Atriplex halimus L. and *Centaurium erythraea* Rafn. Essential Oils: The Phytochemical Profile, Antimicrobial and Antioxidant Properties

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Summary

This study was conducted to determine *in vitro* the antioxidant and antimicrobial potency of essential oils from two medicinal plants known in the Algerian northwest (Mascara city): *Atriplex halimus* L. and *Centaurium erythraea* Rafn. The analysis of essential oils (EOs) chemical compounds was performed by GC/MS. In total, 72 and 35 chemical components were identified for *C. erythraea* and *A. halimus*, which represents respectively 91.89% and 89.17% of the essential oil content. In fact, EO of *A. halimus* abundantly contained viridiflorol (40.23%), phytol (18.24%), germacrene D (6.94%), whereas β-copaen-4α-ol (38.41%), manool (8.2%) and carvacrol (6.43%) were found in OE of *C. erythraea*. Both essential oils were tested for antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis* (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria) and one yeast strain *Candida albicans* using the agar-disc diffusion assay and the microdilution method (Minimum Inhibitory Concentration, MIC). *A. halimus* EO is active against *Escherichia coli*, whereas the essential oil of *C. erythraea* is active against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The antioxidant properties were evaluated using free radical scavenging and ferric reducing power (FRAP) assay. The results obtained showed the existence of an antioxidant activity of the studied essential oils but less effective compared to the standards used (ascorbic acid and catechin).

Key words

antioxidant activity, antimicrobial activity, essential oil, *Atriplex halimus* L., *Centaurium erythraea* Rafn

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Introduction

Infectious diseases were considered as the major cause of worldwide morbidity and mortality at the beginning of the 20th century (Gasu et al., 2018). All the more so as antibiotic use and misuse led to the emerging of antibiotic resistance in human and animal pathogens (García-Salinas et al., 2018), without forgetting to mention that these antibiotics have proven side-effects (Bouyahya et al., 2017). Furthermore, free radicals are the major causes of many chronic diseases such as cancer, atherosclerosis, cardiovascular and inflammatory diseases (Annapandian et Rajagopal, 2017). Moreover, there are potential toxicological and carcinogenic risks related to the use of synthetic antioxidant molecules (Srivastava, 2013). Facing these public health problems, medicinal plants could provide a more appropriate therapeutic response.

A large number of compounds known as "secondary metabolites", such as alkaloids, saponins, tannins, flavonoids, glycosides, betalains, and terpenoids, are produced by different parts of plants during the primary metabolism of plants. These compounds have a defensive role in nature (Khan, 2017). Essential oils, ethereal or volatile oils, are ones of the secondary metabolites of volatile plants and are produced from different parts of plants such as flowers, grains, buds, leaves, fruits, wood, roots, bark and twigs (Kuttan et Liju, 2017). Volatile compounds such as terpenes, phenols, ketones, alcohols, esters, amines and amides are the principal constituents of essential oils. These compounds possess powerful antimicrobial and functional properties (Kumar et al., 2020). Therefore, essential oils have been used in phytotherapy and other medical applications (Zari, 2012; Hanif et al., 2019). They are used effectively in the treatment of pathogenic (viral, fungal and bacterial diseases) or nonpathogenic diseases (For example, hypertension, tumors, leukemia, etc.) (Hanif et al., 2019). As a result of this generalized use, their biological activity has been more explored by scientists.

Our flora is very rich and diversified because of the geographical situation and the climatic diversity of Algeria. It has been used for a long time to treat several diseases (Bouasla et Bouasla, 2017). *Atriplex halimus* L. and *Centaurium erythraea* Rafn, medicinal plants known in North West Algeria, have been widely used in traditional folk medicine for their biological properties. *A. halimus* (Amaranthaceae), known in Algeria as "Guettaf" was used in traditional folk medicine to treat heart diseases and thoracic disorders, as a laxative, to cure muscle and stomach pain and to regulate the excretions of the gallbladder (Chikhi et al., 2014). It is also used to cure diabetes, rheumatism, hypertension, urinary infections (Idm'hand et al., 2020), eczema and scarring and bites (Miara et al., 2019). *C. erythraea* (Gentianaceae), known in Algeria as "Mararet el-hnech" was used as a hypoglycemic, antipyretic, depurative, cardio-regulator (Bellakhdar, 1991) and antihypertensive (Calvo et Cavero, 2014). It was used as a stomach and digestive tract, for diabetes, helminthiasis, for biliary stimulation (Merzouki et al., 2000), to treat asthma (Kultur, 2007), dyspepsia, and kidney calculus (Skidmore-Roth, 2009). In traditional herbal medicine, centaury can treat many diseases in infants and children such as anxiety, insomnia, tension, colic irritable bowel syndrome, topical inflammation and symptoms of attention deficit hyperactivity disorder (Skidmore-Roth, 2009).

