NPC Natural Product Communications

2017
Vol. 12
No. 12
1857 - 1860

Phytochemical Profile and Antibacterial Activity of *Retama raetam* and *R. sphaerocarpa* cladodes from Algeria

Nawal Hammouche-Mokrane[a†], Antonio J. León-González[b†], Inmaculada Navarro[c], Farida Boulila[a], Said Benallaoua[d] and Carmen Martín-Cordero[b*]

[a]*Laboratoire d'Ecologie Microbienne, Faculté des Sciences de la Nature et de la Vie, Université A/MIRA de Bejaia, Route de Targa Ouzemour 06000, Algeria*

[b]*Department of Pharmacology, Faculty of Pharmacy, University of Seville, Seville, Spain*

[c]*Department of Physical Chemistry, Faculty of Pharmacy, University of Seville, Seville, Spain*

[d]*Laboratoire de Microbiologie Appliquée (LMA), Faculté des Sciences de la Nature et de la Vie, Université A/MIRA de Bejaia, Route de Targa Ouzemour 06000 Bejaia, Algeria*

[†] *Joint first authors*

carmenmc@us.es

Received: October 26th, 2017; Accepted: November 3rd, 2017

Retama raetam (*RR*) and *R. sphaerocarpa* (*RS*) are shrubs growing in Algeria desert areas, where are commonly used as healing remedies because of their antiseptic, antipyretic and anti-diarrheal properties. Phytochemical studies have shown that these species are very rich in flavonoids (isoflavones) and alkaloids (quinolizidine and bipiperidyl). The aim of this study was to compare the chemical composition of both *Retama* species by GC/MS and LC/MS and to determinate their antimicrobial activity of two *Retama* species growing naturally in Algeria. Ten alkaloids and seven flavonoids were identified in cladodes of *RR* and *RS.* The quantitative analysis showed that the most abundant flavonoid of both the aqueous extract from *RR* and *RS* was the isoflavone genistein (610.0±2.8 and 408.0±14.1 mg/100 g respectively), whereas sparteine was the predominant alkaloid in *RR* and retamine in *RS.* The antibacterial activity of *Retama* extracts against standard strains was performed by minimum inhibitory concentration (MIC), and by the disc diffusion method (expressed by inhibition zone, IZ). Both *Retama* species showed the best activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), being *RS* aqueous extract more active than *RR* aqueous extract, with MIC 125 µg/mL and bactericidal activity against both strains.

Keywords: *Retama raetam*, *Retama sphaerocarpa*, Quinolizidine alkaloids, Isoflavonoids, Antibacterial activity.

Retama genus constitutes a monophyletic taxon of the family Leguminosae, comprising four closely related species endemic to the Mediterranean region: *R. raetam* (Forssk.) Webb, *R. sphaerocarpa* (L.) Boiss., *R. monosperma* (L.) Boiss., and *R. dasycarpa* Coss. [1-2]. *R. raetam* (*RR*) is common in the North and East Mediterranean region and the Sinai Peninsula and *R. sphaerocarpa* (*RS*) is located in Iberian Peninsula, Atlas regions and the Sahara [2]. They are wild desert shrubs growing in Algeria and they are commonly used as healing plant with antiseptic, antipyretic and anti-diarrheal effects [3]. Decoctions of aerial parts of *Retama* spp. are used in traditional medicine for the treatment of diabetes, hypertension, and rheumatism, as well as anti-inflammatory [4-6]. Moreover, it has been reported in several studies that *Retama* species have different pharmacological activities such as hepato-protective, antibacterial, antifungal, antioxidant, antiproliferative, hypoglycemic, antiulcerogenic, diuretic and antihypertensive [7-14]. Phytochemical studies have shown that these species are very rich in flavonoids (isoflavones) [15-16], alkaloids (quinolizidine and bipiperidyl) [17-18] and the cyclitol pinitol [19]. The aim of this study was to compare the chemical composition of two *Retama* species growing in Algeria by GC/MS and LC/MS and to determinate their antimicrobial activity.

