE) \% inhibition = 100 - (A sample / A control \times 100)$$

Evaluation of antibacterial activity

Microbial strains and inoculums preparation

Seven bacterial strains; *Morganella morganii* (ATCC 25830), *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Citrobacter freundii* (ATCC 8090) and *Proteus vulgaris* (ATCC 29905) were obtained from Pasteur Institute (Algiers, Algeria). Previously conserved strains in glycerol (80%) were revived and inoculated in Nutritive gel (Pasteur institute, Algeria) at 37°C. After 24 h of incubation, suspensions were prepared by introducing 4-5 pure colonies in test tubes containing (0.9% NaCl) and well shaken by vortex. Inocula were set to 0.5 McFarland which corresponds to optical density from 0.08 to 0.10.

Many pathogenic bacteria put human's health in great danger such as: *Staphylococcus aureus* Meticillin-resistant (MRSA) which was first detected in 1961 (Britain) and has been known to be a “common” bacterium until now; *Pseudomonas aeruginosa* which has a fast propagation rate throughout the world, causes complicated skin, hospital-acquired pneumonia and soft tissue infections (cSSTI); *Escherichia coli* which affects the urinary tract and causes intra-abdominal infections (IAI) [8]; *Citrobacter freundii* which causes brain abscess and neonatal meningitis [26], *Proteus vulgaris*, a nosocomially acquired agent, which may cause pyelonephritis [27], *Morganella morganii* which causes systemic infections [28]. Moreover, the inappropriate use of antibiotics leads to drug resistance. For this reason, it will be necessary to focus research on the discovery of new drugs such as secondary metabolites of plants.

Disc diffusion method

Sterile discs of Wattman paper (5 mm) were first soaked with 10 µl of each dilution and then sterilely placed on the surface of Mueller Hinton agar (4 mm of thickness), previously sown with microbial strains. A DMSO-soaked disc was used as a control; the discs were allowed to diffuse for 1 hour at 4°C and all the dishes were incubated for 24h at 37°C [29]. All the extracts were tested against the seven bacterial strains and seven dilutions were realized from 100 mg/ml of plant extracts. All extracts and dilutions were sterilized using micro filters (0.22 µm).

Statistical analysis

All the results were represented as mean $\pm$ SD and were evaluated using paired t-test for antiacetylcholinesterase activity and ANOVA variance analysis for antioxidant activity using GraphPad Prism software, version 7.0. $P < 0.05$ were considered as significant.

Results and Discussion

Phenolics and flavonoids contents

Total phenolic (TPC) and total flavonoid contents (TFC) of the CHCl_3 , EtOAc, and BuOH extracts of *B. mauritanica* Pomel were assessed by Folin-Ciocalteu reagent. CHCl_3 extract contained the highest amount of TPC and TFC followed by BuOH and EtOAc (Table 3).

LC-MS-IT-TOF results

LC-MS-IT-TOF technique is essential for screening of plant constituents (alkaloids, terpenes, saponins... etc). Indeed, its results help to determine and to target contents of analyzed extracts, before carrying out isolation [30]. However, screening of studied plant extracts by LC-MS-IT-TOF is insufficient. Therefore, isolation and structural characterization by NMR are necessary, especially to identify phytochemicals responsible of biological activity. However, this prescreening is useful for the considered species, as it has not been well studied before.

The phytochemical profiles of CHCl_3 , EtOAc and BuOH extracts derived from crude extract of *Bellevalia mauritanica* Pomel were determined by an optimized LC-IT-TOF-MS method (75 min) (Figures 1-3). The analyte peaks having intensities higher than 2,500,000 were evaluated. A total of 91 secondary metabolites belonging to diverse classes were identified in each extract (Figures S6-S96 in the Supplementary file). Formulas, retention times, form of ionization (positive or negative), measured and predicted m/z (with errors) and double bond efficiency (DBE) of supposed phytochemicals are tabulated in Table 2.

Figure 1. LC- MS IT- TOF chromatograms of *B. mauritanica* Pomel CHCl_3 extract

Figure 2. LC- MS IT- TOF chromatograms of *B. mauritanica* Pomel BuOH extract

Figure 3. LC- MS IT- TOF chromatograms of *B. mauritanica* Pomel EtOAc extract

LC-MS/MS results

The phytochemical quantification of the studied extracts was performed by a previously developed and validated LC-MS/MS method using 37 standards of flavonoids, phenolic acids, and aldehydes (Figures 4-6) [18]. The extracts were richer in phenolic acids compared to flavonoid content. EtOAc extract had caffeic acid (1240.7 $\mu\text{g/g}$ extract) and CHCl_3 extract had vanillin (2048.5 $\mu\text{g/g}$ extract), as major compounds. Vanillin is used in cosmetics, foods, beverages and drugs. It exhibits many activities such as antimutagenic, antiangiogenetic, anti-colitis, anti-sickling and antianalgesic effects [31]. Extracts also contained significant amounts of certain phytochemicals such as protocatechuic acid (274.3 and 145.2 $\mu\text{g/g}$ extract, respectively), *p*-coumaric acid (382.8 and 310.9 $\mu\text{g/g}$ extract, respectively), ferulic acid (361.2 and 969.4 $\mu\text{g/g}$ extract, respectively), kaempferol (146.7 and 150.6 $\mu\text{g/g}$ extract, respectively) and syringic acid (138.2 $\mu\text{g/g}$ extract) in CHCl_3 . However, they contained low amounts of salicylic acid (19.2 and 15.5 $\mu\text{g/g}$ extract, respectively), apigenin (12.1 and 5.3 $\mu\text{g/g}$ extract respectively) and luteolin (9.5 and 42.4 $\mu\text{g/g}$ extract, respectively). BuOH extract had low content in these phytochemicals (Table 1).