The aim of the present study is to evaluate *in vitro* the free antiradical activities, antibacterial and anti-*Candida* of essential oils of *A. halimus* and that of *C. erythraea*.

Materials and Methods

Plant Material

A. halimus (grains stage) and *C. erythraea* (flowering stage) were collected respectively in October and March of 2015 from Mascara in the north-west region of Algeria. These plants were identified by a local expert and a Voucher specimen was deposited at the herbarium center of the laboratory of Bioconversion, Microbiological Engineering and Safety Security of the Faculty of Science of the Nature and the Life of Mascara Mustapha Stambouli University for future reference.

Essential Oils Extraction

Dried sample (100 g), from the aerial parts, was submitted for 3 h to water distillation using a Clevenger type apparatus (Kartal et al., 2007). After their extraction, the essential oils were dehydrated with anhydrous sodium sulfate and stored in obscure bottles at 4 °C until use.

The extraction yield was calculated using the following formula (Selvakumar et al., 2012):

$$\% \text{ yield of EO} = (\text{EO mass/dried sample mass}) \times 100$$

GC and GC-MS Spectrometry Analyses

The EOs were analyzed using GC-MS spectrometry To determine the percentage of oil components, a Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific, Agilent, USA), a FID detector regulated at 270 °C, supplied with a H2/air gas mixture and a Split-splitless injector regulated at 240 °C were used. The mode of injection was Split (split ratio: 1/50, flow: 66 mL min^{-1}). Helium was used as the gas with a flow rate of 0.5 mL min^{-1}. The column temperature was programmed from 60 to 250 °C at a rate of 2 °C min^{-1}. The retention indices were calculated using the retention time of the n-alkanes that were injected after the oil under the same chromatographic conditions. The identification of the oil components was based on their Kováts index (KI) and on the comparison of their mass spectral fragmentation patterns with those reported in the literature, and the mass spectra obtained from GC-MS analysis on a Hewlett-Packard HP6890/HP5973 (Hewlett-Packar, USA) instrument equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i. d. x 0.25 µm film thickness); Helium was used as carrier gas at a flow rate of 2 mL min^{-1}. The GC analytical parameters were the same as those listed above, and mass spectrometry was performed in electron impact (EI) at 70 eV.

Antioxidant Activity

DPPH Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured according to the procedure described by Mighri et al. (2010).

Briefly, 1 ml of methanol solution of DPPH was added to the tested essential oil (1ml). After agitation, the mix was incubated in obscurity for 30 min at room temperature and the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (PI %) was calculated as followed:

$$P1\% = [A_{blank} - A_{sample} / A_{blank}].$$

Synthetic antioxidants (ascorbic acid and catechin) were used as positive control and all tests were carried out in triplicate.

Using linear regression, the EC_{50} values (effective concentration or IC_{50}, inhibitory concentration) of essential oils were calculated.

Ferric Reducing Power (FRAP)

The ferric reducing ability was assessed following the method described by Ferreira et al. (2007).

A quantity (2.5 mL) of each dilution of our oils and our reference antioxidants (catechin, ascorbic acid), solubilized in the methanol, was mixed with 2.5 mL of phosphate buffer (0.2 M, PH: 6.6) and 2.5 mL of potassium ferricyanide [K_3Fe (CN) $_6$] (1%). After 20 minutes of incubation at 50 °C, 2.5 mL of trichloracetic acid (10%) was added and the mixture was after that centrifuged at 3000 rpm for 10 min. Finally, 5 mL of the supernatant was mixed with 5 ml of water distillated and 1 mL $FeCI_3$ (0.1%), then the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated stronger reducing power.

