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CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVITIES ESSENTIAL OIL FROM THE NEEDLES OF NORTH AFRICAN *PINUS PINASTER* VAR.

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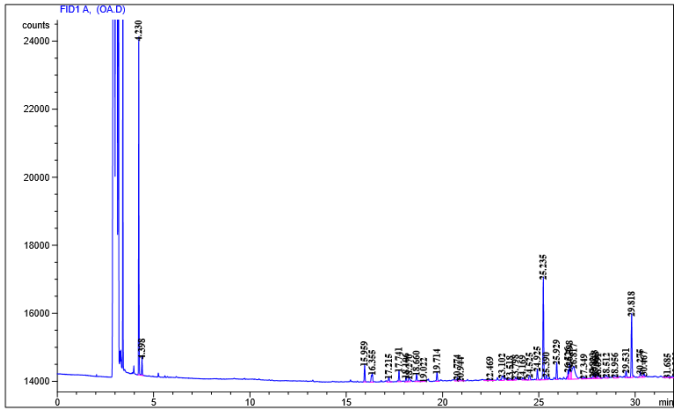
The essential oil from needles of *Pinus pinaster* two varieties, obtained by hydrodistillation, was analysed by GC–MS and GC–FID. Sesquiterpenes represented the main fraction. The major components of Renoui variety essential oil were *trans*-caryophyllene (14.66%), abietane (8.61%), Δ-cadinene (8.43%) and sclarene (8.25%), while, α-amorphene (10.72%), followed by *trans*-caryophyllene (10.74%), β-cadinene (9.73%) were the major constituents found in the essential oil from Maghrebiana variety. The highest antibacterial activity was found against *S. aureus* and *B. cereus*. Based on the two DPPH and ABTS tests, the investigated oils highlighted important in vitro antioxidant capacities.



INTRODUCTION

Essential oils and their components are very demanded by consumers; therefore, they have an interest in the food and pharmaceutical industries as natural antioxidants.[1] Pine oils are widely used as fragrances, flavoring, intermediates in the synthesis and beverages. Traditional therapeutic and pharmaceutical uses of its oils are recorded all around the world.[2] Pine needles essential oils are mainly used in folk medicine for the treatment of cardiovascular and cholesterol lowering benefits, respiratory infection, they have a powerful antioxidant and anti-inflammatory effects and the ability to enhance microcirculation by increasing capillary permeability.[3] Maritime pine (*Pinus pinaster* Aiton) was broadly distributed under Mediterranean climates of Europe, such as Portugal, Spain and Italy, as well as in some north western African countries such as Maroco and Tunisia.[4] In Tunisia, this species represented 68% of the national forests areas[5], the Renoui variety is native to the humid and sub-humid areas, mainly in the regions of Tabarka and Ain Drahem. In Morocco, Maghrebiana variety of *Pinus pinaster*, spontaneous in the humid and sub-humid bio climate, represents approximately 90% of the coniferous plantations of Rif and the oriental Middle Atlas.[6] Several studies have been carried out on composition of *Pinus pinaster* essential oils mentioned that these oils

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were dominated by monoterpene and sesquiterpene hydrocarbons or a combination of both them.

Instead, to our knowledge, this comparison of the essential oils of two varieties of *Pinus pinaster* from two different origin from North African area planted in experimental site of Souiniet (Tunisia: Ain Drahem) have never been described. Therefore, the objective of this study was to determine the phytochemistry of *P. pinaster* varieties endemic from North Africa as well as it biological activities.

MATERIALS AND METHODS

Plant material

Needles of *Pinus pinaster* of two varieties, the first one was from Tunisia (var. Renoui) while the second were from Marocco (var. Maghrebiana), were collected in May 2014 from Souiniet arboretum in Northwest of Tunisia (8° 48 E, 35° 54N, 492 m). The cultivated region is characterized by an annual temperature 15.6 °C and having a mean rainfall of 1534 mm/ year. The identification of the plant material was done by Professor Mohamed Larbi Khouja and a voucher specimen (PPR2014 and PPM2014, for Renoui and Maghrebiana, respectively) of the plant was deposited at the Herbarium of INRGREF (Tunisia).

