The anti-oxidant activity of essential oils from two plants of the Asteraceae family: *Rhetinolepis lonadioides* (Coss.) and *Pulicaria crispa* (Forssk.)

Atividade antioxidante de óleos essenciais de duas plantas da família Asteraceae: *Rhetinolepis lonadioides* (Coss.) e *Pulicaria crispa* (Forssk.)

La actividad antioxidante de los aceites esenciales de dos plantas de la familia Asteraceae: *Rhetinolepis lonadioides* (Coss.) y *Pulicaria crispa* (Forssk.)

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ABSTRACT
Rhetinolepis lonadioides (Coss.) and *Pulicaria crispa* (Forssk.) are two medicinal plants belonging to the Asteraceae family, found in the Bechar region (southwest of Algeria), equipped with various biological virtues and medicinal interests. The potential application of the essential oils of these two plants as natural agents with antioxidant effect is evaluated in this research. The chemical compositions of the essential oils of the aerial parts of these two plants *R. lonadioides* and *P. crispa* were analyzed via chromatographic and spectral methods by GC-FID and GC-MS. The results revealed a richness of these EOs with interesting molecules in which the majority constituents are β-pinene (41.85%) and Caryophyllene E (40.05%) respectively. Antioxidant activity was conducted using a free radical scavenging system by DPPH assay. The results showed that *R. lonadioides* oil gave higher antioxidant activity than *P. crispa* oil with IC50 value: 2.13 µl/ml and 9.11 µl/ml, respectively.

Keywords: *Rhetinolepis lonadioides* (Coss.), *Pulicaria crispa* (Forssk.), essential oil, antioxidant activity, DPPH.

RESUMO
A *Rhetinolepis lonadioides* (Coss.) e a *Pulicaria crispa* (Forssk.) são duas plantas medicinais pertencentes à família das Asteráceas, encontradas na região de Bechar (sudoeste da Argélia), dotadas de diversas virtudes biológicas e interesses medicinais. A potencial aplicação dos óleos essenciais destas duas plantas como agentes naturais com efeito antioxidante é avaliada nesta investigação. As composições químicas dos óleos essenciais das partes aéreas destas duas plantas *R. lonadioides* e *P. crispa* foram analisadas através de métodos cromatográficos e espectrais por GC-FID e GC-MS. Os resultados revelaram uma riqueza destes OEs com moléculas interessantes em que os constituintes maioritários são o β-pineno (41,85%) e o cariofileno E (40,05%), respetivamente. A atividade antioxidante foi conduzida utilizando um sistema de eliminação de radicais livres pelo ensaio DPPH. Os resultados mostraram que o óleo de *R. lonadioides* apresentou uma maior atividade antioxidante do que o óleo de *P. crispa* com um valor de IC50: 2,13 µl/ml e 9,11 µl/ml, respetivamente.

Palavras-chave: *Rhetinolepis lonadioides* (Coss.), *Pulicaria crispa* (Forsk.), óleo essencial, atividade antioxidante, DPPH.

RESUMEN
Rhetinolepis lonadioides (Coss.) y *Pulicaria crispa* (Forssk.) son dos plantas medicinales pertenecientes a la familia Asteraceae, que se encuentran en la región de Bechar (suroeste de Argelia), dotadas de diversas virtudes biológicas e intereses medicinales. En esta investigación se evalúa la potencial aplicación de los aceites esenciales de estas dos plantas como agentes naturales con efecto antioxidante. Las composiciones químicas de los aceites esenciales de las partes aéreas de estas dos plantas *R. lonadioides* y *P. crispa* se analizaron mediante métodos cromatográficos y espectrales mediante GC-FID y GC-MS. Los resultados revelaron una riqueza de estos AE con moléculas interesantes en las que los constituyentes mayoritarios son β-pineno (41,85%) y cariofileno E (40,05%) respectivamente. La actividad antioxidante se realizó utilizando un sistema eliminador de radicales libres mediante ensayo DPPH. Los resultados mostraron que el aceite de *R. lonadioides* proporcionó una mayor actividad antioxidante que el aceite de *P. crispa* con un valor de IC50: 2,13 µl/ml y 9,11 µl/ml, respectivamente.

Palabras clave: *Rhetinolepis lonadioides* (Coss.), *Pulicaria crispa* (Forssk.), aceite essencial, actividad antioxidante, DPPH.