Antimicrobial Activity

Microorganisms

Bacterial strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25853), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis*, obtained from the medical laboratory of Oran Hospital.

Fungi strains: *Candida albicans*, obtained from *L.R.S.B.G.* Laboratory, Mascara Mustapha Stambouli University

Antimicrobial Assays

a) Disc Diffusion Method

1 mL of inoculum adjusted beforehand (10^6 UFC mL^{-1}) was seeded in the Mueller Hinton Agar medium/Sabouraud agar. Then, the sterile discs (6 mm diameter prepared in filter paper) charged by the sterile essential oils, were deposed on the surface of the medium agar. The DMSO was used as negative control and the antibiotics (Tetracycline 30 µg, Gentamicin 10 µg and Ampicillin 10 µg, for the bacteria and Clotrimazole 10 µg for yeast) as positive control. The treated Petri dishes were stored at +4 °C for 30 min, and then incubated at appropriate temperature and incubation time (37 °C for 24h for bacterial strains and 37 °C for 48h for yeast). The diameters (mm) of zones inhibition were than measured (Hajlaoui, 2010).

According to the diameters of inhibition, the essential oil effect can be considered. The diameter of the inhibition classified the sensitivity to essential oils as follows:

- < 8mm: insensitive.
- 8-14 mm: moderately sensitive.
- 14-20: sensitive.
- > 20 mm: extremely sensitive (Ambrosio et al., 2017).

b) Determination of Minimum Inhibitory Concentration (MIC)

The test was carried out as described by Boumediene et al. (2011), 100 µL of sterile nutritive broth (for bacteria) and Sabouraud broth (for yeast) were transferred in the 96-well microplate, then 100 µL of each sterile essential oil were added in the first plate well and binary dilutions in set were affected. Next, sed as negative control. The plates were then incubated at 37 °C for 24h for bacteria, and at 30 °C for 48h for yeast. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Statistical Analysis

The tests were performed in triplicate. The results obtained were expressed as mean ± standard deviation (SD).

Results and Discussion

Yields of Extraction

The results concerning the yield in EOs of the two plants *C. erythraea* and *A. halimus* were low, ranging between 0.04% and 0.022% respectively. In addition, the EO of *C. erythraea* was characterized by a liquid aspect, dark yellow color, while the EO of *A. halimus* was characterized by a light yellow color and liquid appearance. A low yield can be due to different factors. For example, *A. halimus* is known as a halophytic plant that grows in saline soils (Kumari et al., 2019), whereas soil salinity is one of the environmental factors that influence the quality and quantity of essential oils produced by medicinal and aromatic plants (Duarte et al., 2017).

Chemical Composition of Essential Oils

The constituents identified by GC-MS analysis and their percentage in essential oils are listed in Table 1 and 2. Based on the GC-MS results, 35 and 72 components were identified in *A. halimus* and *C. erythraea* essential oil, corresponding to 89.17% and 91.89% of the total oil, respectively.

The chromatographic profile identifying the EO shows that the predominant compound in EO of *A. halimus* is viridiflorol (40.23%), which is a sesquiterpene, followed by phytol (18.24%), which is an acyclic diterpene. The other characteristic constituents determined in this essential oil are viridiflorene (3.23%), myristicin (3.01%), bicyclogermacrene (2.46%), p-acetanisole (1.60%), caryophyllene oxide (1.52%), α-bisabolol B-oxide (1.46%) and cardine-1(10), 4-one (1.25%). The presence of other minor components, including α-muurolol (0.047%), was also noted. There, the *C. erythraea* essential oil is characterized mainly by the presence of β-Copaen-4α-ol (38.41%), manool (8.2%) and carvacrol (6.43%). In parallel other compounds in inferior degrees were identified such as neophytadienne (III) (4.24%), terpinene-4-ol (4.14%), p-cymen-ol (3.13%) and thymol (2.12%). Isomenthol (0.017%) was noted as a minor compound.

However, the present study is the first to identify the chemical composition of *A. halimus* essential oil by GC-MS analysis. Chemical analysis of *C. erythraea* essential oil reveals the presence

of β-Copaen-4α-ol and manool as dominant components. In a later study conducted only β-Copaen-4α-ol was present in traces in Serbian essential oil (Jovanović et al., 2009). However, they were missing in Croatian (Jerković et al., 2012) and Moroccan essential oil (Bouyahya et al., 2019). Also more and similar to our study, carvacrol was also identified among the main constituents in Serbian, Croatian and Moroccan essential oils with a percentage of 7.9%, 6.17% and 8.73%, respectively (6.43% of carvacrol for the studied plant).