Essential oil isolation and analysis

Needles (150 g) were separately grounded and submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The distilled oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis. The essential oil was analyzed as previously described.[7] Identification of constituents was made as elsewhere reported.[8] Component relative concentrations were calculated based on GC peak areas without using correction factors.

Antioxidant activity evaluation

To assess the antioxidant potential of bioactive compounds, the application of at least two different assays varying in their mechanisms of antioxidant action has been recommended.[9] The antioxidant capacity of the studied samples was determined applying the DPPH and ABTS assays.

DPPH assay. The DPPH radical scavenging capacity was measured according to Boukhris *et al.*[10] with some modification. Each experiment was analyzed in triplicate.

ABTS assay. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use.[11] The concentration of the test extract providing 50% inhibition (IC_{50}, expressed in $\mu g.mL^{-1}$) was calculated from the graph plotted with inhibition percentage against the extract concentration.

Microbial strains

The different essential oils of *P. pinaster* var. were tested for their antibacterial and antifungal activities against 10 indicators microorganisms including seven bacteria reference pathogenic (*Escherichia coli* ATCC 8739, *Salmonella typhimurium* NCTC 6017, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC27853, *Aeromonas hydrophila* EI, *Listeria monocytogenes* ATCC 7644, and *Bacillus cereus* ATCC 1247), and three fungi species (*Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* ATCC 2091).[12]

Disk diffusion assay

Antibacterial activity was evaluated using the method described by Choin *et al.*[13] The discs were impregnated with essential oil diluted in hexane. A disc soaked in hexane was used as negative control. We used the bacterial strains for the culture medium Muller-Hinton. At the end of the incubation, 24 hours at 37°C, the diameters of the zone of inhibition were measured.

Statistical analysis

All data were expressed as the average ± standard deviation of the measurements. Quantitative differences was assessed by Tukey's test (at $p < 0.05$) followed by Dunn's post–hoc multiple comparison test (SPSS. v15).

RESULTS AND DISCUSSION

The Chemical composition of essential oils

The yields extracted by hydrodistillation from the dried Needles were 0.02 ± 0.004% and 0.26 ± 0.03% for var. Renoui and var. Maghrebiana, respectively, which Marocco variety has the highest value. *Pinus pinaster* needles exhibited a

low yield of the essential oils when compared with Aleppo pine needles growing in Algeria with a yield of 0.52%.[14]

The chemical composition of the essential oil of needles was investigated using GC-FID and GC-MS apparatus. The chromatograms of *P. pinaster* of two varieties showed that the essential oils were a mixture of numerous compounds; some of them were present in trace amounts. One hundred and fifty components of the aromatic oil of var. Renoui were identified representing 95.91% of the total essential oil composition (Table 1). The major components of *P. pinaster* var. Renoui (Fig. 1) essential oil were trans-caryophyllene (14.66%), abietane (8.61%), Δ-cadinene (8.43%), sclarene (8.25%) and trypethelone (7.29 %). Other important compounds were identified included 3-penten-2-one (4.8%), germacrene D (4.63%), α-humulene (4.45%), isovaleric acid (3.97%), α-cubebene (3.4%), Vilgarol b (1.99%), α- pinene (1.99%), α-bisabolene (1.8%), α-amorphene (1.39%) and β-cubebene (1.3%). On the other hand, eighty-one components of the aromatic oil of var. Magrebiana were identified which 67.04% of the total essential oil composition. The major chemical constituents found in the essential oil from *P.pinaster* var. Maghrebiana needles are α-amorphene (10.72%), followed by trans-caryophyllene (10.74%), β-cadinene (9.73%), abietatriene (4.97%), α-humulene (3.53%), caryophyllene oxide (2.24%), Germacrene D (1.86%), phenethyl isovalerate (1.71%), α-calacorene (1.66%), guaiol (1.62%), muurolol(1.52%), α-cadinol (1.44%) and α-cadinene (0.99%).