Figure 4. LC-MS/MS chromatogram of *B. mauritanica* Pomel EtOAc extract

Figure 5. LC-MS/MS chromatogram of *B. mauritanica* Pomel BuOH extract

Figure 6. LC-MS/MS chromatogram of *B. mauritanica* Pomel CHCl_3 extract

Antioxidant activity

Antioxidant activity was performed using four methods: DPPH[•] free radical scavenging to evaluate the electron transfer to the media, ABTS^{•+} which has the same principle as DPPH[•], whereby the radical is a cation, CUPRAC to evaluate the reduction of a chromogenic oxidizing [22], as O_2^- is produced by mitochondria, by the NADPH oxidase or the xanthine oxidase [6], or by mitochondrial electrons of the transport system. For this reason, DMSO alkaline was chosen. BHA and BHT were used as standard compounds in DPPH, ABTS^{•+} and CUPRAC methods, while α -tocopherol and ascorbic acid were used in superoxide DMSO alkaline method. The results of all the tests, expressed in term of IC_{50} ($\mu\text{g/ml}$), are presented in Table 3.

Among the extracts, the EtOAc derived from BRCE (Bulbs-Roots Crude Extract) was by far the most active in all the studied antioxidant activity methods. The IC_{50} values of the EtOAc extract and standards were obtained in DPPH[•] (IC_{50} : 18.92 ± 0.56 , IC_{50} : 6.14 ± 0.41 , IC_{50} : 12.99 ± 0.41 , and IC_{50} : 13.02 ± 5.17 $\mu\text{g/ml}$ for the extract, BHA, BHT, and α -tocopherol, respectively), ABTS^{•+} (IC_{50} : 5.70 ± 0.07 , IC_{50} : 1.81 ± 0.10 , and IC_{50} : 1.29 ± 0.30 $\mu\text{g/ml}$ for the extract, BHA and BHT, respectively), CUPRAC (IC_{50} : 20.16 ± 0.32 , IC_{50} : 5.35 ± 0.71 $\mu\text{g/ml}$, and IC_{50} : 9.62 ± 0.87 for the extract, BHA and BHT, respectively) and superoxide DMSO alkaline (IC_{50} : 5.59 ± 0.22 , IC_{50} : 1.33 ± 0.04 , and IC_{50} : 0.50 ± 0.004 $\mu\text{g/ml}$ for the extract, α -tocopherol and ascorbic acid, respectively). The EtOAc extract might have anti-inflammatory properties since there is a good correlation between superoxide scavenging and anti-inflammatory activities according to Saldanha et al. [32]. Moreover, it contained saponins [33] that were highlighted by screening on TLC (thin layer chromatography) by sulfuric acid/EtOH (1:9) [34]. Furthermore, the presence of high amounts of caffeic acid and moderate amounts of kaempferol was identified by LC-MS/MS [35, 36]. This extract exhibited the highest antioxidant potential among all studied extracts in all tests.

Table 1. Analytical parameters that belong to the LC-MS/MS method ^[18]