Ten alkaloids were detected in cladodes of *R. raetam* and *R. sphaerocarpa* (Table 1). The percentage of the different alkaloids identified in the two *Retama* species was calculated by comparison of obtained areas with retamine standard. Retention time and fragmentation patterns used for the identification of alkaloids are summarized in table S1 of supplementary data. Total ion chromatograms (TIC) of alkaloids in *R. raetam* and *R. sphaerocarpa* are presented as supplementary data in figure S1.

Table 1: Alkaloids in *Retama raetam* and *Retama sphaerocarpa*

Alkaloid	Rt (min)	*Retama raetam (%)*	*Retama sphaerocarpa (%)*
Sparteine	6.54	43.6±0.4	2.1±0.0
Ammodendrine	7.54	8.7±0.0	20.0±0.1
N-Methylcytisine	8.70	2.9±0.0	n.q.
Retamine	9.00	n.q.	75.6±0.0
Dehydroretamine	10.39	4.5±0.4	n.q.
5,6-Dehydrolupanine	11.18	3.9±0.0	n.q.
Lupanine	11.58	n.q.	0.5±0.0
α-Isolupanine	11.69	7.9±0.0	n.q.
N-Formyammodendrine	12.2	n.q.	1.0±0.0
Anagyrine	14.58	28.5±0.0	0.8±0.0

(n.q.= not quantified). The results are mean values of the replicate measurements (n=2), and expressed as percentage of alkaloid in total alkaloid extract ± standard deviation (% ± SD).

Six tetracyclic quinolizidine alkaloids (sparteine, retamine, dehydroretamine, 5,6-dehidrolupanine, lupanine, α-isolupanine), two tricyclic quinolizidine alkaloids (anagyrine, N-methylcytisine) and two bipiperidyl alkaloids (ammodendrine, N-formyl-ammodendrine) were identified in this study (Table 1). Alkaloid profiles of both *Retama* species were similar, but sparteine was the predominant alkaloid in *R. raetam* and retamine in *R. sphaerocarpa.* These results are according to El-Shazly et al. [18].

Seven flavonoids were identified: the flavonol rutin, the flavones luteolin and apigenin, and the isoflavones daidzin, genistin, daidzein and genistein (Table 2). The quantitative analysis showed that the major flavonoid of the aqueous extract from *R. raetam* and *R. sphaerocarpa* was the isoflavone genistein (610.0±2.8 and

408.0±14.1 mg/100 g respectively). Genistein and daidzein are both isoflavonoids presenting an important antimicrobial activity [20]. Tsuchiya et al. linked the antimicrobial effects of flavonoids to their capacity to form complexes with extracellular and soluble proteins and with the cell wall [21].

The characterization of the flavonoid composition of *R. raetam* and *R. sphaerocarpa* was achieved by LC/MS analysis comparing the retention time and the characteristic fragmentation patterns of the compounds with standards. The retention time (min), molar mass (g/mol) and the selected MRM transitions (*m/z*) from precursor ion to product ion, used for the identification of flavonoids, are summarized in table S2 of supplementary data.

Table 2: Flavonoids in *Retama raetam* and *Retama sphaerocarpa*.

Flavonoid	R_t	*Retama raetam* mg/100 g	*Retama sphaerocarpa* mg/100 g
Daidzin	8.67	2.2±0.0	42.8±0.3
Rutin	8.73	3.1±0.1	9.0±0.3
Genistin	10.10	19.2±0.7	65.2±0.8
Daidzein	10.20	49.3±0.1	165.1±0.1
Luteolin	10.30	98.5±1.3	153.0±1.4
Apigenin	10.80	65.4±1.1	72.1±0.4
Genistein	10.80	610.0±2.8	408.0±14.1

The results are mean values of two replicate measurements (n=2), and expressed as mg of flavonoids per 100 grams of extract ± standard deviation (mg/100 g ± SD).