Table 1

Chemical composition of essential oils of *Pinus pinaster* varieties

N°	Compounds	*P.pinaster* Var. Maghrebiana (%)	*P. pinaster* Var. Renoui (%)	RI	N°	Compounds	*P.pinaster* Var. Maghrebiana (%)	*P. pinaster* Var. Renoui (%)	RI
1	Santene	-	0.02	851	76	β-bourbonene	0.5	-	1594
2	α-pinene	0.21	1.99	931	77	salvial-4(14)-en-1-one	-	0.51	1596
3	Δ- limonene	-	0.03	944	78	guaiol	1.62	-	1601
4	verbenene	-	0.05	951	79	α-selinene	0.18	-	1604
5	β-pinene	0.02	0.22	972	80	geranyl isovalerate	-	0.27	1605
6	myrcene	0.04	-	990	81	3,4-dimethyl-3-cyclohexene-1-carboxaldehyde	0.51	-	1610
7	isoterpinolene	0.01	-	1014	82	globulol	0.45	-	1619
8	α-terpinene	-	0.83	1014	83	Fonenol	-	0.29	1620
9	o-cymene	0.04	0.14	1022	84	cadina-1,4-diene	0.66	-	1628
10	β-phellandrene	0.07	0.18	1026	85	α-elemene	0.42	-	1632
11	bicyclo[3.2.0]hept-6-ene	0.06	-	1067	86	aromadendrene	-	0.14	1634
12	α-terpinolene	0.25	0.55	1069	87	T-muurolol	1.52	-	1643
13	*o*-allyltoluene	-	0.1	1087	88	cedreanol	0.46	-	1647
14	linalool	0.08	0.06	1098	89	β-eudesmol	0.28	-	1652
15	α-campholenal	0.01	0.12	1123	90	α-cadinol	1.44	0.62	1658
16	terpinene-1-ol	-	0.02	1132	91	calacorene	0.47	-	1660
17	*trans*-pinocarveol	0.02	0.11	1137	92	1-deoxycapsidiol	-	0.11	1666
18	α-phellandren-8-ol	-	0.07	1146	93	caryophylla-3,8(13)-dien-5.beta.-o	0.52	-	1673
19	terpinene-4-ol	0.03	0.12	1157	94	γ-costol	-	0.36	1673
20	pinocarvone	-	0.04	1158	95	α-gurjunene	0.21	-	1679
21	borneol	0.03	-	1166	96	Aristol-9-en-3-ol	-	0.2	1686
22	2-methyl-cyclohexa-1,3-iene	-	0.12	1166	97	Isoaromadendrene epoxide	-	0.04	1696
23	Cuminol	-	0.04	1185	98	9,10-dehydro-isolongifolene	0.07	-	1702
24	α- terpineol	0.18	0.32	1189	99	8,9-Dehydro-Neoisolongifolene	-	0.03	1704
25	*trans*-isolimonene	-	0.19	1195	100	1-methoxymethyl-2-methylbenzene	0.08	-	1709
26	cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans-	0.06	-	1196	101	Isospathulenol	-	0.03	1714
27	homomyrtenol	-	0.04	1203	102	Farnesol	0.73	-	1719
28	verbenone	-	0.07	1206	103	Farnesyl alcohol	-	0.17	1719
29	3-cyclohexene-1-cetaldehyde, α,4-dimethyl-	-	0.06	1212	104	isoaromadendrene epoxide	0.11	0.04	1727
30	Δ-5,8-iridadiene	-	0.12	1217	105	caryophylla-2(12),6-dien-5-one	0.07	0.02	1734

Table 1 (continued)