1 INTRODUCTION

Nowadays, the search for new molecules has grown significantly, where the science of ethnobotany and ethnopharmacognosy is used as a guide to lead researchers towards different sources and classes of compounds.Secondary metabolites of medicinal plants such as essential oils, tannins, flavonoids and alkaloids constitute an invaluable resource that has been used to find new bioactive molecules, also plant antioxidants are suggested as an interesting alternative. Essential oils (EOs), also named volatile oils, are concentrated natural plant products that contain volatile aromatic compounds. These compounds are mixtures of volatiles (mainly mono- and sesquiterpenoids, benzoids, phenylpropanoids, etc.).

The Asteraceae or Compositae family (known as the aster, daisy, or sunflower family) is the largest family of vascular plants, in terms of number of species. The species *Rhetinolepis lonadioides* (*R. lonadioides*), also known as *Ormenis lonadioides* (Coss.) Maire, or *Anthemis lonadioides* (Coss.) Hochr., is endemic to Algeria and Morocco, commonly called Kamonate elaachar. Indeed, R. lonadioides is an annual plant, finely acescent, 10-20 cm high, branched from the base with stiff, erect or lying stems with hairy ascending leaves with a blade gradually thinning into a petiole and 3-5 lobed at the apex, with short subobtuse segments, and the bracts are entire and superior.(Battandier, 1888; African Plant Database, 2021). The species *Pulicaria crispa* (Forssk.), also named, *Aster crispus* Forssk; *Inula crispa* (Forssk.); Francoeuria crispa (Forssk.); Inula undulata L.; *Pulicaria undulata* (L.) and *Francoeuria undulata* (L.) (Boulos, 1995). This species is distributed in Egypt, Saudi Arabia, Kuwait, Iraq, Iran, Pakistan, India, Afghanistan and parts of north and west Africa (Bohlmann et al., 1979; Al- Yahya et al., 1984).

We contributed in this paper research to the chemical characterization and biological evaluation of the essential oils of two aromatic plants belonging to the Asteraceae family and growing spontaneously in the Saoura region, located in southwest Algeria, which are *Rhetinolepis lonadioides* Coss and *Pulicaria crispa*, in particular their applications as antioxidant agents.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

The choice of these two plants is based on: these uses in the traditional Algerian pharmacopoeia, Contribute to the study and analysis of chemical composition of their essential oils due to the lack of research in this context, and evaluation of the biological effect of these essential oils.

In addition, each of two plants believes itself to be in two different places in the same ecological niche. Indeed, *Rhetinolepis lonadioides* coss was collected from the Beni Abbes area, while *Pulicaria crispa* was collected from the Kenadsa area in the southwest (Table 1).

They were collected during the flowering period at specific locations under the control of botanists from the Saoura region, who ensured their identification in the field. The flowering period depends on the plant. Indeed, *Rhetinolepis lonadioides* Coss comes into flower in April, while *Pulicaria crispa* in March.

Table 1. Locations of plant samples, geographical location and bioclimate of the different study stations.

Plants	Stations	Floors bioclimatic	Altitude (m)	Latitude (Nord)	Longitude (Ouest)
***Rhetinolepis lonadioides* Coss**	Beni Abbes	Aride, chaud	483	30° 05′	2° 06′
Pulicaria crispa	Kenadsa	Aride, chaud	747	31° 33′	2° 25′

Source: Prepared by the authors (2024).

2.2 EXTRACTION OF ESSENTIAL OILS

Distillation is a separation process based on an L (liquid) _ V (vapor) balance. By heating, the liquid boils and the vapor in equilibrium with the liquid will be richer in the most volatile constituents. The technique involves condensation of vapor and recovery of the resulting liquid fractions. We used the reference extraction technique: hydrodistillation using the Clevenger apparatus (Cocking and Middleton, 1935).

2.2.1 Chemical analyzes of essential oils

The analysis of the samples was carried out using a SHIMADZU GCMSQP 2020 Instrument, equipped with an Rxi®-5ms fused capillary column (Phase: Crossbond® 5% diphenyl/ 95% dimethyl polysiloxane).

Its dimensions are: 30 m × 0.25 mm and 0.25 µm film thickness, this column has a phase similar to the following columns: HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB- 1msUI, Ultra-1, VF-1ms, ZB-1, ZB-1ms, and also considered equivalent to USP phases G1, G2, G38. A volume of 0.5 µL of solution prepared at 10% volume of the sample dilution in n-hexane, was injected in split mode (30:1).