GC-MS analysis also revealed another chemical element, neophytadienne (III) (4.24%). This compound was identified as a major component in the essential oil from Serbia (10.1%) and in small amounts in the essential oils from Croatia (1.4%) and Morocco (0.8%).

In addition, thymol that characterized our essential oil, was also mentioned in the essential oils of Serbia, Morocco and Croatia but with different quantities. The amount of thymol (2.12%) was lower than that of essential oil in Serbia (4.2%) and Croatia (2.6%), but higher than that of Morocco (0.83%). On the other hand, menthol was the principal compound in the Moroccan essential oil (20.82%) and the Croatian essential oil (7%), whereas in this study it accounted for 1.34%.

These variations in the chemical composition of *C. erythraea* essential oil may be due to several factors that can raise or lower the yield as well as the chemical composition of the essential oils. These factors are: mineral nutrition, water, light, temperature, soil, attack of pathogens, pests and herbivores and genetic factors (Boaro et al., 2019). The geographical zone (country of origin), climate, altitude (Shutes et Galper, 2020), harvesting period, equipment and distillation technique also influence this composition (Price L., 2011).

Antioxidant Activity

DPPH Scavenging Method

It can be seen in Fig. 1 that the free radical scavenging actions of *A. halimus* and *C. erythraea* essential oils were concentration dependent. EC_{50} (called, IC_{50}) is the concentration of essential oil required to inhibit 50% of DPPH free radicals, it indicates a high antioxidant capacity if it is low. The EC_{50} values of essential oils obtained using linear regressions were summarized in Table 3. As indicated, the essential oil that had important antioxidant activity was that of *C. erythraea* with an EC_{50} value of 546.61 µg mL^{-1}, lower than that of the standard compounds, ascorbic acid (11.88 µg mL^{-1}) and catechin (14.28 µg mL^{-1}).

FRAP Assay

In the FRAP assay, a high absorption value reflects an increase in the reducing power of the tested essential oil. As shown in Fig. 2, reducing power of essential oils and standard compounds increases with the increase in concentration. in all concentrations (62,5-1000 µg mL^{-1}), ascorbic acid and catechin are more active than essential oils with absorbance value of 0.91±0.056 and 0.98±0.005 at 1000 µg mL^{-1}, respectively. The essential oil of *C. erythraea* shows the highest reducing power when compared to the essential oil of *A. halimus*.

Table 1. The chemical composition *Atriplex halimus* L. essential oil

N°	KI [a]	Compounds	% Area
01	1104	α-Thujone	0.085
02	1141	Camphor	0.246
03	1163	Borneol	0.113
04	1189	α –Terpineol	0.199
05	1129	Citronellol	0.089
06	1293	Thymol	0.257
07	1301	Carvacrol	0.145
08	1331	*p*-Acetanisole	1.604
09	1416	(β)-Caryophyllene	0.413
10	1451	α –Humulene	0.636
11	1475	ɣ-Muurolene	0.081
12	1480	**Germacrene D**	**6.942**
13	1493	Bicyclogermacrene	2.468
14	1518	Viridiflorene	3.239
15	1522	δ-Cadinene	0.328
16	1525	Myristicin	3.016
17	1528	Cardina-1(10), 4-one	1.253
18	1580	Caryophyllene oxide	1.528
19	1590	**Viridiflorol**	**40.235**
20	1600	Ledol	0.624
21	1602	Humulene epoxide II	0.495
22	1616	1,10-di-epi-Cubenol	0.935
23	1626	10-épi-ɣ-Eudesmol	0.200
24	1630	1-épi-Cubenol	0.734
25	1634	(Z)-Nerolidol	0.506
26	1639	épi- α- Cadinol	0.313
27	1640	épi- α-Muurolol	0.239
28	1645	α-Muurolol	0.047
29	1648	β-Eudesmol	0.334
30	1653	α-Cadinol	0.958
31	1656	α-Bisabolol oxide B	1.461
32	1702	Geranyl tiglate	0.306
33	1792	1-Octadecene	0.843
34	1905	Rimuene	0.096
35	2052	Phytol	**18.242**
		Total	**89.17**