The antibacterial activity of *R. raetam* and *R. sphaerocarpa* extracts against standard strains was also investigated in the present work. Results of inhibition zone (IZ) in the disc diffusion method, and minimum inhibitory concentration (MIC) of bactericidal and bacteriostatic activities are given in Table 3. *R. sphaerocarpa* displayed more antibacterial activity against MRSA (IZ=17.5±0.1 mm, MIC=125 µg/mL, bactericidal effect) and *S. aureus* (IZ=16.7±0.2 mm, MIC=125 µg/mL, bactericidal effect) and less important activity against *E. coli*, *B. subtilis*, *V. cholera*, *S. tiphymurium* with IZ zone not exceeding 11.7±0.2 mm and MIC superior to 500 µg/mL. *R. raetam* displayed also an interesting activity against MRSA (IZ=18.3±0.2 mm, MIC=500 µg/mL, bacteriostatical effect) and *S. aureus* (IZ=15.2±0.3 mm, MIC=500 µg/mL, bacteriostatical effect).

Table 3: Antibacterial activity of *R. sphaerocarpa* and *R. raetam* extracts.

	Inhibition zone IZ (mm)				
Strains	***R.sphaerocarpa***	***R. raetam***	**TET**	**GEN**	**C**
E .coli	11.7±0.2	10.3±0.6	21.8	21.4	27.0
P. aeruginosa	6.0±0.1	6.0±0.1	21.0	21.9	13.0
S. aureus	**16.7±0.2**	**15.2±0.3**	29.6	25.6	25.8
MRSA	**17.5±0.1**	**18.3±0.2**	31.3	24.3	26.8
B. subtilis	11.0±0.3	9.0±0 .1	23.1	14.0	25.6
V. cholera	10.1±0.3	9.7±0.3	21.0	18.4	23.2
S. tiphymurium	11.1±0.3	9.7±0.1	23.3	21.7	27.1
	MIC (µg/mL)				
E .coli	2000^{C}	2000^{C}	nt	nt	<2
P. aeruginosa	>2000	>2000	nt	nt	<20
S. aureus	$\mathbf{125}^{C}$	500^{S}	nt	nt	<2
MRSA	$\mathbf{125}^{C}$	500^{S}	nt	nt	<2
B. subtilis	500^{S}	2000^{C}	nt	nt	<2
V. cholera	2000^{S}	2000^{C}	nt	nt	<2
S. tiphymurium	2000^{C}	2000^{C}	nt	nt	<2

MIC: Minimum inhibitory concentration. **C**: bactericidal , **S**: bacteriostatical . **nt**: not tested.
TET: tetracycline 30 µg/disc, **GEN:** gentamycin 10 µg/disc, **C:** chloramphenicol 30 µg/disc

The higher sensitivity to *Retama* extracts of *S. aureus* (IZ=18.3±0.2 mm, MIC=125 µg/mL) and MRSA (IZ=17.5±0.1 mm, MIC= 125 µg/mL) than *P. aeruginosa* (IZ=6.0±0.1 mm, MIC>2000 µg/mL) could be explained by the differences between their cell wall structures, as the Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier [22], while Gram-negative have lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergent, and hydrophilic dyes [23].

The antibacterial activity of the studied extracts is due, at least in part, to their flavonoid composition because flavonoids are known components of the defense mechanism against infection [24]. The flavonoid composition of *Retama* spp. has been also related to other biological activities, as López-Lázaro et al. have shown that a flavonoid-rich aqueous extract of aerial parts of *R. sphaerocarpa,* exerts cytotoxic activity against different cancer cell lines [25].

