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ESSENTIAL OIL FROM AERIAL PARTS OF *RHETINOLEPIS LONADIOIDES* (COSS.): EXTRACTION, CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

In this study, the chemical composition and antimicrobial activity of the essential oil (EO) from aerial parts of *Rhetinolepis lonadioides* collected from southwestern Algeria were assessed. The EO was obtained by hydrodistillation and analyzed by GC/MS. In total, thirty-two compounds were identified representing 80.00% of the total of the EO, withβ-Pinene (41.85%) being the major monoterpenes. Phenanthroquinone (10.39%) and Ethanone, 1-[1,1'-biphenyl]-4-yl- (2.66%) were the main oxygenated monoterpenes, and the diterpene hydrocarbons were mainly represented by m-Camphorene (5.43%). Six EO samples of *R. lionadioides* collected in location exhibited similar chemical composition evidencing a chemical homogeneity. The agar disc diffusion method showed that *R. lonadioides* EO was effective against *Bacillus cereus, Pseudomonas aeruginosa,* and *Staphylococcus aureus* with diameters of inhibition ranging from 17±2.65 to 12.33±1.13 mm, and the minimum inhibitory concentration (MIC) values were between 4 and 10 µL /mL. The lowest MIC values (4.0 µL/mL) were measured for *Bacillus cereus.*
Keywords: antimicrobial activity,chemical composition, essential oil, GC/MS, *Rhetinolepis lonadioides*;

Introduction

Essential oils (EOs) and volatile constituents extracted from aromatic plants are frequently used for diverse purposes especially in folk medicine for prevention and treatment of different human diseases, such as bacterial and viral infections (Rates 2001). Indeed, they have gained an increasing interest in these last years in pharmaceutical and food industries due to their safety aspects as they are natural products. Hence, many investigations of new active EOs for such applications are intensively conducted.

The species *Rhetinolepis lonadioides* (*R. lonadioides*), also known as *Ormenis lonadioides* (Coss.) Maire, or *Anthemis lonadioides* (Coss.) Hochr. is endemic to Algeria and Morocco (Oberprieler, 1998). *Anthemis* L. (*Asteraceae*) is one of the largest genus of the tribe *Anthemideae* which consists of nearly 175 species worldwide. It is distributed widely across Europe, South-West Asia, North and East Africa (Oberprieler *et al*., 2007). South-West Asia is one of the genetic centers of the genus *Anthemis* (Rates, 2001). In fact, *R. lonadioides* is an annual plant, finely canescent pubescent, of 10-20 cm high, branched from the base with stiff stems, erect or lying with ascending hairy leaves with a blade progressively thinned into a petiole and 3-5 lobed at the apex, with short subobtute segments, and the bracts are entire and superior. It is characterized by a synflorescence in loose corymbs, discoid heads, obconic (diameter 5-8 mm), homogamous, on a short peduncle (3-12 mm), involucre with bracts in 1-2 rows, convex receptacle with narrow paleas, acuminate and hairy. Its flowers are tubular (≤ 3 mm)and yellow, with enlarged subailed tube and 5-lobed corolla. Its achenes are less than 2mm, triangular, compressed, glabrous, bald, with numerous fine longitudinal striations and a central vein more or less marked on the ventral surface (Battandier 1888, African Plant Database, 2021).

Anthemis species have several biological activities and are widely used in folk medicine for treatment of gastrointestinal disorders, haemorrhoids, cough, stomach aches and liver failure (Baytop, 1999; Kultur, 2007; Ugurlu and Secmen 2008; Gonenc *et al*., 2011; Korkmaz and Karakus, 2015). In addition, they are able to soothe pains and irritations, clean wounds (Pavlovic *et al*., 2010), used as herb teas, and used in cosmetics as well as in the pharmaceutical industry (Kivcak *et al.,* 2007). Several *Anthemis* spp. have been studied for their essential oils (Uzel *et al*., 2004;

Javidnia *et al*., 2004; Kurtulmus *et al*. 2009; Yusufoglu *et al.,* 2018), secondary metabolites, terpenoids, sesquiterpene lactones, flavonoids and coumarins (Hofer and Greger 1985; Bruno *et al.,* 1997; Vajs *et al.,* 1999; Gonenc *et al*., 2011; Tawaha *et al*., 2015; Orlando *et al.,* 2019) and their neuro protective (Venditti *et al.,* 2016), cytotoxic (Alessandro *et al*., 2016), antioxidant and antimicrobial effects (Kivcak *et al.,* 2007; Albayrak and Aksoy, 2012; Stojkovic *et al.,* 2014; Guragaç Dereli *et al*., 2018).