The injector and detector temperatures were maintained at 250°C and 310°C, respectively the column temperature was programmed at: 60°C fixed for 3min then increased to 310°C with an increment of 2° C/min, then maintained at 310°C for 10 min. The carrier gas used was helium (99.995% pure) with a flow rate of 1mL/min. Mass spectrometer conditions were as follows:

ionization voltage 70 eV, ion source temperature 200 °C, and electron ionization mass spectra were acquired over the mass range of 45 to 600 m/z.

2.2.2 Identification of essential oil components

Linear retention indices (LRI) were calculated for separate compounds relative to serial homologous n-alkanes (n-C7-C20). The components were identified, by comparing their (LRI) calculated with those in the literature (Babushok et al., 2011; Adams, 2017) as well as their mass spectra with those recorded by the NIST (National Institute of Standards and Technology) and Wiley libraries "NIST17.lib, W11N17MAI and FFNSC1.2.lib".

2.2.3 Evaluation of the biological activities of essential oils

2.2.3.1 Study of anti-oxidant activity

In order to evaluate the anti-oxidant activity of the essential oils of the plants Rhetinolepis lonadioides Coss and Pulicaria crispa, a method was used in vitro is the free radical scavenging power of DPPH (Gachkar et al., 2007).

2.2.3.2 Principle

The test used the reduction of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in the presence of volatile compounds from essential oils.

It is based on the decrease in the absorbance of the radical solution according to the reaction DPPH• (purple) + HA → DPPH-H (yellowish) + A•. This radical has been used for many decades to study the mechanism of hydrogen atom donation to free radicals of certain substrates or the antioxidant activity of compounds bearing –SH, –OH and –NH groups. Where (AH) n represents a compound capable of giving up hydrogen to the DPPH radical (purple) to transform it into the DPPH-H molecule.

2.2.3.3 Operating mode

First, we prepared the 0.004% methanolic DPPH solution, where we dissolved 1 mg of DPPH powder in 25 ml of methanol. In order to prepare the stock solutions of each EO, 80 µl of the EO is added to 4 ml of methanol, to obtain a final concentration of the stock solution of 20 µl/ml. The control used is ascorbic acid which is prepared at the same concentration as the essential oils.

From the stock solutions, different concentrations of essential oils were prepared: 10 µl/ml; 5 µl/ml; 2.5 µl/ml; 1.25 µl/ml; 0.625 µl/ml; 0.312 µl/ml and 0.156 µl/ml. A volume of 40 µl of each concentration was added to 960 µl of the DPPH solution. After homogenization by vortexing, the

tubes are placed in the dark at room temperature for 30 minutes. The reading is carried out by measuring the absorbance at 517 nm by a UV-Visible spectrophotometer where methanol is used as a blank. The results were expressed as the average of three measurement separated (Archana et al., 2005; Dung et al., 2008).

2.2.3.4 Expression of results

The anti-oxidant activity is expressed by the reducing power of the methanolic DPPH solution. Lower absorbance values indicate greater free radical scavenging activity than higher ones. The reduction power is determined by applying the following formula:

$$AX(\%) = \left[\frac{Ac - AE}{Ac}\right] * 100$$

$AX(\%)$: Power of the reduction in percentage;
AE: Absorption of the DPPH solution in the presence of essential oil or vitamin C;
AC : Absorption of the DPPH solution in the absence of essential oil and vitamin C;

The activity of an antioxidant can be characterized by a quantity called IC50. This parameter is defined as the concentration of antioxidant required to reduce the initial DPPH concentration to 50%. The IC50 makes it possible to compare the activity of different antioxidant compounds. The lower the IC50, the greater the activity of the antioxidant (Sanchez-Moreno et al., 1998).

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF ESSENTIAL OILS *Rhetinolepis lonadioides* Coss.

The essential oil samples were obtained by hydrodistillation of the dry aerial parts of *R. lonadioides* Coss. The essential oil yield, calculated in relation to dry matter (w/w), varies between 0.90% and 1.40%. Detailed analysis of a raw sample was carried out by a combination of (Ir-GC) and mass spectroscopy (MS). Spectra of essential oils are recorded, and the chemical shift values are compared to those of the reference compounds present in a spectrum library using identification software. It allowed us to identify thirty-six compounds (25 monoterpenes, 4 sesquiterpenes and 2 polyterpenes) representing 80.00% of the entire overall chemical composition (table 4). This essential oil is rich in monoterpenes with a percentage of 68.33%.

