IMPACT OF ENVIRONMENTAL ARIDITY ON THE PHYTOCHEMICAL COMPOSITION OF PHENOLIC EXTRACTS AND ESSENTIAL OIL FROM *Vitex agnus-castus* L. LEAVES ACCLIMATED IN THE ALGERIAN SAHARA

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Abstract. *Vitex agnus-castus* L. (Lamiaceae) is a bushy shrub native to Asian and Mediterranean regions and is often used in traditional and popular medicine for its many biological properties and aromatic virtues. There are many studies devoted to the phytochemistry of species growing in endemic conditions, but little or no information is available on species growing in a subtropical desert climate. In this perspective, a comparative study with works carried out almost everywhere in the world, aims to analyze phenolic extracts and essential oil of leaves harvested in arid regions of the south-west of the Algerian Sahara. The phenolic extracts are characterized by a high content of anthocyanidins (6.1±0.07 $mg \cdot g^{-1}$ DM) and by the presence of 40 components detected by HPLC-DAD, among which five phenolic acids, two flavones and two flavonols are identified. The essential oil is characterized by chemical diversity of volatile substances, 68 (98.97%) are selected by GC-MS of which 52 (89.72%) are identified. Hydrocarbon compounds (25.03%) are less abundant than their oxygenated (64.69%). These were distributed as follows: 25 monoterpenes (32.12%), 3 sesquiterpenes (31.84%), 1 diterpene (0.16%), 2 norisoprenoids (0.29%) and a non-terpene oxygenated compound (0.28%). A unique compound characterizes the oxygenated sesquiterpenes, caryophyllene oxide, which represents one-third (31.5%) of the total area of the foliar essential oil; this molecule is absent or very rare in all the other essential oils indicated in literature. Considering these results, *V. agnus-castus* acclimatized in the arid Saharan zone, presents a particular chemotype compared to those of endemic populations.

Keywords: *Vitex agnus-castus* L.; Saharan arid zone; chemotype profile; analysis; comparative study.

INTRODUCTION

Vitex agnus-castus L., commonly called chaste tree, represents one of the 250 species of the *Vitex* genus, the Lamiaceae family. Native to southern Europe, the species is widely spread in the Mediterranean basin and the Middle East [38]. It is currently distributed worldwide, in Western Asia, on the Anatolian coasts [38], in Latin America [56], in the eastern and southern United States [65, 73], and in the tropical and warm temperate regions of both hemispheres [10].

The species *V. agnus-castus* grows spontaneously in northern Algeria [62]; the species acclimatized in humid sites in the south of the country [36]. These species is known under the vernacular name "Kef Meriem" [62] or "Kherouaa" [36]. To our knowledge, in Algeria, the plant was spotted for the first time in the Kadous region (Béjaïa) [43]. In addition, this plant constitutes the flora of the Edough peninsula located in the Northeast of Algeria [28], the Gorges of Ghouffi (Batna) [2], the Taghit oasis [61], and the Bechar region [4, 25, 49]. We also encountered the shrub in the desert regions in the south-west of the Sahara, in the case of Timimoun and Adrar (personal prospecting), respectively, 1219 and 1430 km from the coastal capital Algiers. These regions characterized by an arid Saharan climate with very high temperatures exceeding 45°C, intense solar radiation, and a short winter with very low rainfall. The chaste tree is a bushy shrub that supports poor draining soils and tolerates drought well; it can reach 5 m in height. The leaves are deciduous palmate [10] and the flowers are fragrant purplish grouped in spikes. The fruit is an edible berry (4 to 5 mm) with a peppery flavor [46].