31	*cis*-geraniol	-	0.03	1228	106	farnesal	0.12	-	1737
32	*cis*-3-hexenyl isovalerate	0.05	-	1230	107	Alloaromadendrene oxide	-	0.05	1742
33	*cis*-3-hexenyl valerate	-	0.21	1232	108	Calamenene	-	0.1	1758
34	hexyl isovalerate	0.02	-	1238	109	diepicedrene-1-oxide	0.06	-	1767
35	piperitone	0.01	0.03	1252	110	4-methyl-1,3-pentadiene	0.04	-	1770
36	geraniol	-	0.08	1255	111	5-Heptenal, 2,6-dimethyl-	-	0.04	1770
37	nerol	0.03	-	1258	112	valencen	0.05	-	1773
38	l-bornyl acetate	0.07	-	1281	113	Isoaromadendrene Epoxide	-	0.02	1775
39	l-bornyl acetate	-	0.05	1281	114	dehydroaromadendrene	-	0.25	1788
40	eudesma-4(14),11-diene	0.15	-	1325	115	15,16-dinorlabd-8(20)-en-13-one	-	0.02	1793
41	1,3-*p*-menthadiene	0.08	-	1330	116	methandrostenolone	-	0.08	1825
42	Δ-1,8-iridadiene	-	0.07	1332		lepidozenal	-	0.04	1797
43	aromadendrene	0.17	-	1333	117	neophytadiene	0.1	0.07	1831
44	germacrene B	-	0.11	1333	118	farnesyl acetate 3	0.43	0.06	1835
45	cyclosativene	-	0.02	1362	119	β-costol	-	0.29	1859
46	α-ylangene	0.33	0.66	1366	120	trans-phytol	0.04		1873
47	α -cubebene	2.23	3.4	1375	121	Benzalpinacolone	-	0.04	1879
48	α-copaene	0.09	0.36	1383	122	isopimara-7,15-diene	0.05		1896
49	longifolene	-	0.3	1401	123	1(2H)-Naphthalenone, 3,4-dihydro-3,3,6,8-tetramethyl-	-	0.06	1897
50	junipen	0.65	-	1402	124	Biformene	-	0.01	1907
51	*trans*-caryophyllene	10.74	14.66	1432	125	geranyl linalool isomer	0.03		1911
52	β-cubebene	0.64	1.3	1434	126	geranyl linalool	-	0.02	1912
53	α-guaiene	0.05	-	1438	127	α-Cedrenal	-	0.02	1917
54	isoledene	0.24	-	1443	128	pimara-8(9),15-diene	-	0.58	1929
55	α-humulene	3.53	4.45	1459	129	manool	0.01		1940
56	γ-gurjunene	-	0.11	1469	130	ent-pimara-8(14),15-diene	-	0.03	1941
57	α-amorphene	10.72	1.39	1484	131	γ-patchoulene	-	0.12	1948
58	Germacrene D	1.86	4.63	1488	132	α-bisabolene	-	0.19	1969
59	unkown	-	3.03	1493	133	cembrene	0.02	-	1983
60	phenethyl isovalerate	1.71	-	1495	134	epimanoyl oxide	0.17	0.29	1988
61	isovaleric acid	-	3.97	1500	135	sclaren	-	8.25	2033
62	β-cadinene	9.73	-	1509	136	alternariol monomethyl ether	-	1	2036
63	Δ-cadinene	-	8.43	1534	137	Pumiloxide	-	0.12	2049
64	α-cedrene	0.38	-	1538	138	abietatriene	4.97	-	2065
65	cadine-1,4-diene	-	0.3	1538	139	abietane	-	8.61	2077
66	α-cadinene	0.99	-	1541	140	trypethelone	-	7.29	2107
67	α-bisabolene	-	1.8	1547	141	3-penten-2-one	-	4.8	2127
	1-methyl[2.2]paracyclophan-1-en	0.13	-	1552	142	androst-5-ene, 4,4-dimethyl-, (13.alpha.)-	0.16	-	2235
68	myrtenal	-	0.32	1556	143	5.β-pregn-11-ene	-	0.38	2237
69	α-calacorene	1.66	-	1565	144	(12S)-15-16-Epidioxy-13-labdene-8,12-diol	-	0.22	2253
70	3-hexenyl benzoate	-	0.22	1573	145	7-oxoabieta-8,11,13-triene	0.22	-	2296
71	β-selinene	0.16	0.32	1576	146	abieta-8,11,13-trien-7-one	-	0.21	2297
72	camphene	-	0.47	1578	147	7-isopropyl-4-[2'-(4'-ethoxyphenyl)ethenyl]-1-methylazulene	0.09	-	2302
73	phenylethyl tiglate	-	0.19	1582	148	*trans*-trismethoxy Resveratrol	-	0.15	2308
74	caryophyllene oxide	2.24	0.09	1587	149	ferruginol	-	0.08	2327
75	vilgarol b	-	1.99	1589	150	calyculone	0.15	-	2334
					151	dehydroabietic acid	-	0.2	2338
					152	methyl abietate	0.04	0.03	2381