The fraction of hydrocarbon monoterpenes (53.94%) was mainly represented by β-Pinene (41.85%). D-Limonene (6.79%), α-Fenchene (3.64%) and α-Pinene (1.01%). hydrocarbon sesquiterpenes (7.99%) were represented by (E)-.beta.-Famesene (3.19%) and. α-Curcumene (1.63%), while mCamphorene (5.43%) was the only diterpene hydrocarbon present in significant

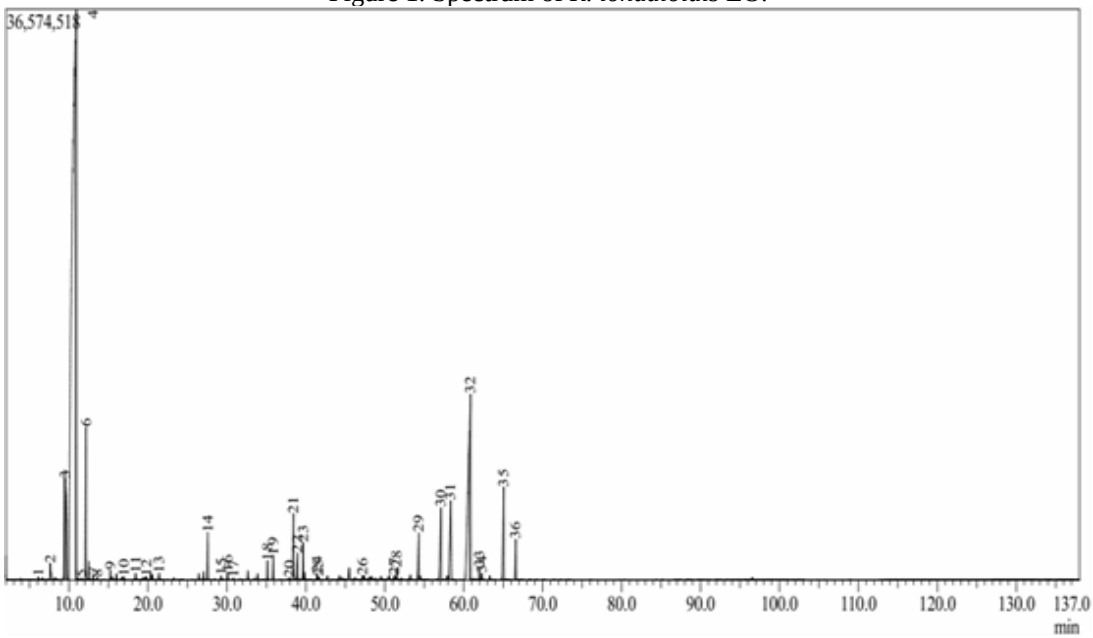
quantity (P<0.05). In addition, this essential oil is characterized by the presence of sesquiterpene oxygenated acids, Eudesm-7(11)-en-4-ol (0.47%) and Nerolidol (0.17%).

Table 2. Compounds identified in the essential oil of *Rhetinolepis lonadioides* Coss. With their corresponding retention time by GC-MS.

	Compounds	RI lit[b]	RI[c]	T.R[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	Bornyl acetate	1285	1284.58	27.57	2.04
15	Guaiacol <4-vinyl->	1293	1310.99	29.26	0.13
16	Myrtenyl acetate	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		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
	Hydrocarbon monoterpenes				53.94%
	Oxygenated monoterpenes				14.39%
	Hydrocarbon sesquiterpenes				7.99%
	Oxygenated sesquiterpenes				3.68%
	Hydrocarbon diterpenes				7.28%
	Total identified (%)				80.00%

Source: Prepared by the authors (2024)

Figure 1. Spectrum of *R. lonadioidis* EO.



Source: Prepared by the authors (2024)

