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Essential Oils Composition, Anticholinesterase and Antioxidant Activities of *Pistacia atlantica* Desf.

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Abstract: Chemical composition and *in vitro* antioxidant and anticholinesterase activities of essential oils obtained from leaves and flowers of *Pistacia atlantica* were studied. GC/GC-MS analyses of the essential oils afforded twenty-two compounds from flower oil and thirty from leaf oil. The antioxidant activity of the essential oils was determined using β-carotene-linoleic acid, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ -scavenging effects. The essential oils exhibited a good antioxidant activity and inhibited the activity of acetyl- and butyrylcholinesterase.

Keywords: *Pistacia atlantica;* essential oil*;* anticholinesterase*;* antioxidant activity.

1. Plant Source

Pistacia atlantica Desf. [1,2] was collected in March 2012 from Berriane-Ghardaia (Algerian Septentrional Sahara). Pr Gérard De Bélair authenticated the plant. A voucher specimen was deposited at the herbarium of the Laboratory of Therapeutic Substances, Faculty of Sciences, University of Constantine 1, Algeria (LOST ZK Pa03.12)

2. Previous Studies

Essential oils of Algerian *P. atlantica*, collected from Laghouat and Aïn-Oussera, [3,4] and from Ouled Djellel (Biskra) and Messaad (Djelfa) [5], have been reported.

3. Present Study

Extraction: Fresh flowers and leaves (100 g each) of *Pistacia atlantica* were hydrodistilled for 3 h in a Clevenger-type apparatus that yielded yellow oils i.e. *Pistacia atlantica* flowers PAF (1.7 %)

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and *Pistacia atlantica* leaves PAL (1.5 %) (w/w). The oils were dried over anhydrous Na_2SO_4, preserved in a sealed vial and stored at +4 °C prior further analyses.

Essential oil analysis: Essential oils were analysed on Shimadzu GC2010 gas chromatogram, coupled to a Shimadzu QP2010 MS, equipped with a DB-5 MS column (30m X 0.25mm; 0.25µm), programmed as 50 °C (5 min) to 300 °C at 5 °C/min and 5 min hold. Helium was used as carrier gas (1.0 mL/min), injected in split mode (1:30), where injector and detector temperatures were 250 °C and 280 °C, respectively. The MS ionization mode was electron impact (EI) at 70 eV with electron multiplier detector at 2500 V. Ion source temperature was 180 °C while mass spectral data were acquired in scan mode in the range of *m/z* 33-450. GC-MS analysis was carried out with the same column and using the same temperature programming as mentioned for GC. Hydrogen was used as carrier gas (1.0 mL/min); injected in split mode (1:60); injector and detector temperatures were 250°C and 280°C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500 V; ion source 280 and 300°C respectively. The essential oil was diluted in hexane (1/30) for the analyses. Identification of individual compounds was made by Kovats method. The compounds assayed by GC were identified by comparing their retention indices with those of reference compounds, Wiley 2005 library (Wiley, New York) and literature [6] and confirmed by comparison of their mass spectra with those of reference substances for major components of the oils.

Antioxidant activity: the antioxidant effect of the essential oils was determined using β-carotene-linoleic acid, DPPH- and $ABTS^{\bullet+}$ scavenging activities [7-10]. For comparison of the antioxidant activity, α-tocopherol and BHA were used as positive controls.

Anticholinesterase activity: Acetylcholinesterase and butyrylcholinesterase enzymes inhibitory activities were assayed by the Ellman and Öztürk methods *in vitro* [11,12]. Galantamine was used as a reference compound.

The chemical composition of the essential oils of *P. atlantica* revealed 30 compounds in PAL and 22 (Table 1) in PAF The amounts of total volatile essential oils were found as 97.8 % in PALand 96.9 % in PAF. The essential oils were rich in monoterpenes and oxygenated sesquiterpenes. The main components found in the essential oils were: *α*-pinene (PAL 18.4 %, PAF 30 %), *α*-phellandrene (PAL 8.9 %, PAF 11 %), *β*-phellandrene (PAL 5.5 %, PAF 13.5 %), *α*-terpinene (PAL 17.3 %), limonene (PAL 5.9 %, PAF 8.9 %), *β*-elemene (PAL 7.5 %), *γ*-gurjunene (PAL 10 %), germacrene-B (PAL 6.2 %) and spathulenol (PAL 10.7 %). Compared to reported essential oils of *P. atlantica* [3-5], *α,β*-phellandrene, *α*-terpinene, *β*-elemene, *γ*-gurjunene and germacrene-B, are reported here for the first time as major components of essential oils of *Pistacia genus*. Morover, *α*-pinene seems to be a chemotype of *P. atlantica*.

Antioxidant and anticholinesterase capacities are two important parameters to decide whether the plant can be used as food supplement or not. As shown in Table 2, PAL was a little more active than PAF in both assays ($ABTS^{\bullet+}$, $DPPH^{\bullet}$ and β-carotene-linoleic acid). The antioxidant activity of the studied essential oils can be attributed to the synergistic effect of monoterpenes and sesquiterpenes content such as limonene (PAL: 5.9 %, PAF: 8.9 %), *α*-pinene (PAL: 18.4 %, PAF: 30.0%), *β*-elemene (PAL: 7.5 %), *γ*-gurjunene (PAL: 10%), germacrene-B (PAL: 6.2%) and spathulenol (PAL: 10.7 %). From the literature, *α*-pinene, limonene and spathulenol were even found to be the main components of reported essential oils of *Pistacia* [9,11]. In addition, previous studies revealed that α-pinene [13] and limonene [14] possess antioxidant activities.