To our knowledge, there are about twenty publications in the literature reporting essential oils from the aerial parts of 17 species and subspecies of plants of the *Anthemis* genus. None concerns *R. lonadioides* (Coss.).

Also, no research has yet been carried out to determine the chemical composition of the essential oil from aerial parts of *R. Lonadioides* or to evaluate its antimicrobial activity. For this reason, our study was undertaken to assess, for the first time, the chemical composition of Algerian essential oil extracted from *R. lonadioides*. Then, the characterized *R. lonadioides* essential oil was evaluated for its *in vitro* antimicrobial effect against some microbial pathogens. The expected results were then used for the valorization of *R. lonadioides* essential oil as a new bio agent for several pharmaceutical or food applications.

Material and Methods

Plant Material

Samples of aerial parts of *R. lonadioides* were collected during the flowering period (April, 2021) in the region of Beni Abbes (southwest of Algeria 30° 05′ north, 2° 06′ west). A voucher specimen has been deposited at the Laboratory Sustainable management of natural resources in arid and semi-arid zones, Department of SNV, Institute of Science and Technology, University Center Salhi Ahmed Nâama Algeria. Dried aerial parts (around 300–400 g) were submitted to hydrodistillation, using a Clevenger-type apparatus for 3 hours. Yields have been calculated from dry material.

Gas Chromatography (GC) Analysis

GC analyses were performed with a BrukerScion SQ. It is equipped with a DB-5 type capillary column (length: 25 m, internal diameter: 0.220 mm, film thickness: 0.25 µm). The column temperature was set at 50°C for 10 min, then raised gradually from 50 to 250°C at a rise rate of 2°C/min, and finally it was maintained at 250°C for 15 min. The injector temperature was set at 250°C.The injection mode was Split (division ratio of 1:100).The carrier gas flow rate (helium) was set at 1 mL/min. the volume of the injected sample was 0.2µL.

Gas chromatography-mass spectroscopy (GC/MS) analysis

The analysis of the *R. lonadioides* essential oil samples was carried out in the Technical Platform of Physico-Chemical Analysis (PTAPC-CRAPC)-Laghouat-Algeria, using a SHIMADZU GCMSQP2020 Instruments, equipped with a fused Rxi®-5ms capillary column (30 m × 0.25 mm, 0.25 µm film thickness, Phase: Cross bond® 5% diphenyl /95% dimethylpolysiloxane). A solution of 0.5µL solution prepared by 10% vol. of the sample dilution in n-hexane, was injected in split mode (30:1). Injector and detector temperatures were maintained at 250°C and 310°C, respectively. The column temperature was programmed at 60°C for 3minthen increased gradually to 310°C at 2°C/min, and finally maintained at 310°C for 10 min. The carrier gas used was Helium (99.995% purity) with a flow rate of 1 mL/min. The mass spectrometer conditions were as follows: ionization voltage 70eV, ion source temperature 200°C, and electronionization mass spectra were acquired over the mass range of 45-600 m/z.

Identification of Essential Oil Composition

Identification of components was performed by comparing their relative retention index (RI) determined with the reference of homologous series of *n*-alkanes (C8 to C24) (Rustaiyan *et al*., 2004 and Toiu *et al.,* 2009).

The fragmentation patterns of the mass spectra were compared with the WILEY and NIST 05 libraries. The linear temperature-programmed RIs of all the constituents were calculated based on the GC through the interpolation between bracketing *n*-alkanes as follows:

$$RI = 100 \times \left[\frac{t_{R(i)} + t_{R(z)}}{t_{R(Z+1)} + t_{R(Z)}} Z \right]$$

Where Z: the number of carbon atoms in the smaller *n*-alkane.

$t_{R(i)}$, $t_{R(Z)}$ and $t_{R(Z+1)}$: the retention time of the desired compound, the smaller *n*-alkane and the larger *n*-alkane, respectively (Jalali-Heravi *et al*., 2015).

Antimicrobial Activity of the Essential Oil

Microbial Strains

Antimicrobial activity of the essential oil extracted from the aerial parts of *R. lonadioides* was evaluated against five Gram-positive bacteria (*Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 21332, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 49452) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853).