3.2 ANALYSIS OF ESSENTIAL OILS *P. crispa*

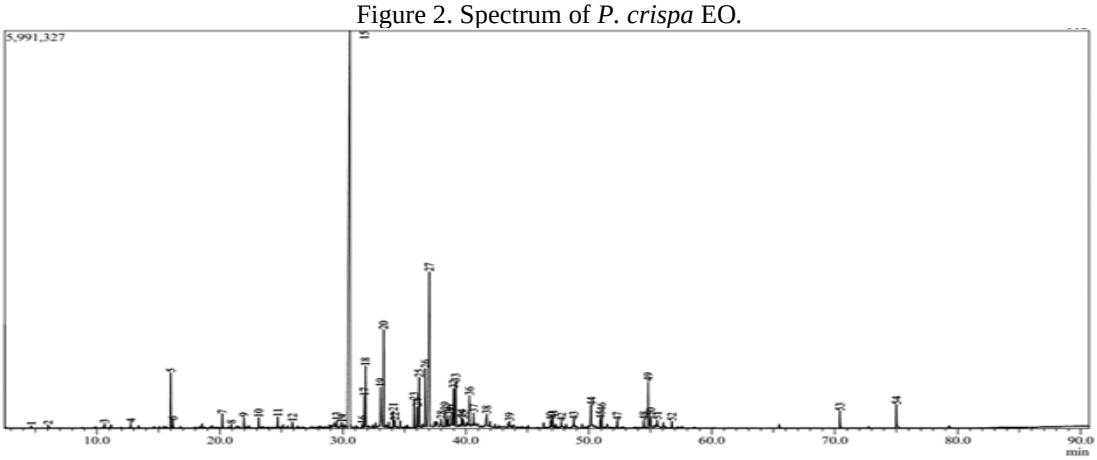
The essential oil obtained by hydrodistillation of *P. crispa* is a pale-yellow color with a strong odor, with a yield varying from 0.1% to 0.3%. Fifty-four compounds were identified representing the majority of *Pulicaria crispa* essential oil (Table 3). The main constituents of P. crispa oil are Caryophyllene E (40.5%), Caryophyllene oxide (11.12%), Neryl isobutanoate (4.94%), Humulene <alpha-> (3.26%), and Nerolidyl acetate (3.11%). The essential oil of P. crispa grouped into six groups which are the group of monoterpene hydrocarbons (HMT)%, the group of oxygenated monoterpenes (MTO)%, the group of sesquterpene hydrocarbons % (HST) and the group of oxygenated sesquterpenes (STO%), the diterpene hydrocarbon group (HDT)% and the oxygenated diterpene group (DTO)%. The data in the same table (Table 5) indicates that HST gave the highest concentrations (45.96%), followed by STO (32.96%), MTO (15.57%), DTO (4.45%), HDT (0.85%) and HMT (0.21%). The group (HST) includes in addition the majority compound Caryophyllene E, other constituents in small proportions: Humulene <alpha->, Alloaromadendrene, Caryophyllene Z and Santalene<beta-, epi. The major constituents of the other groups are Caryophyllene oxide for (STO), Neryl isobutanoate for (MTO), Phytol for (DTO), Hexacosane the only compound in the group (HDT) and Limonene as the unique compound in the group (HMT) with a proportion of 0.21%.

Table 3. Compounds identified in the essential oil of *Pulicaria crispa* with their corresponding retention time by GC-MS.