Notes: RI: retention indices calculated in regard to standards mixture of hydrocarbons (C_8-C_{28}) for 4.790-48.969 min. %: Percentage calculated by GC-FID on HP-INNOWax. RI: retention index.

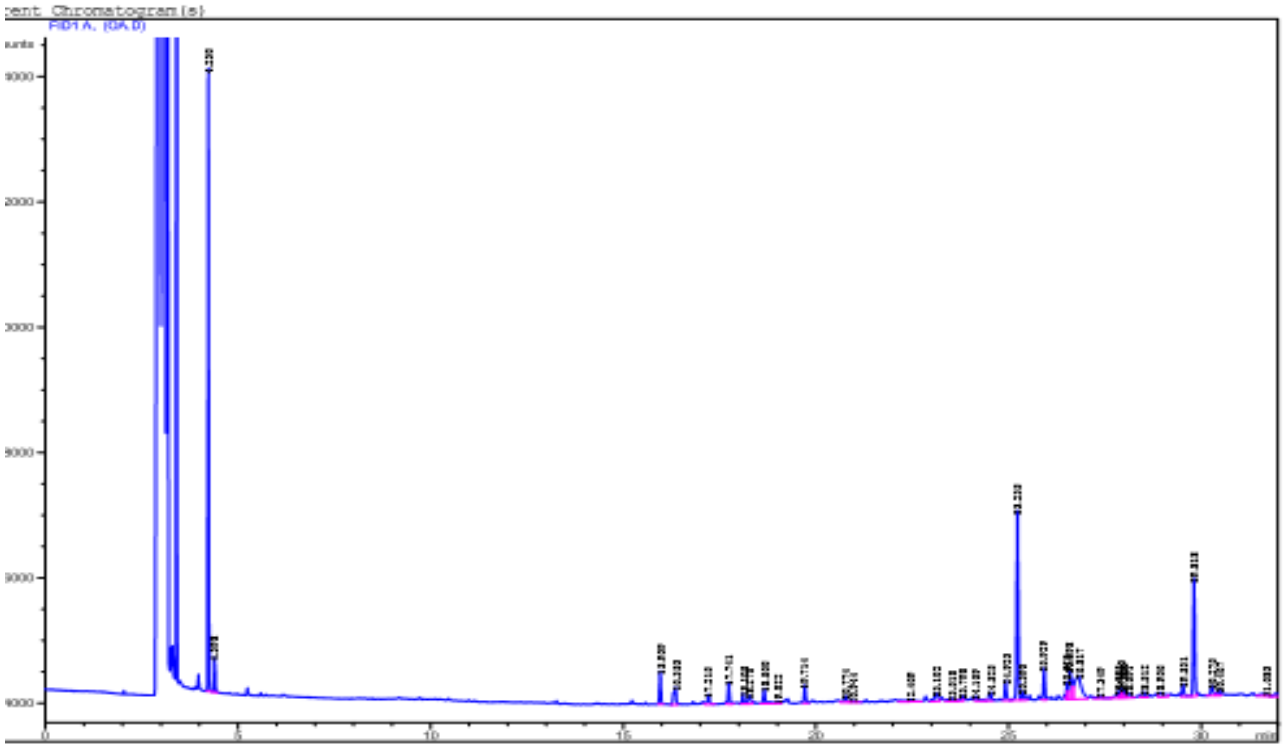

Fig. 1 – GC-FID chromatograms of essential oil of *Pinus pinaster* var. Renoui.

