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Volatiles Profiling and Antioxidant Activity of Moroccan *Artemisia Ifranensis* J. Didier and *Anacyclus Pyrethrum* Link Essential Oils

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Abstract

Moroccan indigenous and spontaneous medicinal plants have been widely used to treatment of a number of diseases. Volatile compounds mixtures isolated from leaves and roots respectively of *Artemisia ifranensis* and *Anacyclus pyrethrum* species, by hydrodistillation, were analyzed by (GC/FID) and (GC/MS). Oils chemical profile of plant species showed the presence of major compounds among the identified constituents, this is respectively β-thujone (41.12 %) and spathulenol (16.9 %). Essential oils studied are screened also for their, *in vitro,* antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The noted results reported that *Artemisia ifranensis* essential oil exhibited promising antioxidant profile, and revealed an antioxidant property more important (IC_{50} = 5.9 mg/ml) than that of ascorbic acid used in food and pharmaceutical industries (IC_{50} = 8.09 mg/ml). This essence can be considered as a valuable source of natural antioxidants. Hence, oil of *A. pyrethrum* has a low efficient antioxidant potency (IC_{50} values 30.05 mg/ml), compared to the tested samples.

The results supported the traditional usage and also possible use of plants volatile oils in combination with other preservatives to protect food materials against a variety of oxidative systems, in pharmaceutical and cosmetic industries.

Keywords: *Artemisia ifranensis J.Didier; Anacyclus pyrethrum Link;* Essential oil; Chemical composition; DPPH$^•$; Antioxidant activity.

1. Introduction

Environmental protection has become an increasingly important global concern in recent decades. Several questions have arisen regarding the efficacy and safety, on human health, of chemicals used in medicine or in the food industry.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in cells by different means [1]. Oxidative stress by free radicals is an important event in cell that can cause aging and degenerative diseases; may cause DNA damage that could lead to mutation and lipid peroxidation in foods that leads to their deterioration. All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damage. However, these natural antioxidant mechanisms can be inefficient, and hence dietary intake of antioxidant compounds becomes important.

Aromatic plants, especially essential oils (EOs), have been used for millennia for their health benefits, well documented in ancient literature [1]. On the other hand, several EOs have been attributed good antioxidant properties. Therefore, the search for natural antioxidants with the virtue of being nontoxic has given rise to a large number of studies on the antioxidant potential of EOs.

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The genus *Artemisia* is one of the largest and most widely distributed genera of the Asteraceae family [2]. Members of this genus have botanical and pharmaceutical interest due to their characteristic scent or taste and are used in the liqueur-making industry [1]. In Morocco, about 12 species have been reported of the *Artemisia* genus [3]. *Artemisia ifranensis*, endemic specie of Morocco, grows naturally in Middle Atlas at an altitude of 1600 to 2100 m [4,5]. Locally known as 'chih', 'izri', and 'ifsi', this specie is regarded as an antiseptic intestinal tonic, stomachic, spasmodic and diuretic. The plant is highly effective against some pathogens thus confirming its use as antirabies [5].

The genus *Anacyclus* (Asteraceae) includes 13 annual and perennial species mainly concentrated in the northwest of Africa [6]. *Anacyclus pyrethrum* is among the plant species, endemic and spontaneous of Morocco, highly valued for the medicinal properties of its roots [5,7]. Locally referred to as Aud el-attas, Akkar Karha, Igendass, and Tigendaste, it is found in the Middle Atlas, High Atlas and Highlands of Eastern Morocco (Jerada region) at altitudes between 1000 and 2500 m and in the Rif to the Chaouène region in the Jebl Assilenh, and the Tizi-n-Lel valley [7]. Previous chemical studies showed that *A. pyrethrum* has immunostimuling, anti-inflammatory, antioxidant, anabolic, and aphrodisiac properties [8]. It has appreciated as a tonic nervous system (paralysis and epilepsy) [9], and it is used for its insecticidal properties [10].

The present study deals with the chemical diversity of their constituents and its influences on the oxidative stability of species EOs, highlighting their potential antioxidant properties.

2. Materials and methods

2.1. Plant material

The leaves and roots parts of Wild *Artemisia ifranensis* and *Anacyclus pyrethrum*, respectively, were collected during June, from the Timahdite region (33° 14' 13" N; 5° 03' 36" W at 1800 m altitude) that is located in the Moroccan Middle Atlas with a semi-arid climate and characterized by an average rainfall of 695 mm/year. Afterward, they were dried in the shade (at room temperature). A botanical identification of the species was carried out by Professor Mohamed Ibn Tattou of the National Herbarium at the Rabat Scientific Institute (Morocco). Voucher specimens have been deposited in the herbarium of the same Institute.