	Compounds	RIc	T.Rd	Area%
1	Hexanal	800	4.646	0.06
2	Hex-2(E)-enal	848	6.029	0.09
3	1-Octen-3-ol	978	10.623	0.18
4	Limonene	1028	12.749	0.21
5	Linalool	1099	15.994	**2.41**
6	Nonanal	1104	16.190	0.34
7	Terpineol <alpha->	1190	20.201	0.63
8	Decanal	1205	20.920	0.15
9	Nerol	1228	21.955	0.57
10	Geraniol	1254	23.155	0.45
11	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2.alpha.,4a.alpha.,8a.alpha.)-	1288	24.694	0.5
12	Theaspirane	1316	25.897	0.28
13	Jasmone <(Z)>	1398	29.493	0.33
14	Caryophyllene Z	1408	29.899	0.21
15	Caryophyllene E	1425	30.569	**40.05**
16	Santalene <beta-, epi->	1449	31.575	0.14
17	Geranylacetone	1453	31.751	1.53
18	α -Humulene	1455	31.839	**3.26**
19	Alloaromadendrene	1485	33.062	**2.3**
20	Neryl isobutanoate	1491	33.318	**4.94**
21	Lavandulyl isobutyrate	1511	34.090	0.87
22	Geranyl isobutyrate	1514	34.225	0.41
23	Caryophyllene oxide	1554	35.790	1.43
24	5-Oxatricyclo[8.2.0.04,6]dodecane, 4,12,12-trimethyl-9-methylene-, (1R,4R,6R,10S)-	1560	36.033	1.02
25	Nerolidol	1565	36.212	**2.46**
26	Nerolidyl acetate	1578	36.707	**3.11**
27	Caryophyllene oxide	1586	37.033	**11.12**
28	Humulene epoxide II	1611	37.993	0.36
29	2-(1-Cyclohexen-1-yl) cyclohexyl acetate	1620	38.322	0.86
30	Geranyl tiglate	1628	38.602	0.49
31	Caryophylla-4(12) ,8(13)-dien-5.alpha.-ol	1635	38.863	0.58
32	Alloaromadendrene oxide-(1)	1638	39.003	1.84
33	Muurolol <alpha-, epi->	1643	39.167	**2.49**
34	α.-Cadinol	1656	39.669	0.43
35	Isoaromadendrene epoxide	1660	39.783	0.41
36	Alloaromadendrene oxide-(2)	1673	40.285	1.46
37	Geranyl tiglate	1682	40.619	0.52
38	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1712	41.688	0.73
39	Benzyl Benzoate	1764	43.548	0.28
40	Pentacyclo [8.4.0.0(3,7).0(4,14).0(6,11)] tetradecane	1862	46.891	0.29
41	Phthalic acid, 2,7-dimethyloct-7-en-5-yn-4-yl isobutyl ester	1867	47.066	0.58
42	Bicyclo [4.4.0] dec-6-en-9.beta.-ol, 1,7-dimethyl-4.alpha.-isopropenyl-	1888	47.761	0.27
43	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	1919	48.769	0.31
44	n-Hexadecanoic acid	1963	50.183	1.28
45	1H-Imidazole, 1-[(4-methylphenyl) sulfonyl]-	1986	50.932	0.4
46	(-)-Spathulenol	1990	51.039	0.84
47	Propanoic acid, 2-methyl-, 2-[3-[(acetyloxy)methyl]oxiranyl]-5-methylphenyl ester	2031	52.325	0.45
48	2(3H)-Furanone, 5-dodecyldihydro-	2101	54.474	0.39
49	Phytol	2113	54.824	**2.43**
50	(7-Methylcyclopenta[c]pyran-4-yl)methyl 3-methylbutanoate	2120	55.027	0.66
51	Linolenic acid	2138	55.557	0.31
52	(12Z)-abienol	2179	56.743	0.3
53	Hexacosane	2700	70.408	0.85
54	Nonacosane	2900	74.999	1.14
55	Hydrocarbon monoterpenes			0.21
56	Oxygenated monoterpenes			15.57
57	Hydrocarbon sesquiterpenes			45.96
58	Oxygenated sesquiterpenes			32.96
59	Hydrocarbon diterpenes			0.85
60	oxygenated diterpenes			4.45
	Total identified (%)			100

Source: Prepared by the authors (2024)

Figure 2. Spectrum of *P. crispa* EO.



Source: Prepared by the authors (2024)

3.3 EVALUATION OF ANTI-OXIDANT ACTIVITY

The anti-oxidant activity of EOs from the aerial part of the *Rhetinolepis lonadioides* Coss and *Pulicaria crispa* plants were evaluated by the DPPH free radical trapping method, where the bioactive molecule has the capacity to act as an atom donor of hydrogen in the transformation of the DPPH radical into its reduced form DPPH.-H. The results are expressed as percentage inhibition of the DPPH free radical according to the concentrations of each essential oil and ascorbic acid (the standard). The absorbance values obtained allowed us to calculate the inhibition percentages.

The IC50 is defined as the concentration of antioxidant required to reduce the initial DPPH concentration by 50%. It was determined from the logarithmic regression equations of plotted curves illustrated in Figures 3, 4 and 5 for ascorbic acid and the essential oils of *R. lonadioides* and *P. crispa* respectively. The IC50 results are shown in Table 4.

Table 4. The results of the antioxidant activity of the samples of plant EOs and ascorbic acid.