No	Analytes	RT ^a	Mother ion (m/z) ^b	Fragment ions	Ion. mode	Equation	R ^{2c}	RSD % ^d		Linearity Range (µg/L)	LOD/LOQ (µg/L) ^e	Recovery (%)		U ^f	EtOAc	BuOH	CHCl ₃
								Interday	Intraday			Interday	Intraday				
1	Quinic acid	1.13	190.95	85.3-93.3	Neg	y=41.06x+10671	0.996	0.00259	0.00274	250-10000	75.8/79.4	1.00288	0.98778	0.0082	19.1	96.3	98.6
2	Malic acid	1.23	133.00	115.2-71.3	Neg	y=316.95x-42041	0.999	0.00477	0.00527	250-10000	55.3/67.5	1.01266	0.99836	0.0113	N.D.	N.D.	N.D.
3	Fumaric acid	1.48	115.00	71.4	Neg	y=64.99x-11592	0.997	0.00536	0.00460	100-5000	28.1/34.5	0.99748	0.99867	0.0124	N.D.	N.D.	N.D.
4	Galic acid	3.00	168.85	125.2-79.2	Neg	y=226.76x+38152	0.998	0.01601	0.01443	250-10000	95.5/106.9	1.00004	1.00454	0.0282	N.D.	N.D.	N.D.
5	Protocatechuic acid	4.93	152.95	108.3	Neg	y=297.75x+30590	0.995	0.01236	0.01296	100-5000	28.2/31.4	0.99404	1.01070	0.0411	274.3	N.D.	145.2
6	Pyrocatechol	6.48	109.00	108.35-91.3	Neg	y=30.61x+14735	0.996	0.01313	0.01339	1000-20000	261.1/278.4	0.99987	0.99936	0.0235	N.D.	N.D.	N.D.
7	Chlorogenic acid	7.13	353.15	191.2	Neg	y=781.36x-18697	0.998	0.00058	0.00076	25-1000	6.2/8.1	1.00806	0.99965	0.0069	1.5	2.5	N.D.
8	4-OH-benzoic acid	7.39	136.95	93.3-65.3	Neg	y=409.03x+112079	0.998	0.01284	0.01538	250-10000	33.2/38.1	0.99662	1.00058	0.0289	N.D.	N.D.	N.D.
9	Vanillic acid	8.57	166.90	152.3-108.3	Neg	y=35.84x-12097	0.999	0.00528	0.00619	1000-20000	122.2/139.7	1.00093	1.04095	0.0508	N.D.	N.D.	N.D.
10	Caffeic acid	8.80	178.95	135.2-134.3	Neg	y=3963.32x+178156	0.998	0.01454	0.01469	25-1000	18.4/22.4	1.00917	0.98826	0.0354	1240.7	14.1	97.0
11	Syringic acid	9.02	196.95	182.2-167.3	Neg	y=42.33x-52547	0.996	0.01049	0.01345	1000-20000	212.5/233.3	0.99922	0.99977	0.0238	N.D.	N.D.	138.2
12	Vanillin	10.87	151.00	136.3-92.2	Neg	y=446.10x+70934	0.998	0.00696	0.00793	250-10000	44.3/53.1	0.99679	0.99611	0.0280	N.D.	N.D.	2048.5
13	Salicylic acid	11.16	136.95	93.3-65.3	Neg	y=5286.26x+309192	0.989	0.01016	0.01242	25-1000	5.0/6.5	1.00989	0.99013	0.0329	19.2	N.D.	15.5
14	p-Coumaric acid	11.53	162.95	119.3-93.3	Neg	y=3199.20x+13002	0.992	0.01820	0.01727	25-1000	7.3/9.1	1.00617	1.01224	0.0516	382.8	N.D.	310.9
15	Rutin	12.61	609.05	300.1-271.1	Neg	y=561.91x-16879	0.997	0.00473	0.00624	25-1000	5.5/6.5	1.00994	0.98017	0.0159	N.D.	N.D.	N.D.
16	Ferulic acid	12.62	192.95	178.3	Neg	y=80.45x-31782	0.997	0.00708	0.00619	250-10000	36.6/42.0	0.99987	1.00289	0.0494	361.2	N.D.	969.4
17	Sinapinic acid	12.66	222.95	208.3-149.2	Neg	y=141.96x-73294	0.992	0.01446	0.01517	250-10000	78.7/86.1	1.00164	0.99962	0.0281	N.D.	N.D.	N.D.
18	Hesperidin	12.67	610.90	303.1-465.1	Poz	y=1340.27x-43769	0.998	0.00945	0.01126	25-1000	3.4/4.2	1.01733	1.01263	0.0262	N.D.	N.D.	N.D.