2.2. Essential oils isolation

In this section, the materials evaluated for their antioxidant activity consist of the essential oils derived from the leaves and roots respectively of *A. ifranensis* and *A. pyrethrum.* The essential oils were extracted by hydrodistillation of 100g leaves and roots of studies species during three hours with a Clevenger-type apparatus. Then, the oils were dried using anhydrous sodium sulfate [11], and stored in dark at 4°C until use.

2.3. Analyses and identification of essential oils chemical composition

2.3.1. Analysis of essential oils constituents by GC-FID and GC-MS

Chromatographic analyses of EOs samples were performed with gas chromatograph Thermo Electron type (Trace GC Ultra) equipped with a column DB-5 (5% phenyl-methyl-siloxane) (30m x 0.25 mm x 0.25 microns film thickness), a FID detector set at 250°C and fed with a gas mixture H2/air. The device was equipped with an injector PVT (Programmed Temperature Vaporization) of split-splitless type. The mode of injection is split (ratio: 1/50, debit: 66 ml/min); the carrier gas used is nitrogen with a flow rate of 1 ml/min. The column temperature is programmed at a rate of 4 mounted °C/min from 50 to 200°C for 5min. GC-MS was carried out on chromatograph Thermo Electron type (Trace GC Ultra) coupled to a mass spectrometer Thermo Electron Trace MS system (Thermo Electron: Trace GC ultra; Polaris Q MS). Fragmentation is performed by electron impact at 70eV. Electron ionization mass spectra were acquired over the mass range 50–350 m/z. The used column was a column DB-5MS (5% phenyl-methyl-siloxane) (30m x 0.25 mm x 0.25 microns film thickness). The column temperature is programmed at a rate of 4 mounted °C/min from 50 to 200°C for 5min. The carrier gas is helium with a flow rate set at 1.5ml/min. The injection mode is split type (ratio: 1/70, flow: ml min^{-1}).

2.3.2. Identification of essential oils constituents

Determination of chemical composition of *A. ifranensis* and *A. pyrethrum* oils has been performed based on the comparison of their retention indices (RI). Theses indices were calculated based on the relation between the compounds and linear alkanes (C_7-C_{40}) injected in the experimental condition and compared to those in the literature [12,13]. The mass spectra were also compared to different references as Adams [13], Nist library (1998).

2.4. Evaluation of antioxidant activity by DPPH˙ free radical scavenging method

The experiment was carried out using the spectrophotometric quantification method cited above. The capacity of prepared extracts to scavenge the 'stable' free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored using a published DPPH˙ radical scavenging activity assay method [14,15] with some slight modifications. The solution of DPPH˙ (1,1-diphenyl-di-picrylhydrazyl) at 6.10^{-5} M is obtained by dissolving 2,4 mg of the powder in 100 ml of ethanol. The samples to be tested were prepared by dissolution in ethanol at a rate of 20 µl/ml. The test is performed by mixing a volume of 2.8 ml of the previous solution of DPPH˙ with 200 µl of essential oil of our samples as well as Ascorbic acid at different final concentrations (0 to 200 µg/ml). The mixtures were well shaken and kept at room temperature in the dark for 30 minutes. The absorbance is read at 515 nm against a blank that contains only ethanol. Ascorbic acid was used as positive controls (Reference). The anti-radical activity is expressed as the percentage of reduction or inhibition (I %) of free radical DPPH˙ according to the following equation [16,17] :

$$I\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

I (%): Percentage of inhibition.
Abs control: Absorbance of the solution containing only the solution of the radical DPPH˙.
Abs sample: Absorbance of the solution of the samples EO to be tested in the presence of DPPH˙.

The IC_{50} values (EO concentration providing 50% inhibition) were determined graphically from the polynomial trend curves of 3rd degree plotted, representing the inhibition percentages as a function of the different doses of the tested EOs. IC_{50} values are expressed in mg/ml, which is inversely related to antioxidant capacity [18,19].

2.5 Statistical analysis

The experimental data obtained were expressed as an average. The statistical analysis of the data, including the correlation coefficient of the antioxidant properties, was carried out using Excel software (Microsoft Office 2010). Values expressed as means ± standard deviation were used to compare averages of essential oils yield among samples.