Note: [a] Kováts indices calculated on DB5 column with reference to n-alkanes injected after the oil at the same chromatographic conditions

Table 2. Chemical composition of *Centaurium erythraea* Rafn. essential oil

N°	KI [a]	Compounds	% Area
01	889	2,5-Dimethylfuran	0.069
02	938	α-Pinene	0.092
03	974	1,2,4-Trimethyl benzen	0.145
04	996	2-Pentyl furan	0.434
05	1030	1,8-Cineole	0.065
06	1100	Linalool	0.134
07	1104	α –Thujone	0.359
08	1115	β-Thujone	0.063
09	1141	Camphor	0.715
10	1163	Borneol	0.064
11	1166	Isomenthol	0.017
12	1173	Menthol	1.345
13	1175	Terpinen-4-ol	4.146
14	1181	*p*-Cymen-8-ol	0.132
15	1184	Naphtalene	0.107
16	1189	α –Terpineol	0.195
17	1200	Decanal	0.098
18	1229	Citronellol	0.258
19	1233	Methyl thymol	0.645
20	1237	Cumin aldehyde	0.065
21	1242	Carvone	0.415
22	1255	Geraniol	0.100
23	1287	*p*-Cymen-7-ol	3.132
24	1293	Thymol	2.125
25	1296	Methyl acetate	0.462
26	1301	Carvacrol	6.435
27	1327	Para-Mentha-1,4-dien-7-ol	0.062
28	1365	Decanoic acid	0.808
29	1374	α-Copaene	0.985
30	1388	β-Bourbonene	0.707
31	1405	Methyl eugenol	0.365
32	1416	(E)-Caryophyllene	1.242
33	1451	α-Humulene	1.443
34	1458	(E)-β-Farnesene	0.070
35	1475	ɣ-Muurolene	0.213
36	1480	Germacrene D	0.129
37	1486	(E)- β-Ionone	0.745
38	1493	Ledene	0.901
39	1499	α-Mnurolene	0.062
40	1506	β-Bisabobolene	0.723
41	1512	ɣ-Cadinene	0.093
42	1522	δ-Cadinene	0.822
43	1541	Calacorene	0.065
44	1548	Selina-3,7(11)-diene	0.061
45	1560	Dodecanoic acid	0.094
46	1580	Caryophyllene oxide	1.752
47	1590	**β-Copaen-4α-ol**	**38.413**
48	1598	Guaiol	0.028
50	1600	Ledol	0.707
51	1606	Humulene epoxide II	0.382
52	1616	1,10-di-epi-Cubenol	0.084
53	1626	10-épi-ɣ-Eudesmol	0.283
54	1630	1-épi-Cubenol	0.605
55	1634	(Z)-Nerolidol	0.504
56	1639	Epi-α-Cadinol	0.204
57	1640	Epi- α-Muurolol	0.123
58	1645	α-Muurolol	0.033
59	1648	β-Eudesmol	0.355
60	1650	α- Eudesmol	0.083
61	1653	α-Cadinol	0.671
62	1656	α-Bisabolol oxide B	0.754
63	1676	Hexyl salicylate	0.396
64	1696	(E,Z)-Farnesol	0.625
65	1702	Geranyl tiglate	0.310
66	1710	(Z,E)-Farnesol	0.462
67	1715	Erythro centaurin	0.716
68	1740	(E,E)-Farnesol	0.442
69	1802	Phytan	0.269
70	1835	Neophytadienne (III)	4.245
71	1905	Rimuene	0.254
72	2052	**Manool**	**8.200**
		Total	**91.89**

Continued. Table 2.