19	Isoquercitrin	13.42	463.00	300.1-271.1	Neg	y=803.23x+4981	0.999	0.00682	0.00515	25-1000	5.4/6.3	1.00594	1.00722	0.0133	N.D.	N.D.	N.D.
20	Rosmarinic acid	14.54	359.00	161.2-197.2	Neg	y=909.67x-201692	0.994	0.02014	0.01751	100-5000	6.6/8.8	0.99206	1.03431	0.0713	N.D.	N.D.	N.D.
21	Nicotiflorin	14.68	593.05	285.1-255.2	Neg	y=498.38x+79274	0.991	0.00737	0.00875	100-5000	22.4/25.5	1.02558	1.00970	0.0276	N.D.	N.D.	N.D.
22	o-Coumaric acid	15.45	162.95	119.4-93.3	Neg	y=1219.34x-10915	0.999	0.02730	0.02566	25-1000	24.4/31.1	0.98344	0.99061	0.0513	N.D.	N.D.	N.D.
23	Rhoifolin	16.11	577.05	269.2-211.1	Neg	y=237.15x+11887	0.999	0.00747	0.01528	100-5000	23.1/27.9	1.01046	1.01739	0.0941	N.D.	N.D.	N.D.
24	Quercitrin	16.41	447.15	301.1-255.1	Neg	y=339.39x+38910	0.999	0.01528	0.02320	100-5000	22.0/25.2	0.99726	1.00620	2.0079	N.D.	N.D.	N.D.
25	Apigetrin	16.59	431.00	268.2-239.2	Neg	y=1775.55x+91121	0.993	0.01797	0.01607	25-1000	5.4/6.1	1.01394	1.00419	0.0597	12.1	N.D.	5.3
26	Coumarin	17.40	147.05	91.0-103.2	Poz	y=33.64x-89700	0.994	0.01306	0.01239	1000-20000	208.4/228.4	0.99947	1.00081	0.0237	N.D.	N.D.	N.D.
27	Myricetin	18.72	317.00	179.2-151.3	Neg	y=583.55x+205727	0.999	0.00652	0.00711	250-10000	53.2/57.2	0.99982	1.00042	0.0126	N.D.	N.D.	N.D.
28	Fisetin	19.30	284.95	135.2-121.3	Neg	y=547.46x+274791	0.991	0.00557	0.00820	250-10000	54.4/61.4	0.99877	1.00031	0.0148	N.D.	N.D.	N.D.
29	Cinnamic acid	25.61	147.00	103.15-77.3	Neg	y=9.06x-12403	0.996	0.00648	0.00816	5000-20000	821.8/859.7	1.00051	0.99927	0.0143	N.D.	N.D.	N.D.
30	Liquiritigenin	25.62	254.95	119.3-135.1	Neg	y=2384.96x+59141	0.996	0.01849	0.01738	25-1000	5.5/6.6	1.00333	0.99957	0.0341	N.D.	N.D.	N.D.
31	Quercetin	28.17	300.90	151.2-179.2	Neg	y=1198.48x+480562	0.990	0.01589	0.01360	100-5000	23.3/28.9	0.98470	1.00103	0.0543	N.D.	N.D.	N.D.
32	Luteolin	28.27	284.75	133.2-151.2	Neg	y=3272.65x+150557	0.997	0.00575	0.00696	25-1000	5.4/6.5	1.00772	0.99524	0.0174	9.5	N.D.	42.4
33	Naringenin	30.68	270.95	151.2-119.3	Neg	y=4315.1x+178410	0.995	0.02054	0.02019	25-1000	5.4/6.4	0.99883	1.01002	0.0521	59.8	N.D.	76.3
34	Apigenin	31.43	268.95	117.3-151.2	Neg	y=4548.36x+295252	0.990	0.02304	0.02204	25-1000	5.4/6.3	1.01444	1.01331	0.0650	34.5	N.D.	73.1
35	Hesperetin	31.76	300.95	164.2-136.2	Neg	y=876.67x+48916	0.997	0.03209	0.02605	25-1000	5.6/6.9	0.98850	0.99435	0.0562	N.D.	N.D.	N.D.
36	Kaempferol	31.88	284.75	255.1-117.3	Neg	y=26.29x+87558	0.992	0.01436	0.01070	1000-20000	206.6/214.3	0.99971	0.99851	0.0209	146.7	N.D.	150.6
37	Chrysin	36.65	252.95	143.3-119.4	Neg	y=2032.13x+95593	0.993	0.00490	0.00630	25-1000	5.4/6.2	1.00338	1.00437	2.0083	N.D.	N.D.	N.D.