Volatile organic materials for two varieties are consisting of complex mixtures of mono-, sesqui-, di-hydrocarbons, and oxygenated materials biogenically derived from them. According to the literature, monoterpenes and sequitepenes are the components found to occur in higher quantities in essential oils from *Pinus* species[15] and *trans*-caryophyllene was identified as the major component in the essential oils of *P. pinaster*. These results were in accordance with previous studies in essential oil collected from north Africa [16,17] while, the needle essential oils of *P. pinaster* from Russia (99.8%), Portugal (97.5%), Argentina (83.2%), Italy (28.9% and 21.7%) and France (44.1% and 29.5%). It is worth noting that the essential oil of *P. pinaster* endemic of Morocco were rich in germacrene D (62.5%) and β-caryophyllene (22.2%).[18] These results were not in accordance with those reported by our research team who found that germacrene D were present only at 1.86% and 4.63 in *P. pinaster* var. Maghrébiana and *P. pinaster* var. Renoui, respectively. On the other hand, Amri *et al.*[19] mentioned that *P. pinaster* oil collected from the National Research Institute of Rural Engineering, Water, and Forests (INRGREF) arboretum (Northeast of Tunisia), was characterized by the high level of α-pinene (31.4%) followed by *trans*-caryophyllene (28%), and α-humulene (6.7%). It is well established that a literature survey showed many differences in the composition of *P. pinaster* essential oil. These variances could be because of the difference between ecotypes, and other environmental parameters.

Moreover, Macchioni *et al.*[20] showed that the major compounds of the *P. pinaster* essential oils of all the three plant parts (needles, branches and cones) were the pinenes, with total percentages more than 50%. In addition, longifolene was also found in important amounts in the essential oils of the branches and the female cones (7.7% and 20.8%, respectively) and decreased to 1.1% in the essential oil of the needles. As previously shown[21], the main components of the essential oil of *P. pinaster* needles were α-pinene, β-pinene, caryophyllene and germacrene D. On the other hand, others studies[22,23] reported high amounts of longifolene, followed by α-pinene. It was noted that the composition of the essential oil varies with climate, season location, soil type, age of plants and leaves, and the method used for drying and extraction.

Determination of Antioxidant activity

The results of the antioxidant activities were determined by two tests namely: DPPH radical scavenging and ABTS radical cation. The two essential oil samples showed antioxidant activity, but

significantly, statistic differences were only observed when they were compared by two varieties. As it was shown in Table 2, Renoui variety oil showed the highest antioxidant activity (IC_{50}= 42 ± 1.31 μg.mL^{-1}) when compared to Magrebiana variety (IC_{50}= 56 ± 1.04 μg.mL^{-1}). ABTS test confirm result obtained by DPPH one which Magrebiana Variety exhibited the lowest level 65 ± 1.2 μg.mL^{-1}. Antioxidant activities of essential oils from medicinal plants are mainly attributed to the active compounds.[24] This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them.[25] In this study, the antioxidant activities were related to the contents of essential oils of *Pinus pinaster* var. Renoui: trans-caryophyllene (14.66%), abietane (8.61%), Δ-cadinene (8.43%), sclaren (8.25%) and trypethelone (7.29 %) and to the presence in high amount of α-amorphene (10.72%), trans-caryophyllene (10.74%), β-cadinene (9.73%) for *Pinus pinaster* var. Magrebiana essential oil. The difference of antioxidant essential oil between two *P. pinaster* varieties may be related to the genotype and geographic origin. The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts.[26] It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods. Our results are in agreement with it was reported by Yongjun *et al.*[27] which showed an important *in vitro* antioxidant activity of the essential oils of *Pinus armandii* cones.[28]