3. Results and Discussions

3.1. Yields and chemical composition of essential oils

Yields of *A. ifranensis* and *A. pyrethrum* EOs obtained during harvest period (June) have been calculated from dry plant material. The yield of *Artemisia ifranensis* (1.11 ± 0.3 %) is higher than that of *A. pyrethrum* (0.07 ± 0.1 %). However, this rate of wormwood EO seem to be higher than those obtained from Moroccan *Artemisia sp.* such as *A. herba alba*, *A. negrei* and *A. mesatlantica* (0.43, 0.38 and 0.36% respectively) [33], and those obtained in other studies on *Artemisia* species [20,21,22]. On the other side, our results are lower than those of *A. herba alba* collected in 2012 from different altitudes (southwest of Tunisia), were obtained a yields (2.70-2.80 %) [23]. In addition, yield of *Anayclus pyrethrum*'s EO is relatively higher than that obtained in Algeria by Selles *et al.*, (2013) (0.019 %) [24]. Likewise, this difference in yields can be explained by the species' nature, the effect of vegetative stage and regional soil conditions.

Chemical composition of theses EOs studied, determined by chromatography-flame ionization detector (GC/FID) and GC-mass spectrometry (GC/MS) analysis, has shown quantitative and qualitative changes (Figure 1, Table 1). The results obtained allowed identifying a total of forty six and thirty six compounds, representing respectively 84.6 and 91.82 %, in *A. ifranensis* and *A. pyrethrum* samples. The EOs chemical examination shows that *A. ifranensis* leaves contain oxygenated monoterpenes and sesquiterpenes, the abundant groups among identified compounds, their level attained respectively 45.7 and 31.1 %. Thus, oxygenated sesquiterpenes (90.58 %) was the most abundant in pyrethrum EO. On the other hand, β-thujone (41.12 %) is the major compound of *A. ifranensis* EO followed at small levels by components namely; α- bisabolone oxide A, and (14.54 %) eremoligenol (4.44 %). Therefore, the spathulenol (16.9 %), was main constituent in *A. pyrethrum* volatile extract. Other components were also relatively identified at low percentages: germacra-4(15),5,10(14)-trien-1-α-ol (12.89 %), Selina-3,11-dien-6α-ol (9.24 %) and caryophylene oxide (7.11 %) (Table1).

EO of *A. ifranansis* mainly composed of *β*-thujone, can be classified as a *β*-thujone-chemotype. In fact, the chemical composition of the studied oils agrees with that reported by some researches previously conducted. *β*-thujone is also the main constituent of *A. mesatlantica* (56.3 %) and *A. arborescens* (68.5 %) [25,20]. However, the chemical composition of *A. herba alba* EOs from eastern Morocco (Guercif), Tunisia and Algeria was totally different than that of our sample, which chrysanthenone (48.4%), *α*-thujone (24.8 %) and *α*-Chrysanthenyl acetate (25.1%) are the major compounds respectively [26, 27]. Moreover, camphor is the major compound of *A. negrie* from Morocco (55%) [28]. EOs of *A. vulgaris* from Lithuania and Iran are mainly dominated respectively by Germacrene-D (15.1 %) and Isobornyl isobutyrate (38.0 %) [29,30].

In *A. pyrethrum* essential oil, spathulenol is the most important compound (Table 1). Thus, the plant EO can be classified as spathulenol chemotype. However, in other studies the results are quite different. *Anacyclus pyrethrum* EO from Algeria is dominated by germacrene-D and defined by the germacrene-D chemotype [31,24]. The study performed by Zardi-Bergaoui et *al*., 2008, reported that the *Anacyclus cyrtolepidioïdes* species, gathered in the south of Tunisia, is dominated by *α*-pinene [32]. Moreover, *A. clavatus species* in Tunisia, contain *Trans*-chrysanthenyl acetate (12.3 %) as major constituent [33].