^aRT: Retention time, ^bMother ion(m/z): Molecular ions of the standard compounds (m/z ratio), ^cR²: Coefficient of determination, ^dRSD: Relative standard deviation, ^eLOD/LOQ (µg/L): Limit of detection/quantification, ^fU (%): percent relative uncertainty at 95% confidence level (k = 2)

Table 2. Screening of chemical constituents in *Bellevia mauritanica* Pomel extracts by HPLC-IT-TOF-MS

N	Elemental composition	Retention time	Measured m/z	Predicted m/z	Error (ppm)	DBE	CHCl ₃	EtOAc	BuOH
1	C ₁₂ H ₂₂ O ₁₁	0.99	365.1055 [M+Na] ⁺	365.1054 [M+Na] ⁺	0.27	2.0	*		
2	C ₁₄ H ₂₀ O ₁₁	0.99	365.1084 [M+H] ⁺	365.1078 [M+H] ⁺	1.64	5.0			*
3	C ₁₇ H ₂₀ O ₁₁	10.55	423.0902 [M+Na] ⁺	423.0898 [M+Na] ⁺	0.95	8.0		*	
4	C ₁₉ H ₁₈ O ₁₁	10.61	421.0773 [M-H] ⁻	421.0776 [M-H] ⁻	-0.3	11.0	*		
5	C ₂₂ H ₂₂ O ₇	14.17	399.1425 [M+H] ⁺	399.1438 [M+H] ⁺	-3.26	12.0		*	
6	C ₂₀ H ₂₄ O ₇	14.30	399.1409 [M+Na] ⁺	399.1414 [M+Na] ⁺	-1.25	9.0	*		
7	C ₂₂ H ₂₂ O ₇	15.37	399.1427 [M+H] ⁺	399.1438 [M+H] ⁺	-2.76	12.0	*		
8	C ₂₁ H ₂₆ O ₈	18.78	429.1528 [M+Na] ⁺	429.1520 [M+Na] ⁺	1.86	9.0	*		
9	C ₂₇ H ₁₂ O ₆	22.59	431.0575 [M+H] ⁺	431.0561 [M+H] ⁺	3.25	22.0	*		
10	C ₁₈ H ₁₆ O ₄	22.85	314.1400 [M+NH ₄] ⁺	314.1387 [M+NH ₄] ⁺	4.14	11.0	*		
11	C ₁₈ H ₁₈ O ₈	22.97	385.0869 [M+Na] ⁺	385.0894 [M+Na] ⁺	-6.49	10.0		*	
12	C ₂₉ H ₁₆ O ₇	23.01	475.0843 [M-H] ⁻	475.0823 [M-H] ⁻	4.21	22.0		*	
13	C ₁₈ H ₁₈ O ₈	23.01	363.1066 [M+H] ⁺	363.1074 [M+H] ⁺	-2.20	10.0		*	
14	C ₂₀ H ₁₆ O ₈	23.07	385.0911 [M+H] ⁺	385.0918 [M+H] ⁺	-1.82	13.0	*		
15	C ₂₂ H ₂₀ O ₁₂	23.08	475.0853 [M-H] ⁻	475.0882 [M-H] ⁻	-6.10	13.0	*		
16	C ₁₈ H ₁₈ O ₈	23.10	363.1083 [M+H] ⁺	363.1074 [M+H] ⁺	2.48	10.0	*		
17	C ₁₉ H ₁₈ O ₅	23.80	344.1502 [M+NH ₄] ⁺	344.1492 [M+NH ₄] ⁺	2.91	11.0	*		
18	C ₁₈ H ₁₈ O ₉	24.41	377.0890 [M-H] ⁻	377.0878 [M-H] ⁻	3.18	10.0		*	
19	C ₂₁ H ₁₈ O ₁₁	24.41	445.0747 [M-H] ⁻	445.0776 [M-H] ⁻	-6.52	13.0		*	
20	C ₂₆ H ₁₀ O ₂	24.41	355.0759 [M+H] ⁺	355.0754 [M+H] ⁺	1.41	22.0		*	
21	C ₁₇ H ₁₆ O ₇	24.43	333.0963 [M+H] ⁺	333.0969 [M+H] ⁺	-1.80	10.0		*	
22	C ₂₁ H ₁₈ O ₁₁	24.52	445.0745 [M-H] ⁻	445.0776 [M-H] ⁻	-6.97	13.0	*		
23	C ₁₇ H ₁₆ O ₇	24.52	333.0962 [M+H] ⁺	333.0969 [M+H] ⁺	-2.10	10.0	*	*	
24	C ₁₀ H ₁₈ O ₁₂	27.02	348.1162 [M+NH ₄] ⁺	348.1137 [M+NH ₄] ⁺	7.18	2.0	*	*	
25	C ₁₈ H ₁₈ O ₇	27.06	369.0930 [M+Na] ⁺	369.0945 [M+Na] ⁺	-4.06	10.0		*	
26	C ₁₈ H ₁₈ O ₇	27.09	369.0946 [M+Na] ⁺	369.0945 [M+Na] ⁺	0.27	10.0	*		
27	C ₁₈ H ₁₈ O ₇	27.13	347.1129 [M+H] ⁺	347.1125 [M+H] ⁺	1.15	10.0	*	*	
28	C ₂₀ H ₁₄ O ₁₁	29.15	429.0434 [M-H] ⁻	429.0463 [M-H] ⁻	-6.76	14.0	*		
29	C ₁₉ H ₂₀ O ₇	33.85	361.1283 [M+H] ⁺	361.1282 [M+H] ⁺	0.28	10.0	*	*	
30	C ₁₈ H ₃₂ O ₅	34.77	351.2139 [M+Na] ⁺	351.2142 [M+Na] ⁺	-0.85	3.0	*	*	
31	C ₁₈ H ₃₄ O ₅	35.73	353.2296 [M+Na] ⁺	353.2298 [M+Na] ⁺	-0.57	2.0		*	
32	C ₁₂ H ₂₀ O ₃	35.81	213.1486 [M+H] ⁺	213.1485 [M+H] ⁺	0.47	3.0	*		
33	C ₁₈ H ₃₄ O ₅	35.82	348.2747 [M+NH ₄] ⁺	348.2744 [M+NH ₄] ⁺	0.86	2.0	*		
34	C ₂₀ H ₃₂ O ₅	35.83	353.2312 [M+H] ⁺	353.2323 [M+H] ⁺	-3.11	5.0	*		
35	C ₁₇ H ₁₄ O ₅	36.46	299.0909 [M+H] ⁺	299.0914 [M+H] ⁺	-1.67	11.0	*		
36	C ₁₆ H ₃₂ O ₂	37.41	274.2738 [M+NH ₄] ⁺	274.2741 [M+NH ₄] ⁺	-1.09	1.0	*	*	*
37	C ₁₈ H ₃₆ O ₃	37.55	318.3002 [M+NH ₄] ⁺	318.3003 [M+NH ₄] ⁺	-0.31	1.0			*
38	C ₂₂ H ₃₈ O	37.55	319.3029 [M+H] ⁺	319.2995 [M+H] ⁺	10.65	4.0			*
39	C ₁₃ H ₂₂ O ₃	37.56	227.1636 [M+H] ⁺	227.1642 [M+H] ⁺	-2.64	3.0		*	
40	C ₁₈ H ₃₀ O ₂	40.02	296.2595 [M+NH ₄] ⁺	296.2584 [M+NH ₄] ⁺	3.71	4.0	*	*	
41	C ₁₈ H ₂₈ O ₂	40.39	277.2152 [M+H] ⁺	277.2162 [M+H] ⁺	-3.61	5.0		*	
42	C ₅ H ₆ O ₂₀	40.42	384.9350 [M-H] ⁻	384.9380 [M-H] ⁻	-7.79	3.0	*		
43	C ₁₆ H ₃₂ O ₂	40.42	279.2309 [M+Na] ⁺	279.2295 [M+Na] ⁺	5.01	1.0	*		
44	C ₁₈ H ₃₀ O ₃	40.45	295.2269 [M-H] ⁻	295.2268 [M-H] ⁻	0.34	4.0	*	*	
45	C ₁₈ H ₃₆ O ₃	40.70	318.3008 [M+NH ₄] ⁺	318.3003 [M+NH ₄] ⁺	1.57	1.0		*	
46	C ₁₈ H ₃₂ O ₂	40.83	298.2738 [M+NH ₄] ⁺	298.2741 [M+NH ₄] ⁺	-1.01	3.0			*
47	C ₂₀ H ₃₀ O ₂	40.84	320.2581 [M+NH ₄] ⁺	320.2584 [M+NH ₄] ⁺	-0.94	6.0			*

DBE: Double Band Efficiency, *: presence.

Table 2. Screening of chemical constituents in *Bellevalia mauritanica* Pomel extracts by HPLC-IT-TOF-MS (Continued)