Screening for Antimicrobial Activity

The agar diffusion method (NCCLS 1997) was used for the determination of the antimicrobial activity of the essential oils. Briefly, 1 mL of the tested microbial suspension (at 10^6 cells/mL) was spread onto the surface of Mueller Hinton Agar (Biokar diagnostics, Beauvais, France) plates. The *R. lonadioides* essential oil was dissolved in dimethyl sulfoxide (DMSO)at 10% to enhance oil solubility. Then, filter paper discs (6 mm of diameter) were impregnated with the prepared mixture of DMSO and *R. lonadioides* essential oil and placed on the surface of the inoculated plates that were afterwards incubated at 37°C for 24 hours. After incubation, observed clear zones of inhibition were measured (mm). DMSO and antibiotics [spiramycin 100 µg and Oflatoxin 5 µg, BioMérieux] were used as controls Set. Each test was performed in triplicate in at least three separate experiments.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of the *R. lonadioides* essential oil were determined by the micro dilution assay in 96-well microplates. The essential oil was dissolved in DMSO then it was first diluted to the highest concentration, followed by serial dilutions made in Mueller-Hinton broth (Biokar diagnostics, Beauvais, France) over the concentration range 1–6 µL/mL. Ten µL of standardized suspension was added.

Inoculated plates were incubated at 37°C for 24 hours for the bacteria. The MIC was defined as the lowest concentration of *R.lonadioides* essential oil resulting in complete inhibition of visible growth after incubation(Ahmed Elkhalifa *et al.,* 2018). Tests were performed three times in triplicate.

Statistical Analysis

All the experimental results were submitted for a variance analysis (ANOVA) using the SPSS software (version 11.5). Means and standard errors were calculated and a probability level of $P<0.05$ was used in testing the statistical significance of all the data. Tukey's post hoc test was used to determine significance of mean values for multiple comparison at $P<0.05$.

Results and Discussion

Essential oil samples were obtained by the hydrodistillation of dry aerial parts of *R. lonadioides* Coss. Yields of essential oil, calculated w/w *versus* dry material, ranged between 0.90% and 1.40%.Theseobtained EO yields are greater than those found for some plants of the same *Anthemis* genus such as *Anthemis nobilis* L. with an EO yield value of 0.67% (Sadiki and Idrissi 2019), *Anthemis maritima* from Corsica (0.015%) (Collu *et al*., 2008) and *Anthemis cretica* sub sp. *carpatic*a (0.22%) (Kürkçüoğluand and Tosun, 2020). Indeed, the yield of an EO depends on many factors (growth stage, pedoclimatic conditions, extraction technique, etc.). One oil sample was analyzed by GC(RI) and GC/MS (Table 1, Fig. 1).