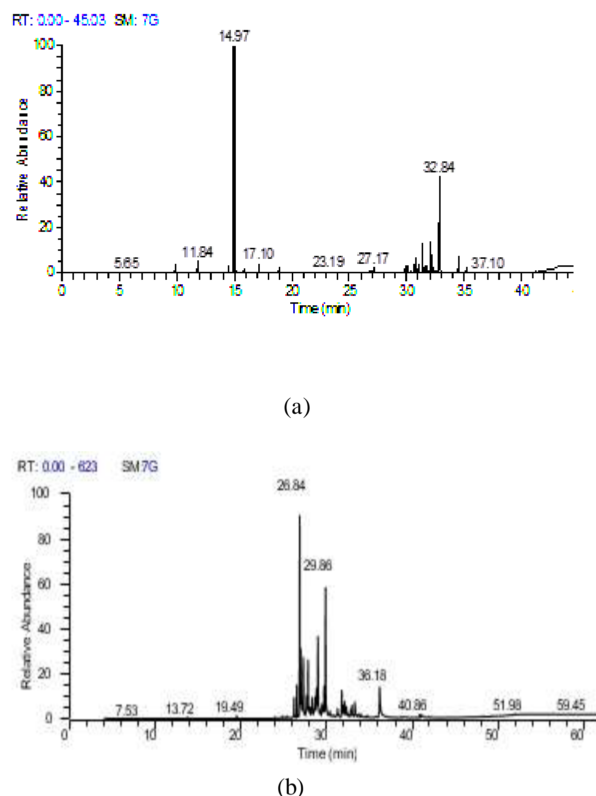


Fig.1. GC-FID and GC-MS chromatograms of *A. ifranensis* (a) and *A. pyrethrum* (b) essential oils.

Table1. Chemical composition of *A. ifranensis* and *A. pyrethrum* essential oils.

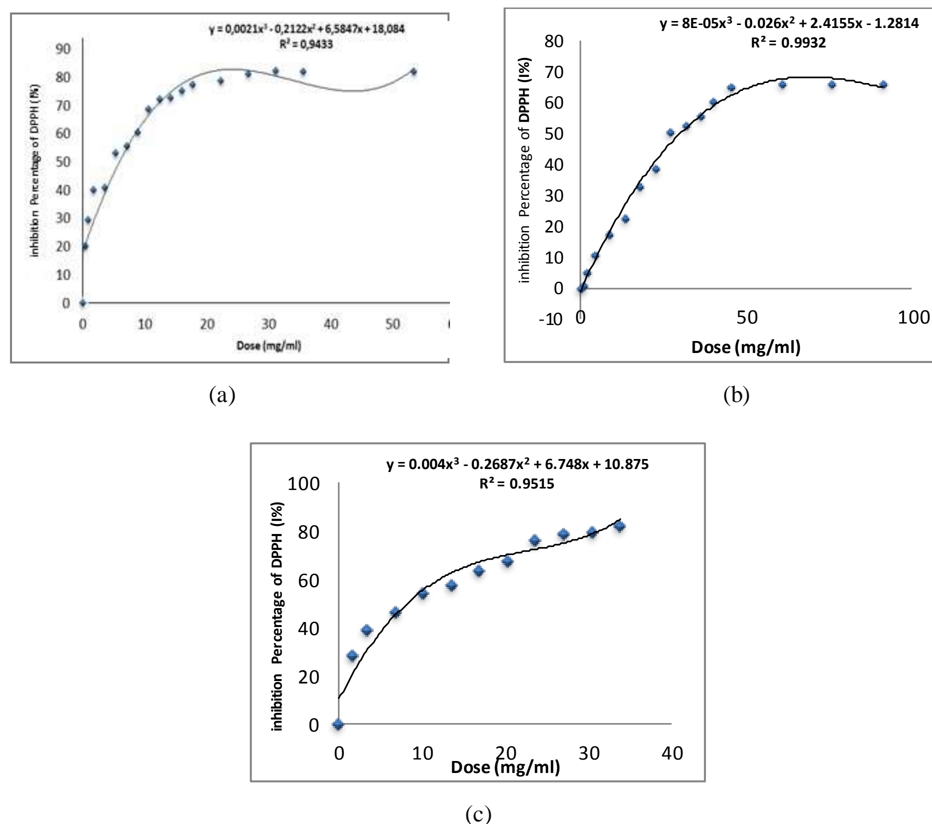
NP	*Artemisia ifranensis*				*Anacyclus pyrethrum*			
	Compounds [a]	**RI$_c$**	**RI$_l$**	**Area (%)**	**Compounds [a]**	**RI$_c$**	**RI$_l$**	**Area (%)**
1	*α*-Pinene	931	939	0.1	α-Neocallitropsene	1470	1476	0.20
2	*β*-Pinene	971	979	1.3	Germacrene D	1479	1481	-
3	Cymene <*ρ*>	1023	1024	0.5	Trans-β-Ionone	1486	1488	-
4	Cineol <1,8>	1030	1031	1.6	Cubebol	1514	1515	0.15
5	Terpinene <*γ*>	1058	1059	0.2	δ-Cadinene	1522	1523	-
6	Sabinene hydtrate <*cis*>	1065	1070	-	Ar-Macrocarpene	1524	1526	0.40
7	Linalool oxide<*cis*>	1074	1072	0.1	Italicence epoxide	1549	1548	-
8	Trans-sabinene hydtrate	1096	1098	0.1	occidentalol	1553	1552	2 .20
9	Thujone <*cis*>	1102	1102	1.1	1α,10α-Epoxy-amorph-4-ene	1568	1572	2.83
10	**Thujone <*trans*>[b]**	**1115**	**1114**	**41.1**	**Spathulenol[b]**	**1577**	**1578**	**16.90**
11	Fenchol <*endo*>	1120	1116	0.3	**Caryphylene oxide[b]**	**1580**	**1583**	**7.11**
12	Thujanol <*iso-3*>	1133	1138	0.1	β-copaen-4-α-ol	1588	1590	-
13	Sabinol<*trans*>	1139	1142	0.8	Salvial-4(14)-en-1-one	1591	1594	4.66
14	Thujanol<*neo-3*>	1149	1153	0.1	Mayurone < cis – dihydro>	1597	1595	-
15	Terpinen-4-ol	1176	1177	1.4	β-Atlantol	1606	1608	2.90
16	Terpineol<*α*>	1190	1188	0.1	β-Biotol	1611	1613	5.16
17	Myrtenol	1196	1195	0.2	E-Isoeugenol acetate	1619	1615	0.36
18	Dihydrocarveol< *iso*>	1214	1214	-	Trans-Isolongifolanone	1623	1626	0.45
19	Fragranol	1216	1215	0.1	Muurola-4,10(14)-dien-1-β-ol	1628	1631	1.81
20	Dihydrocarveol<*neo iso*>	1228	1228	1.0	β-Acorenol	1635	1637	2.58
21	Sabinyl acetate<*trans*>	1293	1290	0.1	Caryophylla-4(18), 8(13)-dien-5α-ol	1640	1640	-
22	Dihydrocarveol acetate<*neo iso*>	1357	1359	0.2	3-iso-Tujopsanone	1638	1642	1.54