Chaste tree is often used in traditional and popular medicine for its many biological properties and aromatic virtues; much research has focused in recent decades on its use in various pharmacological and medical fields [14, 51, 53, 76, 77]. This work has confirmed its efficacy for treating a wide variety of female hormonal disorders, in particular the symptoms associated with premenstrual syndrome, corpus luteum insufficiency, and hyperprolactinemia [3, 27, 54, 64, 68, 71]. Commonly used to relieve disorders of the female hormonal cycle and stimulate lactation [6, 74], chaste tree is now a safe bet among the plants used in herbal medicine against cyclical breast pain, menstrual irregularities, and menopause [1, 13]. The fruits have a diuretic, antiparasitic and former effect; they treat stomachaches, headaches and syphilis [50]. They relieve headaches and treat ulcers, abscesses, and rheumatoid arthritis [74]; they can work favorably to prevent or treat osteoporosis [60]. *In vitro*, *V. agnus-castus* extracts exhibit cytotoxic activity and induce apoptosis of cancer cells of the cervix, ovary, and colon [58], prostate [75], lung and breast [15].

Previous phytochemical investigations have shown the presence of flavonoids [26, 32, 38], diterpenoids [26, 45], and iridoids [39, 63] in leaves, fruits, and flowers. Flavonoids are predominantly represented by luteolin [31], casticin and artemetin [32, 38]; these secondary metabolites have antioxidant [26, 40] and cytotoxic potential [31]. The essential oil of *V. agnus-castus* is aromatic; this property is due to the presence of terpenes [9]. According to the literature, the essential oil is rich in eucalyptol (or 1,8-cineole) [12, 21, 56, 71]. Eucalyptol is a cyclic monoterpene compound, the percentage of which can vary

depending on the geographical position of the species; it can reach up to 50% [80].

The biological properties of the essential oil of *V. agnus-castus* are multiple; it presents an antibacterial [24] and antifungal [78] activity as well as a molluscicidal [17], nematicidal [57], insecticidal [59] and acaricidal [56] action.

This study is a contribution to a research project aiming at the valorization of natural resources in the Algerian arid zones for a sustainable development in the region. Although *V. agnus-castus* growing in its natural environment has aroused great interest in phytochemical and biological research for few studies have focused on this acclimate species in an arid environment. Therefore, the objective of the present study is to quantify and identify the phytochemical constituents of the phenolic extracts and essential oil of *V. agnus-castus* leaves from the Algerian Sahara using different techniques: spectrophotometry UV-Vis, HPLC-DAD and GC-MS. In addition, a quantitative and qualitative comparison was conducted considering the results obtained in different regions of the world.

MATERIAL AND METHODS

Plant material

The leaves of *V. agnus-castus* collected in March 2018 from plants grown in Ouled Aïssa municipality, located in the Cherouine district of Timimoun province (Algeria) (fig. 1).

The geographical coordinates and main physicochemical and pedological characteristics of the study station are summarized in table 1. The climatic characteristics of the study area place it in the arid Saharan bioclimatic stage of Emberger with a temperate winter, a dry season all year round and an aridity index of 0.37. The soil is sandy (79.65%), slightly alkaline (pH=7.4) and poor in organic matter (1.19%) (Personal data). Professor A. Hirche (Faculty of Biological Sciences USTHB, Algiers) identified the species, and his description is in perfect agreement with that established by Quezel and Santa [62]. The voucher specimen (MP 12-22-1/2018) was deposited at the Research Laboratory on Arid Zones (USTHB, Algiers, Algeria).

The samples dried at room temperature, away from bright light and humidity. Once dry, they reduced to a

Figure 1. The geographical location of the study area: Ouled Aïssa municipality, province of Timimoun in Algeria, country the African continent

Table 1. Main geographical coordinates and physicochemical and pedological characteristics of the study station

Geographical coordinates and climatic characteristics					
Latitude	Longitude	Altitude (m)	Tm (°C)	Rain (mm/an)	AI (mm/°C)
29°25'06" N	0°05'15" W	355	25.84	13.36	0.37
Physicochemical and pedological parameters (Personal data)					
Rm (%)	pH	Om (%)	$CaCO_3$ (%)	Fs (%)	Cs (%)
1.50	7.40	1.19	7.97	44.50	35.15

Note: Tm (°C): Mean annual temperature; Rain (mm/an): mean annual precipitation; AI (mm/°C): aridity index [Rain/(Tm + 10)]; Rm (%): residual moisture; pH: potential hydrogen; Om (%): organic matter; $CaCO_3$ (%): Carbonate de calcium; Fs (%): fine sand; Cs (%): coarse sand

fine powder and then analyzed during the same year in order to study the possible influence of the extreme aridity of the environment on the quality and quantity of the phenolic compounds and the essential oil in the leaves.