Determination of Antimicrobial activity

Table 3 summarized the qualitative (diameters of inhibition zones) of the *in vitro* antimicrobial effect of the essential oil of *Pinus pinaster* varieties on the ten microbial strains. The diameters of inhibitory zones recorded in millimeter. It was observed that the oil was efficient against some tested microbial strains. The size of all inhibition zones was between 6 to 20 mm. Our results showed an antibacterial activity against *S. aureus*. and *B. cereus* of essential oil of two *P. pinaster* varieties. The same result is found[16] in the essential oil of *P. pinaster* from Algeria. Thus Gram-positive found a sensibility against the essential oil of *P. pinaster* while the Gram-negative strains of bacteria were more resistant as it was reported by Lambertn *et al*.[29] and Wilkinson *et al*.[30]

The essential oils of *Pinus pinaster* did not inhibit the growth *Candida Albicans*, *Aspergillus flavous* and *Aspergillus niger*. Antimicrobial activity of essential oils might be attributed to variation in chemical compounds. In the same context, our result mentioned that antifungal activity of *Pinus* essential oil can be attributed to the higher amount of α-pinene, as well the oil from *Pistacia lentiscus*.[31]

CONCLUSION

Our finding revealed significant variations in the yields, chemical content and antioxidant activity of essential oils between *P. pinaster* varieties endemic of North Africa growing in North West of Tunisia. No significant antimicrobial potentials difference was found between the two studied samples. The difference of chemical composition and biological activities of the two *P. pinaster* varieties may be related to the genotype and geographic origin. Finally, this study suggests that *P. pinaster* needles essential oil of North African area will be a good resource as natural antioxidant and an antibacterial agent.

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Table 2

Antioxidant activities of *Pinus pinaster* varieties essential oils

	DPPH (μg.mL^{-1})	ABTS (μg.mL^{-1})
Pinus pinaster var Magrebiana	**56±1.04** [a]	**65±1.2**[i]
Pinus pinaster var Renoui	**42±1.31**[b]	**52±1.45**[ii]

Results are expressed as mean ± standard deviation of 3 determinations. Means with different symbols and letters were significantly different at $p< 0.05$.

Table 3

Antimicrobial activity of *P. pinaster* essential oils (growth inhibition zones in mm)

		ID (mm)	
		***Pinus pinaster* var. Magrebiana**	***Pinus pinaster* var. Renoui**
Gram-negative Bacteria	*E. coli*	8	10
	S. typhimurium	7	8
	A. hydrophila	6	5
	P. aeruginosa	9	7
Gram-positive Bacteria	*S. aureus*	18	20
	L. monocytogenes	6	8
	B. cereus	10	13
Fungus	*Asp. Flavus*	-	-
	Asp. niger	-	-
	C. albicans	-	-

Note: No activity found.