Quinolozidine alkaloids (QA) and QA-containing plant extracts also have been shown to have antimicrobial activity by several researchers [26-27]. In a study by Wink [28], sparteine was reported to possess antimicrobial activity against bacteria and phytopathogenic fungi; Tyski et al. [29] reported that pure QA isolated from *Lupinus angustifolius* var. Mirela including lupanine, 13αhydroxylupanine, sparteine, and angustifoline showed bacteriostatic effects on *S. aureus, B. subtilis, E. coli, P. aeruginosa,* and *Bacillus thuringiensis.* Thus, we can suppose that the high antibacterial activity of *R. sphaerocarpa and R. raetam* is also related to these QA.

Paris and Dillemann [30] reported that the toxicity action of retamine is nearly twice as strong as that of sparteine. That could explain that antibacterial activity of *R. sphaerocarpa,* which contains 75.60±0.02% of retamine, is higher than *R. raetam* which contains very few quantity of retamine.

In conclusion, ten alkaloids were identified by GC/MS analysis. Alkaloid profiles of both *Retama* species are similar, sparteine was the predominant alkaloid in *R. raetam* and retamine in *R. sphaerocarpa.* Seven flavonoids were identified by LC/MS, the major flavonoid of the aqueous extract from *R. raetam* and *R. sphaerocarpa* was the isoflavone genistein. Both *Retama* species showed the best activity against MRSA and *S. aureus.* Aqueous extract of *R. sphaerocarpa* was more active then aqueous extract of *R. raetam*, with MIC 125 µg/mL and bactericidal activity against both stains.

Experimental

Plant material: Aerial parts of *Retama raetam* (L.) Boiss. were harvested in Lota, Souk El Ténine (Béjaïa, Algeria), on April 2011 and *Retama sphaerocarpa* (L.) Boiss in Bousselam, Bouandas (Setif, Algeria), on March 2011.

Preparation of the extracts: Plant samples were dried at room temperature. Then, 2 grams of areal parts were powdered and extracted with water at 70°C, under agitation using magnetic stirrer, during one hour. The water extract was lyophilized to provide a crude water extract with 20.32% yield for *R. raetam* and 25.49% yield for *R. sphaerocarpa*. For antibacterial activity test, each extract was dissolved in dimethyl sulfoxide (DMSO) to make a 20 mg/mL stock solution.

A selective extraction was performed prior to analysis of phytochemical composition on alkaloids, achieved by GC/MS. Five grams of dried and powdered branches were extracted for 1 hour in 100 mL CH_2Cl_2/MeOH/NH_4OH (15:5:1), at 30°C. The obtained extract was filtered and concentrated under reduced pressure and taken to 20 mL with 0.5N HCl. The resulting acid solution was then alkalinized with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 fraction was collected in anhydrous sodium sulphate and evaporated under reduced pressure, providing a 0.99% of total alkaloids (0.0493 g) for *R. raetam* and 0.94% of total alkaloids (0.0469 g) for *R. sphaerocarpa*.

Chemicals: Methanol, dichlorometane, acetonitrile and formic acid (high-performance liquid chromatography [HPLC] grade) were purchased from Panreac (Barcelona, Spain). The standards compounds: daidzin, rutin, genistin, daidzein, luteolin, apigenin, genistein, were procured from Extrasynthese (Lyon, France), and retamine was obtained by isolation from *R. sphaeroca*rpa [31].

LC/MS conditions: The water extract was dissolved in MeOH to obtain a concentration of 0.5mg/mL and then filtered. Analysis was performed by LC/MS/MS in negative multiple reaction monitoring (MRM) scanning mode, using Perkin Elmer Series 200 HPLC system (Wellesley, USA) equipped with an auto-sampler, binary pump system. Chromatographic separation was achieved by using a Zorbax Eclipse 2.1 x 150 mm, 3.5µm particle size XDB-C18column (Agillent). The mobile phase for chromatographic separation was composed of solvent A (0.1% of formic acid in water) and solvent B (0.1% of formic acid in acetonitrile) under gradient elution, starting at 10% B, isocratic for 5 min, then increased linearly to 100% B over 15 min, held constant for 5 min, then reduced linearly to 10% B for 5 min and then held constant for an additional 5 min, at a flow rate of 0.2 mL/min. The injection volume was 20 µL. Mass spectrometer used for the detection was QTRAP system (Applied Biosystems, Foster City, USA), consisting of an hybrid triple quadrupole linear ion trap (QqQ_{LIT}), equipped with electrospray ionization (ESI) interface, and the acquisition data processor Analyst® 1.4.2 software.