PN	Compound	RIc	RIl	%	Compound	RIc	RIl	%
23	Caryophylene<*E*>	1419	1419	0.1	**Selina-3,11-dien-6α-ol[b]**	**1646**	**1644**	**9.24**
24	Ionone<isomethyl-*α (E)*>	1473	1479	0.7	cis-guai-3.9-dien-11-ol	1647	1649	-
25	Germacrene-D	1481	1481	0.2	Cedr-8(15)-en-9-α-ol	1650	1651	-
26	Ionone<methyl-*γ*>	1482	1481	0.8	Himachalol	1651	1653	-
27	Cubebol<*epi*>	1494	1494	0.1	3-Thujopsanone	1661	1654	0.42
28	Cubebol	1515	1515	0.1	E-Caryophylene-14-hydroxy-9-epi	1670	1669	-
29	Cubebol<10-*epi*>	1533	1535	0.1	Z-α-Santalol	1672	1675	1.86
30	Italicene epoxide	1550	1548	-	Khusinol	1675	1680	3.29
31	Patchouli alcool	1562	1558	t	**Germacra-4(15), 5, 10(14)-trien-1-α-ol[b]**	**1682**	**1686**	**12.89**
32	Palustrol	1567	1568	0.2	Eudesma-4(15) ,7-diene-1-β-ol	1685	1688	-
33	Dendrolasin	1572	1571	-	Nootkatol<epi>	1698	1699	-
34	Spathulenol	1578	1578	1.0	Eudesm−7(11)−en−4−ol	1699	1700	-
35	Caryophyllene oxide	1582	1583	1.2	Amorpha-4,9-dien-2-ol	1704	1700	0.12
36	Salvial-4(14)-en-1-one	1593	1594	0.2	γ-Gurjunenepoxide	1706	1704	0.62
37	Guaiol	1603	1600	1.2	(+)-Trans-Nootkatol	1717	1715	0.28
38	Cedrol<*epi*>	1618	1619	1.6	Vetiselinenol	1734	1731	-
39	**Eremoligenol[b]**	**1630**	**1631**	**4.4**	Isobicyclogermacrenal	1737	1734	1.41
40	Caryophylla-4(12),8(13)-dien-5*β*-ol	1640	1640	-	Khusimol	1746	1742	2.88
41	Hinesol	1642	1641	1.4	β-acoradienol	1762	1763	1.58
42	Khusilol	1650	1648	0.1	Cedryl acetate[b]	1763	1767	-
43	*α*-Cadinol	1652	1654	2.1	14-oxy-α-Muurolenee	1769	1768	1.49
44	Patchouli alcohol	1562	1656	t	Amorpha-4,11-diene<2-α-hydroxy>	1777	1775	-
45	Bisabolol oxide B<*α*>	1656	1658	-	14- hydroxy-α-muurolene	1780	1780	0.86
46	Isobornyl isobutanoate<*5-hydroxy*>	1661	1663	2.7	Hinesol acetate	1783	1784	-
47	Valeranone	1673	1675	0.3	8-Cedren-13-ol acetate	1790	1788	0.29
48	Guaia-3,10(14)-dien-11-ol	1674	1677	-	Isovalencenol	1791	1793	0.14
49	**Bisabolone oxide A<*α*>[b]**	**1684**	**1685**	**14.5**	α-Eudesmol acetate	1797	1795	0.68
50	Curcumen-12-ol<*γ-(z)*>	1730	1729	0.1	14-Hydroxy-δ-cadinene	1802	1803	1.26
51	Cedr-8(15)-en-9- *α* -ol acetate	1737	1742	t	Vetivenic acid	1815	1811	2.18
52	Bisabolol oxide-A<*α*>	1746	1749	2.7	Khusinol acétate	1827	1823	-
53	Lanceol<*z*>	1767	1760	0.1	8-hydroxy-eremphilone	1848	1847	0.59
54	Cedryl acetate	1770	1768	0.9	Chenopodiol<α>	1855	1856	-
55	Cedren-13-ol acetate<*8*>	1783	1788	0.1	Murolan-3,9(11)-diene-10-peroxy	1875	1876	0.21
56	Chenopodiol<*α*>	1856	1856	0.1	Carissone	1920	1927	-
57	-	-	-	-	Selorene	1976	1974	-
58	-	-	-	-	Kaurene	2044	2043	0.28
	Oxygenated monoterpenes[b] (%)			**45.7**	Sesquiterpene hydrocarbons (%)			0.6
	Monoterpene hedrocarbons			2.2	**Oxygenated Sesquiterpenes[b] (%)**			**90.58**
	Sesquiterpene hydrocarbons (%)			0.7	Diterpenes hydrocarbones (%)			0.28
	Oxygenated Sesquiterpenes[b] (%)			**31.1**	Phenylpropanoide (%)			0.36
	Other (%)			4.9	-			-
	Total (%)			**84.6**	**Total (%)**			**91.82**