Note: [a] Kováts indices calculated on DB5 column with reference to n-alkanes injected after the oil at the same chromatographic conditions

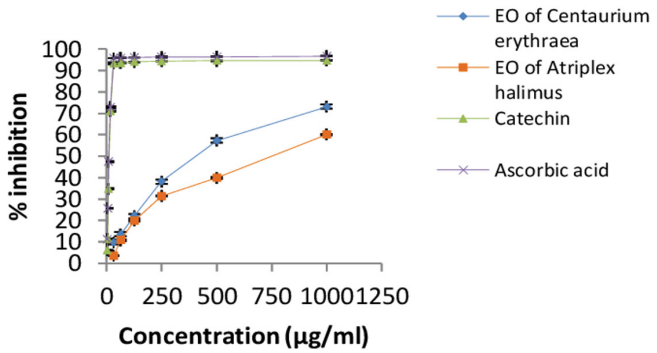

Figure 1. Free radical scavenging activity of *Atriplex halimus* L. and *Centaurium erythraea* Rafn essential oils and standard compounds

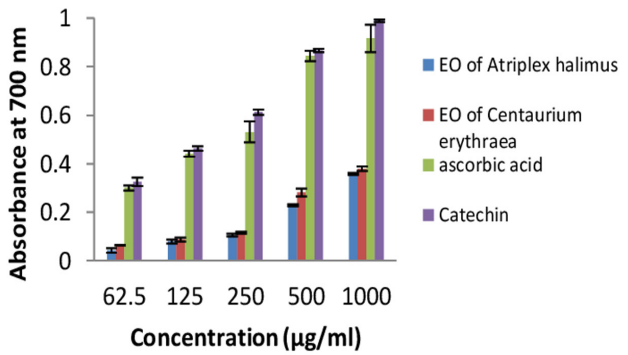

Figure 2. Ferric reducing power of *Atriplex halimus* L. and *Centaurium erythraea* Rafn essential oils and standard compounds

The antioxidant activity was related to the chemical composition of the essential oils. As it is known in the literature, the components that have a phenolic fraction (such as carvacrol and thymol) are those that give antioxidant activity to an essential oil (Amorati et Foti, 2017), due to their ability to stop or suspend the oxidation reaction through the presence of oxygen (Moosavi-Nasab et al., 2020). As shown in Table 1 and 2, the amount of thymol and carvacrol (0.25%, 0.14%, respectively) was low in the essential oil of *A. halimus* which had low antioxidant activity compared to the essential oil of *C. erythraea* (2.12%, 6.43%, respectively). The antioxidant activity may also be the result of other minor components or a synergistic effect among them (Bhatnagar, 2020).

Table 3. EC_{50} (µg mL^{-1}) of essential oils from *Atriplex halimus* L. and *Centaurium erythraea* Rafn in DPPH scavenging assay

Antioxidants	EC_{50} (µg mL^{-1})	Coefficient of correlation (R^2)
EO of *A. halimus*	754.97	0.92
EO of *C. erythraea*	546.61	0.91
Ascorbic acid	11.88	0.91
Catechin	14.28	0.91

Antimicrobial Activity

Disc Diffusion Method

The antimicrobial activity of *A. halimus* and *C. erythraea* essential oils was evaluated against Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and a fungal strain (*Candida albicans*), the results obtained are grouped in Table 4. The results show that *C. erythraea* essential oil has inhibitory activity on *E. coli* with an inhibition diameter of 10±1.41 mm, which is in accordance with the results of Jerković et al. (2012). Contrary to our results Bouyahya et al. (2019) indicated that there was no activity. The essential oil of *A. halimus* showed the highest antibacterial effect with an inhibition diameter of 13.5±2.12 mm, higher than the ATB tested.

Our results showed also that *Pseudomonas aeruginosa* was resistant to both essential oils. This result confirms that of the bibliography, which reports that this gram-negative bacteria is all the less sensitive to the action of essential oils (Barry-Ryan et Bourke, 2012). This negative response could be due to the hydrophilic outer membrane, rich in lipopolysaccharides, which prevents the penetration of essential oils (Khunkitti, 2010).

The results presented indicate that *C. erythraea* essential oil is active against *Staphylococcus aureus* with an inhibition diameter of 13± 2.82 mm which is higher than that of tetracycline (10±2.82 mm) and gentamicin (12±1.41 mm).

The antibacterial activity of this essential oil against this strain was confirmed in previous studies with inhibition diameters of 8±0.28 mm (Jerković et al., 2012) and 28±1.5 mm as reported by Bouyahya et al. (2019).