REFERENCES

1. R. A. Mothana, M. S. Alsaidn, S. S. Hasoon, N. M. Al-Mosaiyb, A. J Al-Rehaily and M. A. Al-Yahya, *J. Med. Plants. Res*., **2012**, *6*, 3066.
2. P. Schauenberg and F. Paris, *Neuchatel.*, **1977**, 386.
3. S. Iravani and B. Zolfaghari, *Res. Pharm. Sci*., **2011**, *6*, 1.
4. J. S.Carrión, C. Navarro, J. Navarro and M. Munuera, *Holocene.*, **2000**, *10*, 243.
5. DGF, General Directorate of Forestry. Results of the first national forest inventory Tunisia. *Ministry of Agriculture.*, **1995**, 87.
6. B. Belghazi and M. Ezzahiri, *Ann. For. Sci*. (Rabat)., **1995**, *28*, 17.
7. M. Boukhris, M. Bouaziz, I. Feki, H. Jemai, A. El Feki and S. Sayadi, *Lip. Heal. Dis*., **2012**, *11*, 81.
8. J. Valente, M. Zuzarte, R. Resende, M. J. Goncalves, C. Cavaleiro, C. F. Pereira, M. T. Cruz and L. Salgueiro, *Ind. Crop. Prod*., **2015**, *65*, 361.
9. G. Rigane, R. Ben Salem, S. Sayadi and M. Bouaziz, *J. Food. Sci*., **2011**, *76*, 965.
10. G. Rigane, S. Ben Younes, H. Ghazghazi and R. Ben Salem, *Int. Food. Res. J*., **2013**, *20*, 3001.
11. N. R. Re, Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. A. Rice-Evans, *F. Ra. Biol. Med.*, **1999**, *26*, 1231.
12. C. Trakhna, A. Harf-Monteil, A. Abdel-Nour, Maaroufin and P. Gadonna-Widehem, *Lett. Appl. Microbiol.*, **2009**, *49*, 186.
13. Y. M. Choin, D. O. Noh, S. Y. Cho, H. J. Suh and J. M. Kim, *Food. Sci. Tech*., **2006**, *39*, 756.
14. I. Touihri, M. Boukhris, N. Marrakchi, J. Louisn, B. Hanchi and O. Kallech-Ziri, *J. Olea. Sci*., **2015**, *64*, 869.
15. I. Zafar, A. Fatima, S. J. Khan, Z. Rehman and S. Mehmud, *Pak. J. Biochem. Mol. Biol*., **2011**, *44*, 36.
16. A. T. Nouara, A. M. Djouher and Y. Aziza, *J. Coast. Life. Med*., **2013**, *1*, 54.
17. T. Dob, T. Berramdane and C. Chelghoum, *Chem. Nat. Compd*., **2005**, *4*, 545.
18. M. Hmamouchi, J. Hamamouchi, M. Zouhdi and J. M. Bessière, *J. Essent. Oil. Res*., **2001**, *13*, 298.
19. I. Amri, M. Hanana, S. Gargouri, B. Jamoussi and L. Hamrouni, *Chil. J. Agr. Res*., **2013**, 73.
20. F. Macchioni, P. L. Cioni, G. Flamini, G. I. Morelli, S. Maccioni and M. Ansaldi, *Fla. Frag.*, **2003**, *18*, 139.
21. P. V. Petrakis, C. Tsitsimpikou and O. Tzakou, *Flav. Fra. J*, **2001**, *16*, 249.
22. M. Valero and E. Frances, *Food. Microbiol*, **2006**, *23*, 68.
23. D. R. Batish, H. P. Singh, R. K. Kohli and S. Kaur, *For. Ecol. Manag*, **2008**, *256*, 2166.
24. S. Burt, *Food. Microbial.*, **2004**, *94*, 223.
25. S. Y. Wang, C. T. Chen, W. Sciarappa, C. Y. Wang and M. J. Camp, *Agric. Food. Chem.*, **2008**, *56*, 5788.
26. L. C. Mao, X. Pan, F. Que and X. H. Fang, *Europ. Food. Res. Tech.*, **2006**, *222*, 236.
27. T. Yongjun, H. Kidokoro, T. Watanabe, Y. Igeta, H. Sakaji and S. Ino, *J. Mar. Sys.*, **2012**, *19*, 1.
28. X. Yang, H. T. Zhao, J. Wang, Q. Meng, H. Zhang, L. Yao, Y. C. Zhang, A. J. Dong, Y. Man, Z. Wang, D. C. Xu and Y. Ding, *J. Med. Plants. Res.*, **2010**, *4*, 1668.
29. R. L. Lambertn, P. N. Skandamis, P. J. Coote and G. J. Nychas, K. G. Lee, *Shibamoto.,* **1983**, *74*, 443.
30. J. M. Wilkinson, M. Hipell, T. Ryan and H. M.Cavanagh, *Agric. Food. Chim*, **2003**, *51*, 76.
31. M. Krauze-Baranowsk, M. Mardarowicz, M. Wiwart, L. Poblocka and M. Dynowska, Z *Naturforsh* C., **2002**, *57*, 478.