The biochemical analysis of phenolic compounds

Extraction and separation of flavonic extracts

Pulverized dry plant matter (1 g) was suspended in 80 mL of hydrochloric acid (2N) (ACS reagent 37%/Sigma aldrich), after cold contact for 5 min; the mixture was boiled for 40 min in a water bath with oxygen insufflation every 10 min. Oxygen allows the oxidation of proanthocyanidins into the corresponding anthocyanidins [44]. The aqueous and organic phases was obtained by three successive extractions with diethyl ether (2 x 30 mL, 1 x 20 mL) (analytical standard/Sigma aldrich) using a glass separating funnel. The aqueous phase containing anthocyanidins characterized by a red color; their basic structure is a flavylium ion, initially colorless; it turns red during acid hydrolysis in the presence of oxygen. The organic phase containing flavonic aglycones (flavones, flavonols) and phenolic acids has a shiny bright red color [20]. This phase evaporated under a ventilated hood; the dry residues collected in 3 mL of absolute ethanol (ACS analysis/Sigma aldrich).

Determination of flavonoids content

Three classes of flavonoids have been quantified: anthocyanidins, flavones and flavonols. The contents of these secondary metabolites were determined by UV-Vis spectrophotometry (Junway 7300 model).

The volume of the aqueous phase is measured; the anthocyanidins then quantified at 520 nm, their content expressed in cyanidin equivalents $mg \cdot g^{-1}$ of dry weight and calculated using the following formula [44].

$$T\ (mg \cdot g^{-1}) = (\eta \times Abs / \varepsilon) \times (M_w \times V \times D) / P$$

where, T: anthocyanidins content ($mg \cdot g^{-1}$); η: corrective factor corresponding to the yield of transformation of proanthocyanidins into anthocyanidins, it is 17% and equal to 6; Abs: absorbance at 520 nm; ε: molar absorption coefficient of cyanidin, equal to 34700 $L \cdot mol^{-1} \cdot cm^{-1}$; M_w: molar mass of cyanidin equal to 306 $g \cdot mol^{-1}$; V: volume of aqueous phase (mL); D: dilution factor; P: dry weight (DW) of plant powder (in this study equal 1 g). From where:

$$T\ (mg \cdot g^{-1}) = 5{,}2 \times 10^{-2} \times (Abs \times V \times D) / P$$

From the ethanolic extract corresponding to the organic phase, we carried out a dilution with ethanol 95° (a) and another with aluminum chloride 1% (anhydrous, powder/Sigma aldrich) in ethanol 95° (b). The differential absorbance (ΔAbs) of flavonols and flavones, respectively, measured at 420 and 340 nm. Their contents are expressed respectively in quercetin (mg) for flavonols and in luteolin (mg) for flavones per gram of dry weight according to the following formulas [44].

$$T\ (mg \cdot g^{-1}) = (\Delta Abs / \varepsilon) \times (M_w \times V \times D) / P$$

where, T: flavonic aglycones (flavonols or flavones) content ($mg \cdot g^{-1}$); ΔAbs: differential absorbance (b - a) at 420 nm for flavonols and 340 nm for flavones; ε: molar absorption coefficient of quercetin (23000 $L \cdot mol^{-1} \cdot cm^{-1}$) or of luteolin (20428 $L \cdot mol^{-1} \cdot cm^{-1}$); M_w: molar mass of quercetin (302 $g \cdot mol^{-1}$) or of luteolin (286 $g \cdot mol^{-1}$); V: volume of flavonic aglycones (mL); D: dilution factor, P: dry weight (DW) of plant powder (in this study equal 1 g). From where:

$$T\ (mg \cdot g^{-1}) = 1{,}3 \times 10^{-2} \times (\Delta Abs \times V \times D) / P$$

Qualitative analysis of phenolic compounds by HPLC-DAD

The chromatographic analysis of ethanolic leaf extract by high performance liquid chromatography (HPLC) was carried out at the Central Laboratory of the Scientific Police of Algiers (operator: 4752-CHIM-0002093-18), using an Agilent model Technologies 1100 series (Location: Vial 1 injection) equipped with a quaternary pump and an automatic injector.