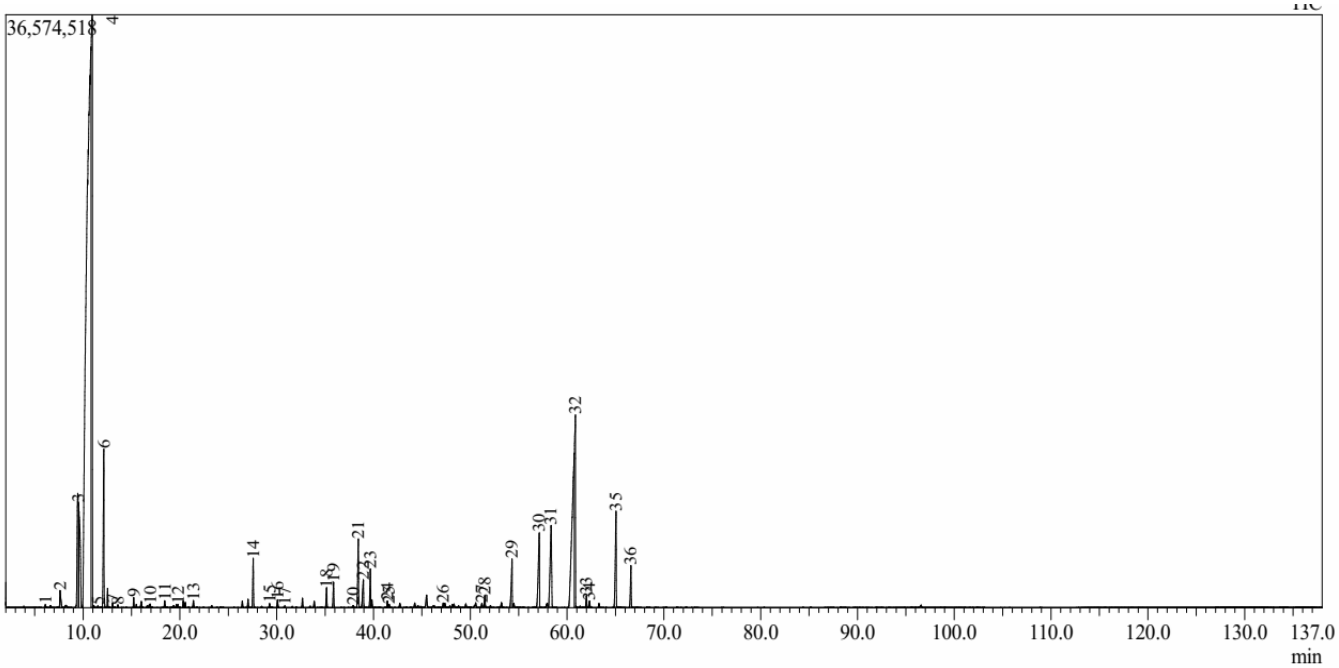

Fig. 1 : Gas chromatogram GC/SM of *Rhetinolepis lonadioides* Coss. essential oil The numbered peaks are the identified components as listed in Table 1

Chemical Composition of *R. lonadioides* (Coss.) Essential Oil

The detailed analysis of a bulk sample was carried out by a combination of chromatographic and spectroscopic techniques. One oil sample was analyzed by GC(RI) and GC(MS) (Table 1). In total, thirty-two compounds were identified, accounting for 80.00% of the whole composition, and were dominated by monoterpenes (68.33%).The hydrocarbon monoterpenes (53.94%) fraction was mostly represented by β-Pinene (41.85%). D-Limonene (6.79%), α-Fenchene (3.64%) and α-Pinene (1.01%). Hydrocarbons sesquiterpene (7.99%) were well represented by (E)-.beta.-Famesene (3.19%) and. α-Curcumene (1.63%), while, m-Camphorene (5.43%)was the only diterpene hydrocarbons present in a significant (P<0.05) amount. Moreover, this essential oil is characterized by the presence of oxygenated sesquiterpene of, Eudesm-7(11)-en-4-ol(0.47%)and Nerolidol (0.17%).

According to our results, the composition of the essential oil isolated from Algerian *R. lonadioides* differs drastically ($P<0.05$) from those of other *Anthemis* species dominated by oxygenated compounds, *A. montana* (α-Thujone 46.9%, β-Thujone 16%, Trans chrysanthemyl 11.3%) (Bulatovic *et al*., 1998), *A. tinctoria* L. (1,8 cineole 7.9%, β-Pinene 7.3%, α-Pinene4.4%) (Holla *et al*., 2002) and *A. talyshensis* A. (α-Eudesmol 18.2%,Borneol 13.3%, Hexadecanoic acid 9.5%) (Aghajani *et al*., 2005). On the other hand, it presents some analogies with *Anthemis* essential oils that contained appreciable contents of monoterpene hydrocarbons: 2,4-*Thujadiene*,β-Pinene or Paracymene. For instance, *A. melampodina* (Grace, 2002) contained β-Pinene (6.4%) and Paracymene (11%), *A. altissima* (Javidnia *et al*., 2004) (2,4-*Thujadiene at 27% and*α-Pineneat 4%) and *A. melanolepis* (Saroglou *et al*., 2005) (β-Pinene 11.7%).

Generally, the difference in chemical composition of our essential oil comparing to others from different *Arthemis* plants could be attributed to many factors such as the plant species, the part of the plant from which was extracted the essential oil, the genetic factors as well as the physiological, environmental and geographic variations (Dridi *et al*., 2020).