GC/MS conditions:The total alkaloid extract was analyzed by gas chromatography on an Agilent 6890N chromatograph with Autospec-Q detector, using a TBR-1 HP6890 column (25 m x 0.25 mm x 250 µm) and helium as mobile phase. A 1.1 mg/mL solution of retamine in CH_2Cl_2 was used as standard.

Antibacterial activity

Disc diffusion method: The antibacterial activity of the extract was carried out by the disc diffusion method according to the National Committee for Clinical Laboratory Standards [32] against seven bacteria strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Bacillus subtilis* ATCC 6633, *Vibrio cholera* ATCC 14034, and *Salmonella typhimurium* ATCC 13311. It was performed using an 18h culture growth at 37C° and adjusted to 0.5 Mac Farland standards (10^8 CFU/mL).

The sterile filter paper disks (6 mm in diameter, Whatman No. 1), deposited on the surface of Mueller-Hinton agar plates, were inoculated by sterile cotton swabs with 10 µL of extract (20mg/mL). The plates were left 30 min at 4°C to allow the diffusion of the extract, and then they were incubated at 37°C for 24 h. At the end of this period, the inhibition zones were measured. All the experiments were performed in triplicate. Positive (gentamycin 10 µg/disc, tetracycline 30 µg/disc, chloramphenicol 30 µg/disc) and negative controls (10 µl of DMSO) were also included in the test.

MICs: Various concentrations (75 µg/mL up to 2000 µg/mL) of plant extracts were used to determine MICs with National Committee for Clinical Laboratory Standards (NCCLS) reference methods [32, 33]. Overnight both cultures were adjusted to 0.5 Mac Farland standards. The minimal inhibitory concentrations (MICs) were determined on Mueller-Hinton (MH) agar plates. One mL of each extract previously dissolved in dimethyl sulfoxide (DMSO) were mixed for each concentration with 19 mL of MH agar at 40°C and poured over Petri dishes. Plates containing only medium with DMSO were used as controls. After 18 h of incubation at 37°C, the minimum inhibitory concentration (MIC) was defined. Each test was performed in triplicate.

In order to determine the nature of activity, a minor amount of medium was withdrawn from the plate containing the MICs and deposited in a tube of nutritive broth. The variation of turbidity was observed after 24 h of incubation at 37 °C [28].

Statistical analysis: Experimental results were expressed as the mean ± standard deviation (S.D.) of two replicates (n=2) for chemical analysis and three replicates (n=3) for inhibition zones. ANOVA test comparison of means was performed by XLstat pro 7.25

Supplementary data: Figure S1. Total ion chromatograms (TIC) of alkaloids in *Retama raetam* and *Retama sphaerocarpa.* Table S1. Retention time (R_t) and fragmentation patterns used for the identification of alkaloids. Table S2. Retention time (R_t) and fragmentation patterns used for the identification of flavonoids.

Acknowledgments - The authors are grateful to Mass Spectrometry Service at "Centro de Investigación, Tecnología e Innovación" (CITIUS) of University of Seville for the GC-MS and LC-MS analysis. AJLG was founded by Alfonso Martín Escudero postdoctoral fellowship.