In recent years, AChE inhibitory activity, induced by essential oils, has gained importance, because it is potentially relevant to the treatment of Alzeimer's disease [15]. Monoterpenoids have been shown to be acetylcholinesterase inhibitors such as 1,8 cineole, *α*-pinene, *β*-pinene, borneol, camphor, geraniol, *α*-terpineol, *γ*-terpinene, caryophyllene epoxyde and linalool [16]. From our results, both of the essential oils (PAL and PAF) are mainly characterized by monoterpenoids such as α-pinene (18.4%, 30.0% respectively) which has been reported to possess AChE inhibitory and antioxidant activities [16]. In addition, previous studies have showed that limonene was found to be a potent AChE inhibitor [17] as well as *β*-phellandrene [18] which mainly characterized PAL and PAF (8.9 %, 11.0 %, respectively).

Table 1. Chemical composition of essential oils from *P. atlantica*

Compounds	RI[a]	PAL[b] %	PAF[c] %	Identification methods
α-Thujene	930	0.9	4.4	MS, RI
α-Pinene	939	**18.4**	**30.0**	MS, RI
Camphene	954	0.4	2.7	MS, RI
Sabinene	975	1.7	2.7	MS, RI
α-Phellandrene	1003	**8.9**	**11.0**	MS, RI
α -Terpinene	1017	0.4	**17.3**	MS, RI
Limonene	1029	**5.9**	**8.9**	MS, RI
β-Phellandrene	1030	**5.5**	**13.4**	MS, RI
α-Terpinolene	1089	0.4	0.4	MS, RI
Linalool	1091	0.3	0.5	MS, RI
l-Terpineol	1134	-	0.5	MS, RI
Terpinen-4-ol	1177	-	0.7	MS, RI
Pulegone	1237	0.2	-	MS, RI
Limonene dioxide	1294	2.7	-	MS, RI
Thymol	1290	0.7	-	MS, RI
β-Elemene	1391	**7.5**	-	MS, RI
Caryophyllene	1409	1.0	1.5	MS, RI
Aromadendrene	1441	-	0.5	MS, RI
Ledene	1473	0.7	-	MS, RI
γ-Gurjunene	1477	**10.0**	-	MS, RI
Germacrene D	1485	4.8	0.4	MS, RI
β –Guiaiene	1493	0.9	-	MS, RI
Germacrene-A	1508	0.6	-	MS, RI
δ-Cadinene	1514	0.4	0.3	MS, RI
Germacrene B	1561	**6.2**	0.4	MS, RI
Spathulenol	1578	**10.7**	0.5	MS, RI
Caryophyllene oxide	1583	-	0.4	MS, RI
Globulol	1585	0.7	-	MS, RI
Guaiol	1601	1.0	-	MS, RI
β-Eudesmol	1651	0.8	-	MS,RI
α-Eudesmol	1654	1.5	-	MS,RI
α-Cadinol	1654	4.6	-	MS,RI
Guaiol acetate	1727	-	0.2	MS,RI
Sclareol	2223	-	0.2	MS,RI
Total (%)		**97.8%**	**96.9%**	

[a]Retention index calculated based on Kováts indices; [b]*Pistachia atlantica* leaves; [c]*Pistachia atlantica* flowers

Table 2. Antioxidant activities by β-carotene-linoleic acid, DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and Acetylcholinesterase (AChE), butyrylcholineterase (BChE) inhibitory activities of *P. atlantica* essential oils[a].

Sample	β-carotene-linoleic acid assay	DPPH$^{\bullet}$ assay	ABTS$^{\bullet+}$ assay	AChE assay	BChE assay
	IC_{50} (µg/mL)[b]				
PAL	17.0±0.3	23.9±0.3	4.7±0.20	18.5±0.5	79.7±2.1
PAF	19.1±1.7	28.5±0.3	6.5±0.54	20.5±0.5	107.0±1.6
BHA[c]	2.1±0.1	7.3±0.1	4.3±0.10	-	-
α-Tocopherol[c]	1.3±0.04	45.3±0.4	4.1±0.06	-	-
Galantamine[c]	-	-	-	5.0±0.1	50.8±0.9

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).
[b] Inhibitions were determined between 200 µg/mL and 800 µg/mL.
[c] Standard: BHA (butylatedhydroxylanisole), α-Tocopherol, EDTA, Galantamine.

PAL and PAF exhibited a good AChE (IC_{50}: 18.5±0.5 µg/mL and IC_{50}: 20.5±0.5 µg/mL, respectively) and BChE (IC_{50}: 79.7±2.1 µg/mL and IC_{50}: 107±1.6µg/mL, respectively) inhibitory activities at 500 µg/mL (Table 2).

In another hand, the higher antioxidant and anticholinesterase activity of PAL, compared with PAF, may be due to the major presence of *β*-elemene (7.5%), germacrene B (6.2%) and monoterpene rich essential oils which have been previously reported as potent antioxidant and cholinesterase inhibitors [19-24]. *γ*-Gurjunene, and spathulenol were also mainly found in PAL, they may have a synergic effect with *α*-terpinene which could explain the higher inhibition of AChE and BChE and antioxidant activity observed in PAL.

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