Antimicrobial Activity

The antimicrobial activity of *R. lonadioides* EO was assayed against seven bacteria using the agar disc diffusion method and measuring the minimum inhibitory concentration (MIC) (Table 2).The results showed that the EO had

substantial antimicrobial activity. In fact, it was effective against *B. cereus* and *P. aeruginosa* with inhibition zone diameters ranging from 13.67±7.37 to 17.00±2.65 mm and MIC values of 4-6µL/mL. *B. subtilis* and *S. aureus* were less sensitive (diameters of the zone of inhibition: 11.00±2.00 and 12.33±1.13 mm respectively, MIC: 8-10 µL/mL). On the other hand, the *R. lonadioides* EO showed weak activity against *L. monocytogenes*, *E. faecalis* and *E. coli* with diameters of inhibition zones ranging from 6.00±0.00 to 8.00±0.00 mm. According to these founds, it could be observed that the sensitivity of the microbial strains to our *R. lonadioides* essential oil was not correlated to the bacterial types (Gram-positive or Gram-negative). Hence, the studied essential oil could affect both of the bacterial membranes. Generally, the mechanisms of action of EOs and their selectivity towards certain bacteria have so far remained poorly understood (Hammer *et al*., 1999, Bagamboula *et al*., 2004). Accordingly, this selectivity is the result of the varied composition of the active fractions of the EOs, which often exhibit synergistic actions. It seems that their mechanisms of action are essentially linked to the structure of the wall and to the membrane permeability of Gram-positive and Gram-negative bacteria. In this context, the work of (Burt, 2004) showed that an active EO will exert its antimicrobial effect by its interference with the lipid bilayer of the target cell thanks to its hydrophobic property, which leads to a disturbance of the permeability and loss of the constituents of the cell. In addition, this reaction varies depending on the nature of the lipid bilayer, which explains the resistance of Gram-negative bacteria (Mahmoud *et al.* 2004). In addition, (Debbah *et al.,* 1970) demonstrated the high sensitivity of Gram-positive bacteria compared to Gram-negatives. In the same study approach, (Gordon *et al*., 1973) and (Mahmoud *et al*., 2004) suggested that the antimicrobial effect exerted by EOs could be explained by the destruction of certain enzymatic systems including those which participate in the production of cellular energy and the production of structural compounds. Moreover, (Mahmoud *et al*., 2004), (Guesmi and Boudabous, 2006) for their part, put forward the hypothesis of inactivation and destruction of genetic material and, finally (Caillet *et al*., 2007) reported that EOs prevent the multiplication of bacteria, their sporulation and the synthesis of their toxins.

In the whole, as it was previously reported by many researchers that the antimicrobial activity of the EOs depends on their chemical composition, the obtained antimicrobial activity of our *R. lonadioides* essential oil towards the tested bacteria could be attributed to one or more of its major biological components: β-Pinene (41.85%), Phenanthroquinone (10.39%), D-Limonene (6.79%). However, α and β-Pinene have been reported to exhibit slight activity against a range of microorganisms with MIC values ranged from 7.5 to 20.0 mg/mL against *E. coli*, *S. aureus* and *E. faecalis* (Dorman and Deans, 2000; Sonboli *et al*., 2006; Leite *et al*., 2007; Jung, 2009; Runyoro *et al.,* 2010). Indeed, it is important to notice that synergistic interactions could occur between major or minor components of the EO thus playing a key effect on its antimicrobial activity (Dridi *et al.,* 2020).

By understanding the antibacterial activity of the Eos extracted from some plants of the *Anthemis* genus, the *Anthemis xylopoda* O. Schwarz EO (Borneol, 31.80%; β-pinene, 12.67%) showed a weak antibacterial effect against *B. subtilis*, *E. coli*, and *P. aeruginosa* with inhibition zone diameters ranging from 7.6 to 10 mm, while the antimicrobial activity against *S. aureus* was remarkably higher (23.2 mm) (Dridi *et al*., 2020).

In parallel, *Anthemis stiparum* subsp. *Sabulicola* EO (with Germacrene D(11.13%) and t-Cadinol(11.01%) as major compounds), also showed low antimicrobial activity against the same bacterial strains with MIC values between 50 and 100 µL/mL (Šarac *et al*., 2014).

In short, it could be summarized that oxygenated terpenes, alcohols, aldehydes and ketones are active but with different specificity and activity levels, which could be linked firstly to the functional group but also to hydrogen binding parameters (Panizzi *et al.,* 1993; Adam *et al.,* 1998).

Table 1: GC/SM Components of *Rhetinolepis lonadioides* Coss.essential oil from Algeria.