References

[1] Belmokhtar Z, Kaid-Harche M. (**2012**) Evaluation of genetic diversity in three species of *Retama* genus: *R. monosperma* (L) Boiss*, R. raetam* (Forssk) Webb and *R. sphaerocarpa* (L) Boiss. (Fabaceae) based on SDS-PAGE. *Current Research Journal of Biological Sciences*, ***4***, 202-205.

[2] Greuter W, Burdet HM, Long G. (**1989**) Med-Checklist: A critical inventory of vascular plants of the circum-mediterranean countries. Vol. *4,* Conservatoire et Jardins botaniques de la Ville de Gèneve, Switzerland.

[3] Rebbas K, Bounar R, Gharzouli R, Ramdani M, Djellouli Y, Alatou D. (**2012**) Plantes d'intérêt médicinal et écologique dans la region d'Ouanougha (M'Sila, Algérie). *Phytothérap*ie, ***10***, 131–142.

[4] Benítez-Cruz G. (**2007**). El uso de las plantas a través de la cultura tradicional Lojeña. Fundación Ibn al-Jatib de Estudios de Cooperación Cultural, Granada, Spain.

[5] El Beyrouthy M, Arnold N, Delelis-Dusollier A, Dupont F. (**2008**) Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. *Journal of Ethnopharmacology*, ***120***, 315–334

[6] Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B. (**2007**) Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province) *Journal of Ethnopharmacology*, ***110***, 105–117

[7] Hayet E, Maha M, Samia A, Mata M, Gros P, Raida H, Ali MM, Mohamed AS, Gutmann L, Mighri Z, Mahjoub A. (**2008**) Antimicrobial, antioxidant, and antiviral activities of *Retama raetam* (Forssk.) Webb flowers growing in Tunisia. *World Journal of Microbiology and Biotechnology*, ***24***, 2933−2940.

[8] Edziri H, Mastouri M, Mahjoub MA, Mighri Z, Mahjoub A, Verschaeve L. (**2012**) Antibacterial, antifungal and cytotoxic activities of two flavonoids from *Retama raetam* flowers. *Molecules*, ***17***, 7284-7293.

[9] El-Toumy SA, Farrag AH, Ellithey MM, Korien KM. (**2011**) Effect of plant derived-phenolic extracts on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. *Journal of Pharmacy Research*, ***4***, 189-192.