Notes: PN: Peak number .RI_c: Retention indices calculated against *n*- alkanes (C_7-C_{40}) mixture on the DB-5 column. RI_l: Literature retention indices [13]. [a] : Compounds area order of their elution from a DB-5 column and their percentage were obtained by flame ionization detector peak-area normalization. [b] : Major compounds. t : trace (<0.1%).Value in bold bold character: High value percentage.

3.2. Scavenging effect of the free radical DPPH• by essential oil plant extract

A. ifranensis and *A. pyrethrum* EOs, were subjected to screening for their possible antioxidant activity using the DPPH assays. This radical is generally one of the most widely used substrates for the rapid and direct assessment of antioxidant activity due to its radical form stability and the simplicity of analysis [34]. Results from these assays are presented in Figure 2:



(a) (b)

(c)

Fig.2. Percentage inhibition of the free radical DPPH˙ as a function of different concentrations used of *Artemisia ifranensis* (a), *Anacyclus pyrethrum* essential oils(b) and Ascorbic acid (c).

The results reported above demonstrate the DPPH˙ radical scavenging activities (% inhibition), caused by different concentrations of *A. ifranensis*, *A. pyrethrum* essential oils and vitamin C used as a positive control, which increased with increasing its concentration, until reaching a plateau that corresponds to the almost total containment of the DPPH˙ present in the medium. The reduction of this radical is accompanied by its passage from the violet color (2.2 diphenyl-1-picryl hydrazyl (DPPH˙)) to the yellow color (2,2 diphenyl -1-picryl hydrazine (DPPH-H) [35,36]. According to the obtained profiles (Figure 2), *A. ifranensis* essential oil show a significant activity in the DPPH-scavenging test and 83 % maximal inhibition was achieved at a concentration of 31 mg/ml. From the same concentration, where ascorbic acid exerted an antiradical effect reaching 77 % inhibition, the oil of *A. pyrethrum* show less efficient antioxidant power and only 62% inhibition percentage has been registered.