N	Elemental composition	Retention time	Measured m/z	Predicted m/z	Error (ppm)	DBE	CHCl ₃	EtOAc	BuOH
48	C ₁₈ H ₂₈ O ₃	41.03	293.2143 [M+H] ⁺	293.2111 [M+H] ⁺	10.91	5.0	*		
49	C ₂₄ H ₃₄ O ₃	41.15	369.2469 [M-H] ⁻	369.2435 [M-H] ⁻	9.21	8.0			*
50	C ₁₉ H ₃₈	41.20	284.3315 [M+NH ₄] ⁺	284.3312 [M+NH ₄] ⁺	1.06	1.0	*		
51	C ₁₉ H ₃₂ O ₂	41.49	293.2478 [M+H] ⁺	293.2475 [M+H] ⁺	1.02	4.0		*	
52	C ₁₉ H ₃₂ O ₃	41.54	309.2417 [M+H] ⁺	309.2424 [M+H] ⁺	-2.26	4.0	*	*	
53	C ₂₂ H ₂₈	41.55	310.2481 [M+NH ₄] ⁺	310.2529 [M+NH ₄] ⁺	-15.47	9.0		*	
54	C ₂₀ H ₃₄ O ₂	41.98	307.2644 [M+H] ⁺	307.2632 [M+H] ⁺	3.91	4.0	*		
55	C ₂₀ H ₃₄ O ₃	42.03	323.2579 [M+H] ⁺	323.2581 [M+H] ⁺	-0.62	4.0		*	
56	C ₂₁ H ₄₄ O ₈	42.05	442.3352 [M+NH ₄] ⁺	442.3374 [M+NH ₄] ⁺	-4.97	0.0			*
57	C ₁₂ H ₁₆ O	42.08	177.1268 [M+H] ⁺	177.1274 [M+H] ⁺	-3.39	5.0			*
58	C ₁₂ H ₂ O ₁₅	42.37	384.9343 [M-H] ⁻	384.9321 [M-H] ⁻	5.72	12.0		*	
59	C ₂₁ H ₃₈ O ₄	42.43	372.3117 [M+NH ₄] ⁺	372.3108 [M+NH ₄] ⁺	2.42	3.0	*	*	
60	C ₁₂ H ₂ O ₁₅	42.43	384.9342 [M-H] ⁻	384.9321 [M-H] ⁻	5.46	12.0	*		
61	C ₂₁ H ₃₈ O ₄	42.44	377.2665 [M+Na] ⁺	377.2662 [M+Na] ⁺	0.80	3.0	*		
62	C ₁₈ H ₂₈ O	42.65	278.2434 [M+NH ₄] ⁺	278.2478 [M+NH ₄] ⁺	-15.81	5.0			*
63	C ₃₃ H ₄₆ O ₄	42.73	505.3315 [M-H] ⁻	505.3323 [M-H] ⁻	-1.58	11.0			*
64	C ₄₃ H ₄₂ O	42.74	619.3224 [M+HCOO] ⁻	619.3218 [M+HCOO] ⁻	0.97	23.0			*
65	C ₂₆ H ₅₂ O ₁₄	42.84	633.3364 [M+HCOO] ⁻	633.3339 [M+HCOO] ⁻	3.95	1.0			*
66	C ₃₄ H ₄₈ O ₄	42.87	519.3461 [M-H] ⁻	519.3480 [M-H] ⁻	-3.66	11.0			*
67	C ₁₂ H ₂ O ₁₅	42.93	384.9349 [M-H] ⁻	384.9321 [M-H] ⁻	7.27	12.0		*	
68	C ₃₉ H ₅₀ O ₇	43.05	629.3454 [M-H] ⁻	629.3484 [M-H] ⁻	-4.77	15.0			*
69	C ₁₉ H ₃₂ O ₂	43.07	293.2484 [M-H] ⁻	293.2475 [M-H] ⁻	3.07	4.0		*	
70	C ₃₅ H ₄₈ O ₃	43.20	515.3519 [M-H] ⁻	515.3531 [M-H] ⁻	-2.33	12.0	*		
71	C ₃₁ H ₄₀ O ₃	43.21	459.2901 [M-H] ⁻	459.2905 [M-H] ⁻	-0.87	12.0	*		
72	C ₅ H ₆ O ₂₀	43.44	384.9355 [M-H] ⁻	384.9380 [M-H] ⁻	-6.49	3.0	*		*
73	C ₃₅ H ₄₈ O ₄	43.54	531.3480 [M-H] ⁻	531.3480 [M-H] ⁻	0.00	12.0			*
74	C ₁₈ H ₃₄ O	43.59	284.2964 [M+NH ₄] ⁺	284.2948 [M+NH ₄] ⁺	5.63	2.0		*	
75	C ₃₃ H ₅₄ O ₈	43.62	577.3724 [M-H] ⁻	577.3746 [M-H] ⁻	-3.81	7.0		*	
76	C ₁₈ H ₃₄ O	43.65	284.2961 [M+NH ₄] ⁺	284.2948 [M+NH ₄] ⁺	4.57	2.0	*		
77	C ₁₆ H ₃₈ O ₉	43.79	392.2865 [M+NH ₄] ⁺	392.2854 [M+NH ₄] ⁺	2.80	-2.0	*		
78	C ₂₂ H ₃₈ O	44.02	336.3251 [M+NH ₄] ⁺	336.3261 [M+NH ₄] ⁺	3.87	4.0	*		*
79	C ₄₅ H ₄₄ O	44.05	645.3393 [M+HCOO] ⁻	645.3374 [M+HCOO] ⁻	2.94	24.0	*		
80	C ₃₅ H ₅₈ O ₈	44.30	605.4027 [M-H] ⁻	605.4059 [M-H] ⁻	-5.29	7.0	*		
81	C ₂₄ H ₃₈ O	44.43	360.3228 [M+NH ₄] ⁺	360.3261 [M+NH ₄] ⁺	-9.16	6.0		*	
82	C ₁₂ H ₂ O ₁₅	44.43	384.9347 [M-H] ⁻	384.9321 [M-H] ⁻	6.75	12.0		*	
83	C ₂₁ H ₄₈ O	44.49	339.3452 [M+Na] ⁺	339.3597 [M+Na] ⁺	-42.73	-2.0		*	
84	C ₂₂ H ₃₆ O ₃	44.53	347.2581 [M-H] ⁻	347.2592 [M-H] ⁻	-3.17	5.0		*	
85	C ₂₂ H ₄₀ O	44.57	338.3428 [M+NH ₄] ⁺	338.3417 [M+NH ₄] ⁺	3.25	3.0	*		*
86	C ₅ H ₆ O ₂₀	44.86	384.9367 [M-H] ⁻	384.9380 [M-H] ⁻	-3.38	3.0	*	*	
87	C ₂₂ H ₄₂ O	45.11	340.3573 [M+NH ₄] ⁺	340.3574 [M+NH ₄] ⁺	-0.29	2.0	*	*	*
88	C ₂₄ H ₄₄ O	45.18	366.3744 [M+NH ₄] ⁺	366.3730 [M+NH ₄] ⁺	3.82	3.0	*	*	*
89	C ₃₃ H ₅₄ O ₅	45.33	531.4077 [M+H] ⁺	531.4044 [M+H] ⁺	6.21	7.0	*		*
90	C ₂₄ H ₄₆ O	45.93	368.3917 [M+NH ₄] ⁺	368.3887 [M+NH ₄] ⁺	8.14	2.0			*
91	C ₁₆ H ₂ O ₁₂	45.36	384.9456 [M-H] ⁻	384.9473 [M-H] ⁻	-4.42	16.0			*