[10] Bremner P, Rivera D, Calzado MA, Obón C, Inocencio C, Beckwith C, Fiebich BL, Muñoz E, Heinrich M. (**2009**) Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-Kappa B and other pro-inflammatory mediators. *Journal of Ethnopharmacology*, ***115***, 295-305
[11] Algandaby MM, Alghamdi HA, Ashour OM, Abdel-Naim AB, Ghareib SA, Abdel-Sattar EA, Hajar AS. (**2010**) Mechanisms of the antihyperglycemic activity of *Retama raetam* in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology*, ***48***, 2448-2453.
[12] González-Mauraza H, Martín-Cordero C, Alarcón-de-la-Lastra C, Rosillo MA, León-González AJ, Sánchez-Hidalgo M. (**2014**) Anti-inflammatory effects of *Retama monosperma* in acute ulcerative colitis in rats. *Journal of Physiology and Biochemistry*, ***70***, 163-172.
[13] Maghrani M, Zeggwagh NA, Haloui M, Eddouks M.(**2005**) Acute diuretic effect of aqueous extract of *Retama raetam* in normal rats. *Journal of Ethnopharmacology*, ***99***, 1331−1335.
[14] Eddouks M1, Maghrani M, Louedec L, Haloui M, Michel JB. (**2007**) Antihypertensive activity of the aqueous extract of *Retama raetam* Forssk. leaves in spontaneously hypertensive rats. *Journal of Herbal Pharmacotherapy*, ***7***, 65-77.
[15] López-Lázaro M, Martín-Cordero C, Ayuso MJ. (**1999**) Flavonoids of *Retama sphaerocarpa*. *Planta Medica*, ***65***, 777-778.
[16] Kassem ME, Mosharrafa SAM, Saleh NAM, Wahab SMA. (**2006**) Flavonoids of *Retama raetam*, and *in vitro* antitumor screening of two isoflavones. *Egyptian Journal of Pharmaceutical Sciences*, ***47***, 1-11.
[17] Sandberg F. (**1958**) The alkaloids of *Retama raetam*. *Pharmaceutisch Weekblad*, ***93,*** 8-10.
[18] El-Shazly A, Ateya AM, Witte L, Wink M. (**1996**) Quinolizidine alkaloid profiles of *Retama raetam*, *R sphaerocarpa* and *R monosperma*. *Zeitschrift Fuer Naturforschung*, ***51,*** 301-308.
[19] González-Mauraza NH, León-González AJ, Espartero JL, Gallego-Fernández JB, Sánchez-Hidalgo M, Martin-Cordero C. (**2016**) Isolation and Quantification of Pinitol, a Bioactive Cyclitol, in *Retama* spp. *Natural Product Communications*, ***11***, 405-406.
[20] Dixon RA, Sumner LW. (**2003**) Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiology*, ***131***, 878-885.
[21] Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. (**1996**) Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology*, ***50***, 27-34.
[22] Scherrer R, Gerhardt P. (**1971**) Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. *Journal of Bacteriology*, ***107***, 718-735.
[23] Nikaido H, Vaara M. (**1985**) Molecular basis of bacterial outer membrane permeability. *Microbiology Reviews*, ***49,*** 1-32.
[24] Dixon RA, Paiva NL. (**1995**) Stress-Induced Phenylpropanoid Metabolism. *Plant Cell*, ***7***, 1085-1097.
[25] López-Lázaro M, Martín-Cordero C, Cortés F, Piñero J, Ayuso MJ. (**2000**) Cytotoxic activity of flavonoids and extracts from *Retama sphaerocarpa* Boissier. *Zeitschrift Fuer Naturforschung*, ***55***, 40-43.
[26] Erdemoglu N, Ozkan S, Duran A, Tosun F. (**2009**) GC-MS analysis and antimicrobial activity of alkaloid extract from *Genista vuralii*. *Pharmaceutical Biology*, ***47***, 81–85
[27] Korir E1, Kiplimo JJ, Crouch NR, Moodley N, Koorbanally NA. (**2012**) Quinolizidine alkaloids from *Sophora velutina* subsp. zimbabweensis (Fabaceae: Sophoreae). *Natural Product Communications*, ***7***, 999-1003.
[28] Wink M. (**1984**) Chemical defense of Leguminosae: Are quinolizidine alkaloids part of the antimicrobial defense system of lupins? *Zeitschrift Fuer Naturforschung*, ***39c***, 548-552.
[29] Tyski S, Markiewicz M, Gulewicz K , Tawardowski T. (**1988**) The effect of lupine alkaloids and ethanol extracts from seeds of *Lupinus angustifoliuson* selected bacterial strains. *Journal of Plant Physiology*, ***133***, 240-242.
[30] Paris R, Dillemann G. (**1960**) Pharmacological aspect. In *Medicinal plants of the arid zones*. Part two. Oberthur. UNESCO, Rennes, France, 72.
[31] Martín Cordero C, Gil Serrano AM, Ayuso González MJ. (**1991**) Variations des alcaloïdes de *Retama sphaerocarpa* Boissier. *Plantes médicinales et Phytothérapie*, ***25***, 157-160.
[32] NCCLS, National Committee for Clinical Laboratory Standards (**1997**) Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 4th ed. NCCLS approved standard, NCCLS document M7-A4
[33] Laouer H, Zerroug MM, Sahli F, Cker AN, Valentini G, Ferretti G, Grande M, Anaya J. (**2003**) Composition and Antibacterial Activity of Ammoïdes pusilla (Brot) Breistr. Essential Oil. *Journal of Essential Research*, ***15***, 135-138.