In the DPPH test, the analysis of the antioxidant activity results is also evaluated by comparison of the IC_{50} values, the lowest value corresponds to the highest efficiency. This parameter has been used by several researchers groups to present their results [19]. Therefore, 50 % inhibitory concentration results are given below (Figure 3):

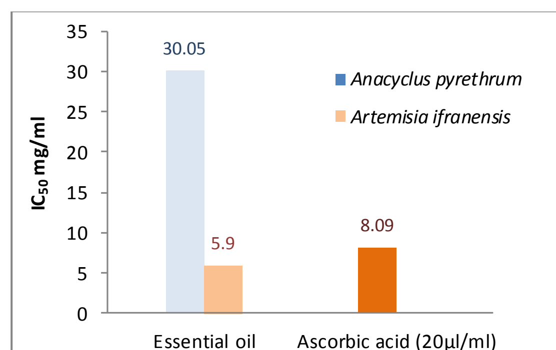


Fig.3. ***IC_{50} values illustration of A. ifranensis and A. pyrethrum essential oils, and ascorbic acid.***

Data processing of the obtained results allowed us to deduce that tested *A. ifranensis* EO was the most active (IC_{50} = 5.9 mg/ml) followed by the reference antioxidant (IC_{50} = 8.09 mg/ml) (Figure 3), a solution containing a pure molecule of ascorbic acid, which was less effective. In other words, this oil is a good source of natural antioxidants. The essence of *A. pyrethrum* was found, however, less interesting (IC_{50} values 30.05 mg/ml), it revealed poor radical scavenging activity compared to other tested samples.

This study is an original work that treats and highlights endemic species of Morocco. In the literature, no data have been previously published on antioxidant activity of *Artemisia ifranensis* and *Anacyclus sp*. EOs, including *A. pyrethrum* species. In fact, it is an original work which treats and highlights endemic species of Morocco. On the other hand, the antioxidant effect of certain *Artemisia* genus oils has been recorded in several works. Pradeep et *al*. (2013) reported similar results, they found that the Indian *A. nilagirica* oil has a significant trapping effect compared to vitamin C (EC_{50} = 65.4 ± 0.03 and 125 ± 0.022 µg/ml, respectively) [37]. It also seems that *A. arborescence* EO has a powerful antioxidant effect interpreted by an IC_{50} (6.26 mg/ml) very close to that of vitamin C (5.33 mg/ml) [38]. Similarly, in another study of *A. campestris* EO, it has a significant trapping property since it acts at a low dose (IC_{50} = 3.5 mg/ml) [39]. In contrast, *A. absintium* and *A. spicigera* EOs (from India and Iran successively) reveals IC_{50} values of 0,014 mg/ml and 0.086 ± 2.23 mg/ml respectively. These values remain modest compared to that recorded for ascorbic acid (0.003 mg/ml and 0.0021 ± 0.23 mg/ml, respectively) [40, 41]. In other works, researchers have tested the antioxidant activity of *A. herba alba* essence collected in different regions of Tunisia: Sidi Bouzid and Kairouan. They deduce that oil sample from Sidi Bouzid region is more efficient to reduce the $DPPH^{\cdot}$ radicals (IC_{50} = 0.05 mg/ml) than that from the Kairouan region (IC_{50} = 5.03 mg/ml). Comparison of the DPPH scavenging activity of the investigated essential oils from Tunisia and those expressed by BHT showed that the oils possessed weaker antioxidant effects than BHT standard (0.037 and 0.015 mg/ml respectively) [42, 43]. In another study, EOs radical trapping effect of various populations of *A. chamaemelifolia* collected in vegetative and flowering stages was evaluated. In both stages, IC_{50} values varied between 0.310 ± 11.9 and 0.809 ± 42.2 mg/ml respectively. Then, the positive control tested, the most efficient antioxidant, has value (IC_{50} = 0.076 ± 11.3 mg/ml) [44].