The weakest antioxidant potential was observed for the BRCE and APCE extracts in all the tested methods (Table 3). However, one study reported the high antioxidant potency of aerial parts extracts of *Bellevia gracilis* Feinbrun, in total antioxidant status (TAS) ranging from 4,169 to 1,301 mmol Trolox Equiv./L [37].

Anticholinesterase activity

Regarding the enzymatic inhibitory effect, the anticholinesterase activities were evaluated for all extracts using two enzymes: AChE and BChE (Table 3) which decrease neurotransmitter Ach involved in Alzheimer disease [38]. The only active extract able of inhibiting AChE, was EtOAc (IC_{50} : 7.09 ± 0.04 μ g/ml) which was close to galantamine (IC_{50} : 6.27 ± 1.15 μ g/ml) that was used as standard. Antiacetylcholinesterase activity might be due to the presence of triterpenoids in the EtOAc fraction [39], confirmed by phytochemical screening on TLC using Anisaldehyde. Furthermore, the EtOAc extract contained phenolic acids (high amounts of caffeic acid and moderate amounts of ferulic acid, p-coumaric acid and protocatechuic acid) that have acetylcholinesterase inhibitory activity solely or together [40]. There might be a synergy between these compounds. As for BChE, none of the extracts had an inhibitory effect on this enzyme.

Table 3. Antioxidant activity by DPPH[•], ABTS^{•+}, Superoxyde DMSO alkaline, and CUPRAC assays^a, anticholinesterase activity^a and total phenolic and flavonoids of the different extracts of *B. mauritanica* Pomel

Extracts	[IC ₅₀] (μg/ml)		Total phenolic compounds content ^[a] (μg GAE/mg)	Flavonoids content ^[a] (μg QE/mg)	DPPH ^{•a}	[IC ₅₀] (μg/ml)		[A _{0.5}]
	BChE assay ^[a]	AChE assay ^[a]				ABTS ^{•+} [a]	superoxyde DMSO alkaline ^[a]	
APCE	NA ^[c]	NA ^[c]	24.59 ± 0.55	8.15 ± 0.25	150.17 ± 10.25	95.64 ± 1.92	110.74 ± 7.36	105.08 ± 1.12
BRCE	NA ^[c]	NA ^[c]	2.68 ± 0.30	3.01 ± 0.80	281.33 ± 2.35	101.09 ± 3.22	47.08 ± 1.76	231.24 ± 4.35
CHCl ₃	NA ^[c]	NA ^[c]	118.27 ± 1.30	44.75 ± 0.98	52.53 ± 3.39	40.20 ± 1.75	20.1 ± 1.25	50.24 ± 0.14
BuOH	NA ^[c]	NA ^[c]	91.65 ± 0.82	17.05 ± 0.92	29.28 ± 2.53	20.85 ± 1.07	10.0 ± 0.08	45.17 ± 0.04
EtOAc	NA ^[c]	7.09 ± 0.04	65.67 ± 1.10	10.78 ± 0.55	18.92 ± 0.56	5.70 ± 0.07	5.59 ± 0.22	20.16 ± 0.32
Galantamine ^[b]	6.27 ± 1.15	34.75 ± 1.99	-	-	-	-	-	-
BHA ^[b]	-	-	-	-	6.14 ± 0.41	1.81 ± 0.10	NT	5.35 ± 0.71
BHT ^[b]	-	-	-	-	12.99 ± 0.41	1.29 ± 0.30	NT	9.62 ± 0.87
α-tocopherol ^[b]	-	-	-	-	13.02 ± 5.17	NT	1.33 ± 0.04	NT
ascorbic acid ^[b]	-	-	-	-	NT	NT	0.5 ± 0.004	NT

BHA: Butylatedhydroxyanisole, BHT: Butylatedhydroxytoluene. NT: not tested. ^[a]Values expressed are means±S.D. of three parallel measurements (n=3). ^[b]Reference compounds. ^[c]NA: Not active.