According to the literature, it is indicated that due to the direct interaction of cells with the hydrophobic compounds of essential oils, gram-positive bacteria are more sensitive to essential oils than gram-negative bacteria. These compounds have the potential to alter cytoplasmic membranes, to interfere with the cell energy production system (ATP), to perturb the proton motive force and to change the permeability of microbial cells which can cause cell death (Villalobos-Delgado et al., 2019).

On the contrary, *Staphylococcus aureus* was insensitive to *A. halimus* essential oil and *Enterococcus faecalis* was resistant to both essential oils. This resistance can be attributed either to the chemical composition of the essential oils tested poor in compounds known for their antibacterial power, or to the insufficient concentration of the active component.

The results indicated also that *Candida albicans* was resistant to the essential oil of *A. halimus* and sensitive to the essential oil of *C. erythraea* with the inhibition diameter of 18.5±3.53mm.

The antibacterial and antifungal activity of our essential oils against certain germs can be explained by the lipophilic characteristic of the monoterpenes present in them, which could be responsible for this action (Cristani et al., 2007), by partitioning the lipids from the bacterial cell membrane and mitochondria, causing a disruption of the cell structures, and making them more permeable, which leads to cell death (Prabuseenivasan et al., 2006).

Table 4. Diameter of inhibition zones of essential oils of *Atriplex halimus* L. and *Centaurium erythraea* Rafn. (expressed in mm, X ± σ)

Microorganism	Diameter of inhibition zone (mm)					
	EO of *A. halimus*	EO of *C. erythraea*	Antibiotics			
			Amp	TC	Gn	Cl
S. aureus		13 ± 2.82		10 ± 2.82	12 ± 1.41	nd
E. coli	13.5 ± 2.12	10 ± 1.41	9±1.41	11 ± 1.41	10 ± 1.41	nd
P. aeruginosa	-	-	-	-	7.5 ± 0.7	nd
E. faecalis	-	-	-	-	-	nd
C. albicans	-	**18.5 ± 3.53**	nd	nd	nd	0

Note: EO - essential oil; AMP – Ampicillin; TC – Tetracycline; Gn – Gentamicin; Cl – Clotrimazole; nd - not tested

Table 5. Results of CMI of tested essential oils (µL mL^{-1}) against the studied microbial strains

Strains	MIC				
Essential oil	*S. aureus*	*E. coli*	*P. aeruginosa*	*E. faecalis*	*C. albicans*
EO of *A. halimus*	-	25	-	-	-
EO of *C. erythraea*	25	50	-	-	12.5
Negative Control (DMSO)	-	-	-	-	-
Positive Control (ATB)	nd	Nd	nd	nd	nd

Note: ATB - antibiotics, nd: not tested, (-): the resistance

However, the activity of a complex mixture is not easily attributed to a unique or specific constituent. Major or minor compounds present in the essential oil may cause antimicrobial activity. Possible synergistic and antagonistic interactions between compounds in the oil should be considered (Lopes-Lutz et al., 2008).

Viridiflorol and phytol are the main components of *A. halimus* essential oil; their antimicrobial activity has been reported in previous studies (Pejin et al., 2014; Trevizan et al., 2016). According to Hamiche et al. (2019), manool, a labdane-type diterpene, which characterizes *C. erythraea* essential oil, has different biological activities, such as antibacterial activity. Other minor components present in our oils, such as 1,8-cineol, camphor, terpinen-4 ol, linalool, α-terpineol and borneol are oxygenated monoterpenes that have been signaled in other studies as having antimicrobial activity (Lopes-Lutz et al., 2008).

Minimum Inhibitory Concentration (MIC)

The MIC values of the essential oils tested are given in Table 5. These results reveal that *A. halimus* essential oil was effective against *E. coli* with a MIC value of 25 µl mL^{-1}. Thus, the essential oil of *C. erythraea* was also active against *S. aureus*, *E. coli* and *C. albicans* with MIC values ranging from 2.5 µl mL^{-1} to 50 µl mL^{-1}. *C. albicans* was the most susceptible germ tested with a lowest MIC.

Conclusion

This preliminary work confirmed the presence of antioxidant molecules in both essential oils. These results obtained *in vitro* are only a starting step in the search for active natural antioxidant and/or antimicrobial products. It would be important to isolate, identify and determine the chemical structure of the active compounds responsible for these effects as well as their mechanisms of action.

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