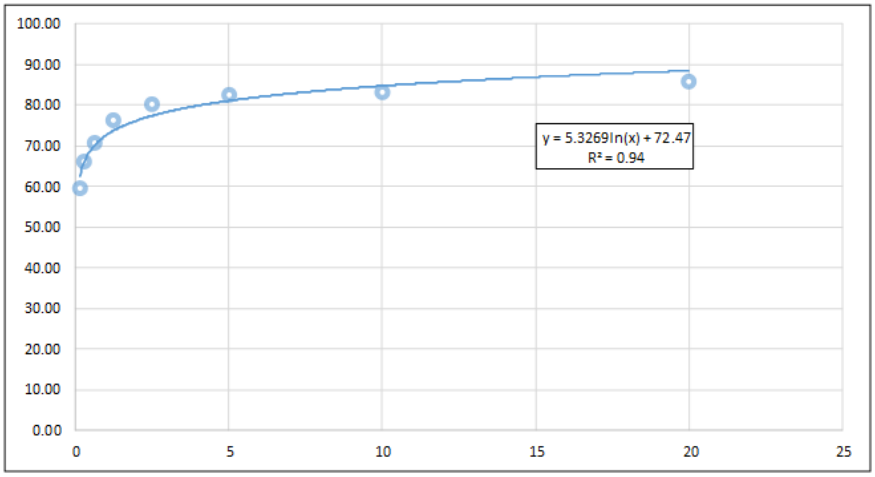
Samples	Concentration (µg/mL)	Trapping effect on DPPH(%)	IC 50 Concentration (µg/mL)
ascorbic acid	20	86.76	0.014
	10	80.93	
	5	73.36	
	2.5	61.94	
	1.25	56.74	
	0.625	48.94	
	0.312	45.94	
***Rhetinolepis lonadioides* Coss. EO**	20	75.18	2.13
	10	74.15	
	5	68.48	
	2.5	35.70	
	1.25	31.60	
	0.625	30.18	
	0.312	21.75	
***Pulicaria crispa* EO**	20	52.01	9.11
	10	50.35	
	5	48.23	
	2.5	33.96	
	1.25	29.94	
	0.625	22.85	
	0.312	8.04	

Source: Prepared by the authors (2024)

The DPPH free radical scavenging capacity of the essential oils of both plants also increased with increasing concentration. The two essential oils showed a significant trapping effect, with different contents. The lowest effect (8.04%) was presented by the essential oil of *Pulicaria crispa*, at a concentration of 0.312 µg/mL, while the highest activity (86.76%) was presented by the ascorbic acid at a concentration of 20 µg/mL. In the same concentrations tested previously, the inhibition rate was estimated at 75.18% and 52.01% for the essential oils of *Rhetinolepis lonadioides* Coss and *Pulicaria crispa* respectively. Therefore, it was concluded that the antioxidant effect of *Rhetinolepis lonadioides* Coss was higher than that given by *Pulicaria crispa*.

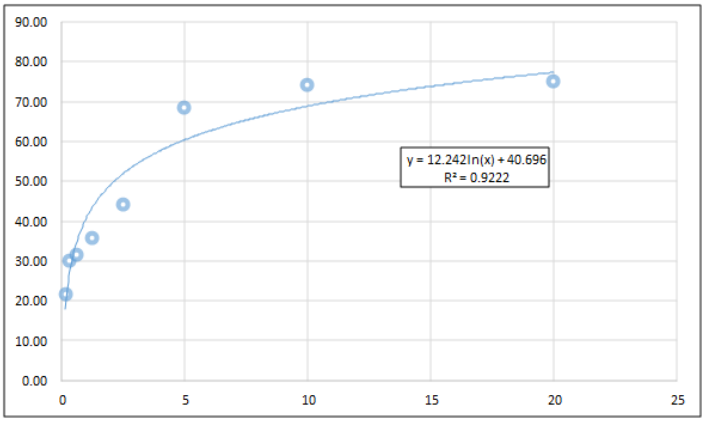
For comparison between the DPPH trapping activity of the essential oils of the samples tested and those expressed by ascorbic acid showed that the essential oil had a weak effect compared to vitamin C, this is explained by the big difference between the IC 50 of ascorbic acid at 0.014 µg/mL and that given by the EOs of *Rhetinolepis lonadioides* Coss and *Pulicaria crispa* with concentrations of 2.13 µg/mL and 9.11 µg/mL respectively (Fig. 6).

Figure 3. Percentages of DPPH free radical inhibition as a function of ascorbic acid concentrations.



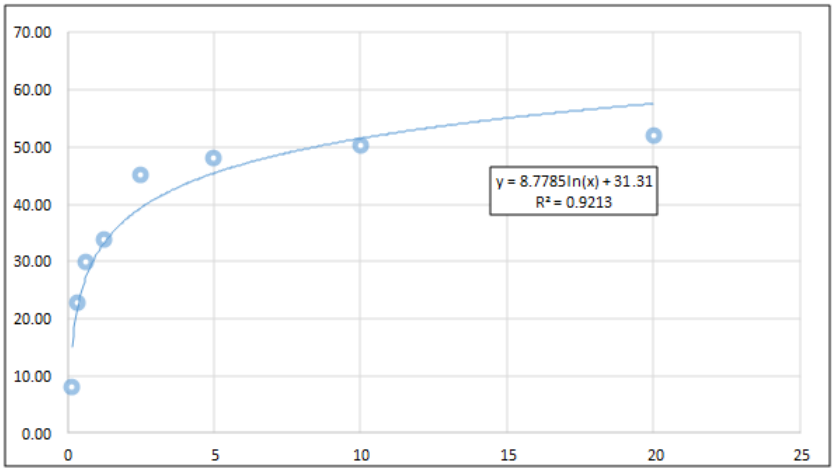
Source: Prepared by the authors (2024)

Figure 4. Percentages of DPPH free radical inhibition as a function of EO concentrations from the plant *Rhetinolepis lonadioides* Coss.