	Components[a]	RI lit[b]	RI[c]	R.T[d]	Area%
1	styrene	870	890.94	6.09	0.11
2	α-Pinene	930	931.67	7.61	1.01
3	α-Fenchene	959	977.81	9.50	3.64
4	β-Pinene	972	980.00	10.93	**41.85**
5	α-Terpinene	1017	1020.18	11.52	0.03
6	D-Limonene	1018	1031.44	12.13	**6.79**
7	beta.-Ocimene	1041	1048.07	13.04	0.12
8	γ-Terpinene	1047	1057.99	13.58	0.07
9	Terpinolene	1080	1087.93	15.22	0.32
10	Hotrienol	1115	1116.63	16.91	0.12
11	.D-Camphor	1141	1140.76	18.42	0.22
12	Borneol	1148	1149.66	19.78	0.10
13	α-Terpineol	1172	1188.40	21.39	0.25
14	Bornylacetate	1285	1284.58	27.57	2.04
15	Guaiacol<4-vinyl->	1293	1310.99	29.26	0.13
16	Myrtenylacetate	1314	1324.01	30.08	0.32
17	δ-EIemene	1334	1335.83	30.83	0.05
18	Methyleugenol	1402	1400.65	35.15	0.82
19	Caryophyllene	1421	1416.21	35.87	1.07
20	α-Humulene	1454	1449.75	37.90	0.06
21	(E)-β-Famesene	1448	1458.38	38.42	3.19

22	β-Selinene	1469	1467.02	38.95	1.61
23	α-Curcumene	1472	1478.90	39.67	1.63
24	α-Farnesene	1499	1508.29	41.43	0.24
25	δ-Cadinene	1514	1511.44	41.61	0.14
26	Nerolidol	1605	1564.00	47.20	0.17
27	α-Bisabolol	1683	1681.11	51.19	0.14
28	Eudesm-7(11)-en-4-ol	1681	1686.59	51.49	0.47
29	Ethanone, 1-[1,1'-biphenyl]-4-y	1716	1739.56	54.30	2.66
30	NI	-----	1793.37	57.12	5.26
31	NI	-----	1817.50	58.34	**6.93**
32	Phenanthroquinone	1880	1867.70	60.85	**10.39**
33	NI	-----	1890.70	62.00	0.52
34	Farnesylacetone	1895	1897.07	62.32	0.24
35	m-Camphorene	1944	1954.24	65.05	**5.43**
36	α-Springene	1969	1969.42	66.59	1.85
	Hydrocarbonmonoterpenes				53.94%
	Oxygenatedmonoterpenes				14.39%
	Hydrocarbonsesquiterpenes				7.99%
	Oxygenatedsesquiterpenes				3.68%
	Hydrocarbonditerpenes				7.28%
	Total identified (%)				80.00%

Notes:
[a]Order of elution and percentages of individual components are given on an apolar column (DB-5).
[b]RI lit, retention indices taken in the literature, ref. (60 ; 61), [c]RI: Retention index calculated from relative retention times, [d]RT : retention times.

Table 2: Antimicrobial activity of *Rhetinolepis lonadioides* essential oil (aerial parts): Inhibition zones and MICs.

Bacteria	Inhibition zones (mm)			MIC of *R. lonadioides* **essential oil (µL/mL)**
	Essential oil 15µL	Spiramycin (at 100 µg/d)	Oflatoxin (at 5 µg/d)	
Gram-positive bacteria				
Bacillus cereus	17.00±2.65	17.67±2.08	22.00±1.73	4
Bacillus subtilis	11.00±2.00	16.67±1.53	20.67±1.15	10
Staphylococcus aueus	12.33±1.13	17.33±1.15	24.00±1.00	8
Enterococcus faecalis	8.00±1.5	22.67±2.08	26.67±1.53	--
Gram-negativebacteria				
Listeria monocytogenes	6.00±0.00	30.20±2.00	21.67±2.08	--
Escherichia coli	6.00±0.00	6.00±0.00	41.00±5.57	--
Pseudomonas aeruginosa	13.67±7.37	20.67±5.03	26.67±3.06	6

Conclusion

The present study investigated, for the first time, the chemical composition of *R. lonadioides* essential oil endemic of Algeria. The studied essential oil is dominated by monoterpene hydrocarbons (53.94%), oxygenated monoterpenes (14.39%), oxygenated sesquiterpenes (3.68%) and sesquiterpene hydrocarbons (7.99%). The main compounds are β-Pinene (41.85%), Phenanthroquinone (10.39%) and D-Limonene (6.79%). Furthermore, our results showed that the essential oil of *R.lonadioides* possessed interesting antimicrobial properties which potentially suggest its exploitation as a new natural antimicrobial agent in pharmaceutical applications for treating or preventing human infectious diseases and/or in food industries.

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