Antibacterial activity

Furthermore, antibacterial activity was evaluated using the disc diffusion method. Among the tested extracts, the most active one was the EtOAc followed by CHCl₃ (Table 4). Against *Staphylococcus aureus* ATCC 43300, EtOAc extract had medium activity (11 ± 0.58 mm), and (8.5 ± 0.58) mm at 100 mg/ml and 50 mg/ml respectively), and such was the case for CHCl₃ extract (9.5 ± 0.58 mm) at 100 mg/ml). Against *Bacillus subtilis* ATCC 6633, EtOAc extract had a mild activity (11 ± 0.58 mm) at 100

mg/ml and a weak activity (8 ± 0.58 mm) at 6.25 mg/ml and 50 mg/ml, and (7 ± 0.58 mm) at 12.5 mg/ml and 25 mg/ml) followed by the CHCl_3 extract which had medium activity at 100 mg/ml (9.5 ± 0.58) and (8.5 ± 0.58 , 6.5 ± 0.58 , 9 ± 0.58 mm) at 50, 25, and 12.5 mg/ml respectively. BRCE showed a weak activity (7.5 ± 0.58 mm) at 50 mg. Only the two Gram+ve strains were sensitive to the active extracts: *S. aureus* ATCC 43300 and *B. subtilis* ATCC 6633 (Table 4). This activity might be due to Kaempferol [35]. However, the BuOH (1-butanol of bulb-roots crude extract) and aerial part extract showed no sensitivity against all tested pathogens. This antimicrobial action of plant secondary metabolites is due to their diffusion through cellular membranes and their interaction with intracellular constituents [41].

No studies on extracts of the species *Bellevallia* have so far been performed. Therefore, our results were compared with those of other works carried out on the genus *Muscari* which is the closest to *Bellevallia* Lapeyr. We found that APCE had similar effects to cyclohexane, dichloromethane, methanolic and aqueous extracts of *Muscari caucasicum* Baker against which the two common strains *S. aureus* and *Escherichia coli* were not sensitive [42]. Besides, APCE had a similar effect to the ethanolic extract of *Muscari neglectum*. The latter had no antibacterial effect against *S. aureus*, while it exerted a good antibacterial effect on *Escherichia coli* [43].

CHCl_3 extract had a similar composition to EtOAc but it was less active, in terms of antioxidant and antibacterial effects. However, it had no inhibitory effect on acetylcholinesterase, despite the presence of enzymatic inhibitors of phenolic acids (Table 3). This could be explained by the fact that the CHCl_3 extract contains more metabolites such as primary ones and may interfere antagonistically with secondary metabolites [44]. Furthermore, secondary metabolites may undergo antagonistic effects.

Supplementary material

HPLC-MS-IT-TOF Figures (S1 to S96) are available in the Supplementary file.

Table 4. Zones of inhibition of *Bellevia mauritanica* Pomel active extracts^a and antibiotics^b tested against pathogen bacterial strains (mm)

	Gram +ve					Gram –ve				
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Citrobacter freundii</i>	<i>Morganella morganii</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus vulgaris</i>			
	ATCC 43300	ATCC 6633	ATCC 8090	ATCC 25830	ATCC 25922	ATCC 27853	ATCC 29905			
EtOAc ^[a] mg/ml										
100	11 ± 0.58	11 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
50	8 ± 0.58	8 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
25	0.00 ± 0.00	7 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
12.5	0.00 ± 0.00	7 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
6.25	0.00 ± 0.00	8 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
CHCl ₃ ^[a] mg/ml										
100	9.5 ± 0.58	9.5 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
50	0.00 ± 0.00	8.5 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
25	0.00 ± 0.00	6.5 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
12.5	0.00 ± 0.00	9 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
BRCE ^[a] mg/ml										
100	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
50	0.00 ± 0.00	7.5 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00			
Oxa ^[b]	NA ^[c]	NT	NT	NT	NT	NT	NT			
E ^[b]	22	NT	NT	NT	NT	NT	NT			
C ^[b]	28	NT	23	NT	NT	NT	25			
Pi ^[b]	NT	NT	NT	NA	18	NT	NA ^[c]			
PRL ^[b]	NT	NT	NT	NA ^[c]	NA ^[c]	NA ^[c]	NT			
AK ^[b]	NT	NT	23	27	28	24	NT			
CD ^[b]	NT	NA ^[c]	NT	NT	NT	NT	NT			
CIP ^[b]	NT	27	NT	NT	NT	30	NT			
AMC ^[b]	NT	NT	NA ^[c]	NT	NT	NT	NT			

Ox: oxacillin (1µg/disc); Pi: pipemidic acid (20µg/disc); E: erythromycin (15µg/disc) ; PRL: piperacilline (100µg/disc); AMC: augmentin (30µg/disc); C: chloramphenicol (30µg/disc); CD: clindamycin (2µg/disc); AK: Amikacine (30µg/disc); CIP: ciprofloxacin (5µg/disc). NA: Not active. Nt: Not tested. N.B. negative control: DMSO (dimethyl sulfoxide) was no active. ^[a]Values expressed are means±S.D. of three parallel measurements (n=3). ^[b]Reference compounds. ^[c]NA: Not active.

Conclusion

The present study deals for the first time with phytochemical screening, antioxidant, antibacterial and anticholinesterase activities of *Bellevallia mauritanica* Pomel. According to the high-resolution LC-MS-IT-TOF analysis results, 91 phytochemicals were qualitatively determined in each fraction of the studied species. Moreover, quantitative screening of phenolic acids and flavonoids, accomplished by using a previously developed and validated LC-MC/MS method, revealed the presence of different classes of flavonoids and phenolic compounds, in addition to the presence of triterpenes and saponins highlighted by prescreening on plates.

The ethyl acetate fraction of bulbs-roots showed good antioxidant and antiacetylcholinesterase activities, with a medium antibacterial potent which can serve as a natural antioxidant and an antiacetylcholinesterase. However, *in vivo* tests must be conducted in order to derive its toxicological and therapeutical properties. An isolation of phytoconstituants of this species must be carried out so as to identify the compound(s) responsible for each activity and to affirm synergetic or antagonist effect of components, present in each extract and EtOAc in particular.

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