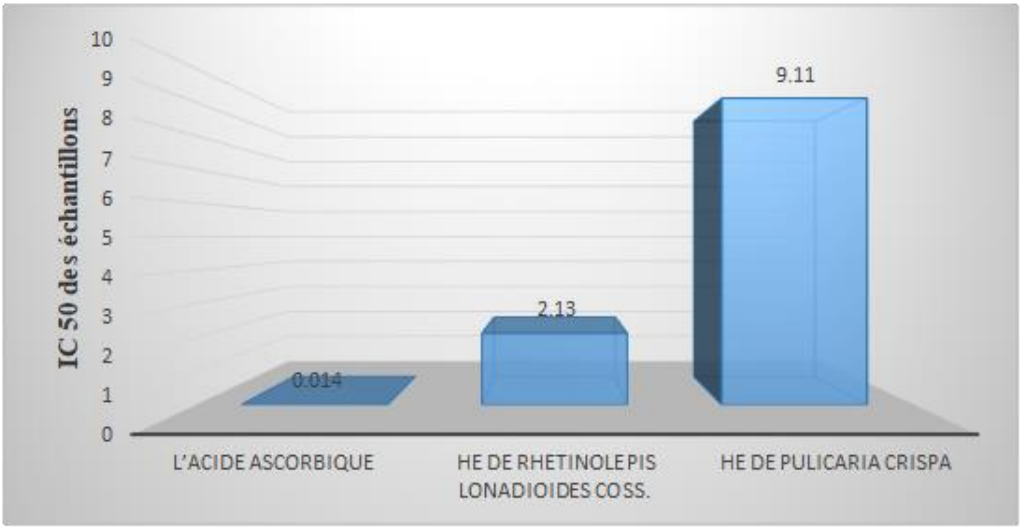
Source: Prepared by the authors (2024)

Figure 5. Percentages of inhibition of the free radical DPPH depending on the concentrations of the EO of the *Pulicaria crispa* plant.



Source: Prepared by the authors (2024)

Figure 6. The IC50 values (in µg/ml) of ascorbic acid and the EOs of the plants studied.



Source: Prepared by the authors (2024)

The anti-oxidant effect could be attributed to the main constituents of EOs, such as β-Pinene, D-Limonene and Phenanthroquinone for Rhetinolepis lonadioides Coss., and Caryophyllene E, Neryl isobutanoate and Linalool for *Pulicaria crispa*. Additionally, oxygenated sesquiterpenes could act individually or synergistically as antioxidants. The activity of oxygenated compounds could be attributed to free electrons due to the high oxygenation (Abd-Elgawad et al., 2020).

Furthermore, the resulting antioxidant activity can be explained by the high content of mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes for the oils of the plants studied. It is reported in the literature that sesquiterpenes exhibit free-radical scavenging activity. The structure and concentration of sesquiterpenes determine their mode of action as an antioxidant (Bartikova et al., 2014).

4 CONCLUSION AND PERSPECTIVES

The present study provides new information on the biological activities of plant essential oils that have not been reported previously, in order to discover new natural bioactive products. First of all, the volatile constituents of the essential oils of two plants of the Asteraceae family, which grow spontaneously in the Saoura region located in southwest Algeria, as well as their diverse biological effects.

We evaluated the anti-oxidant activity of the two essential oils. The results showed that *R. lonadioides* oil exhibited higher free radical scavenging activity than *P. crispa* oil, with IC50 values of 2.13 µl/ml and 9.11 µl/ml, respectively. These results suggest that these EOs can be considered as potential new sources as natural free radical scavengers.

Finally, the results obtained constitute only the first step of research into the biological activity of essentials oils studied; in research for larger and more in-depth work, it would be desirable to purify and isolate the molecules contained in essentials oils of the plants studied, especially the major compounds, then elucidate the mechanisms of action, effectiveness and synergistic effects of the identified compounds, down to advisable to increase in vivo research through histological studies, to carry out studies on cell culture to better refine the effect of these essential oils and their components.

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