Moreover, in the literature the antioxidant activity of essential oils formed from complex mixtures of chemical compounds, having various functional groups; can be attributed to various mechanisms that due to their redox properties, the compounds act as reducing agents donors of hydrogen and singlet oxygen [45, 46]. Thus, it has been established in large works that the activity of an essential oil relates to the majority compounds, but there may also be another minority compounds which can interact in a synergistic (or antagonistic) manner to create a system effective against free radicals [47, 48, 49]. In general, EOs rich in oxygenated compounds exhibit more marked antiradical activity than those with hydrocarbon terpenes [43,50,51,52]. It also seems that the antioxidant performance of EOs is due to the presence of phenolic compounds, known to be good antioxidants [48,53]. Indeed, strong antioxidant activity observed in *A. ifranensis* essential oil should be attributed to their main components. From the results on chemical composition above, *A. ifranensis* oil contain higher amount of oxygenated monoterpenens and sesquiterpenes (45.7 % and 31.1 % respectively), which promotes its property of being a radical scavenging agent. Therefore the intervention of main oxygenated monoterpene compound β-thujone (41.12 %), known for its antioxidant potential [54,55], or even major oxygenated sesquiterpenoids such as α- bisabolone oxide A (14.54 %) and eremoligenol (4.44 %), expresses the powerful antioxidant activity. However, the antioxidant activity of the major compounds tested separately often gives lower results, compared to the activity of all of the essential oil. In general, the synergistic interactions between the different constituents of the oil are responsible for a much greater antioxidant power [56,57]. Indeed, the synergistic (co-activity) effect of more than one oil compound can be held, which confirms the above hypothesis. In fact, it is assumed that the contribution of minor and major compounds exhibited this activity and not only one or few active molecules. However, one last study have also reported similar results, β-thujone (41.9 %) is in fact responsible for the potent antioxidant activity of *A. herba-alba* essential oil [43]. Thus, other researchers have reported that the antioxidant capacity of *A. nilagica* and *A. absinthium* EOs was attributed to the major compounds β-thujone, which is in concord with our present data [37,58]. In contrary, Sbayou et *al*. (2016) have reported different results [59]. The essential oil of Moroccan *A. herba alba,* is characterized by its weak antioxidant activity (IC_{50} = 77 ± 3.69 mg/ml)

compared to the positive control (IC_{50} = 0.14 ± 0.001 mg/ml); it is in agreement with a previous study [58]. Therefore, thujone present in high concentrations (59.07 %), in oil of *A. herba alba*, has lower antioxidant potency [57].

About *Anacyclus pyrethrum*, EO demonstrated moderate antioxidant activity in the model assayed. The chemical profile of EO, is characterized by a high abundance of oxygenated sesquiterpene compounds (90.58 %). Quite obviously, the modest antioxidant activity of species can be linked to the majority phenolic compound spathulenol (13.31 %). These results have been reported by another work, which attribute the weak scavenger effect to the presence of main constituents, especially spathulenol [60]. On the other hand, this compound is also may account, in part, for the good antioxidant potential reported in else studies [61,62]. In fact, the weak antioxidant activity of oil can be attributed to the absence of some components that have DPPH radical scavenging effect, also with the antagonistic effect created by the minority compounds of the essence.

These results lead to conclude that it is very difficult to attribute the antioxidant effect of an essential oil to one or a few active principles, because an essential oil always contains a mixture of different chemical compounds. In addition to the major compounds, also minor compounds may make a significant contribution to the oil activity.

4. Conclusion

This study characterized and provided data on the chemical composition and antioxidant activity of *A. ifranensis* and *A. pyrethrum* essential oils. The chemical composition of Moroccan species, respectively, confirms the predominance of β-thujone and spathulenol chemotypes respectively. Subsequently the antioxidant potentialities were analyzed in terms of DPPH radical scavenging assays. This *in vitro* assay indicates that the essential oil of wormwood possesses a strong antioxidant potency, enough to reduce DPPH radicals, than of synthetic antioxidant and pyrethrum oil.

From the obtained results, it is obvious that the essential oils chemical composition has an important impact on the antioxidant activity. However, this antioxidant activity cannot be attributed to the sole presence of the major compounds, synergistic interactions between the various constituents of essential oil are also to be taken into consideration. Thus, antioxidant activity of studied essential oils, especially that of *A. ifranensis*, constitutes a potential source of natural antioxidants in foods in order to find possible alternative to synthetic antioxidant, and the pharmaceutical industry for the prevention the progress of numerous oxidative stresses and the treatment of various human diseases.

5. Compliance with ethical standards

The authors declare no conflict of interest.

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