The column is of the Hypersil™ BDS-C18 type with dimensions of 5 µm, 250 × 4.6 mm at a temperature of 30°C. The mobile phase was formed of water acidified by acetic acid in solution (0.2%) (suitable for HPLC/Sigma aldrich) at pH=3.1 and acetonitrile (suitable for HPLC/Sigma aldrich) in a linear elution gradient for 30 min at 1 $mL \cdot min^{-1}$, starting with 95% water and ending with 100% acetonitrile. Two volumes of the phenolic extract were tested 10 µL (test A) and 30 µL (test B). Substances are detected using a diode array detector (DAD). The latter makes it possible to measure the absorbance on several wavelengths (230, 254, 280, 340 and 360 nm) at the same time, which are chosen according to the maximum absorbance of the target molecules.

In the case of our extract, DAD detected the purity and absorbance of the peaks at 280 nm; the detector placed at the outlet of the column gives a plot called chromatogram. The levels of the molecules detected expressed as distribution area (percentage), obtained by dividing the area of each peak by the sum of the areas of all the peaks in the chromatogram. The identification of phenolic compounds retained in the organic phase was performed by comparison of the retention time (RT) and UV-Vis absorption spectrum with reference standard substances.

The biochemical analysis of essential oils

Extraction and determination of yield

The leaf essential oil was extracted using a Clevenger-type apparatus by hydrodistillation for 3 hours of 30 g of dry plant matter in 500 mL of distilled water. The collected oil was dried over anhydrous Na_2SO_4 (ACS reagent/Sigma aldrich) ; its weight was measured and then stored at 4°C in the dark. The percentage of its yield is determined by the weight of dry leaf matter according to the formula:

$$Yield\ (\%) = (OW / DW) \times 100$$

where, OW: oil weight (g); DW: leaf powder dry weight (g).

Qualitative and quantitative analysis by GC-MS

Gas chromatography coupled with mass spectrometry (GC-MS) was carried out at the Organometallic and Molecular Materials Engineering

Laboratory of the University of Fez (Morocco), using a Trace GC ultraapparatus, with a running time of 60.02 min for each test. The gas chromatograph was equipped with a non-polar capillary TR-5 column (60 m x 0.32 mm ID x 0.25 µm); with the following temperature program: 40°C for 2 min, ramp of 5°C·min^{-1} to 280°C. The final temperature was maintained for 10 min, injector temperature at 220°C. The injection volume was 1 µL (dilution factor 1) in the Split mode, opening the split at 0 min. The mass spectrometer model Polaris Q was operating in electron ionization (EI) mode at 70 eV; the mass spectra were recorded using an MS quadrupole detector; source and transfer line temperatures 270°C. The carrier gas was helium at a flow rate of one mL·min^{-1}. Volatile compounds are detected according to their order of elution, retention time (RT) and recognition rate; the identification of separated compounds is validated by mass spectra compared with Wiley Registry/NIST 2011 edition mass spectral library.

Statistical analysis

Samples of chaste tree (phenolic extracts and essential oils) were prepared and analyzed in triplicate. The results expressed as mean values and standard deviation (SD), this results are considered significantly different at $p<0.05$.

RESULTS

Biochemical results of phenolic compounds

Flavonoid contents

Anthocyanidins and flavonic aglycones from the leaves of *V. agnus-castus* quantified by UV-Vis spectrophotometer assay (fig. 2). The results indicate that the contents of anthocyanins taken from the aqueous phase are high (6.1±0.07 mg·g^{-1} DW) compared to those of the flavonic aglycones contained in the organic phase; the difference between them is very significant. Flavones (0.80±0.01 mg·g^{-1} DW) are more concentrated than flavonols (0.49±0.02 mg·g^{-1} DW); the difference between these two classes is not significant.

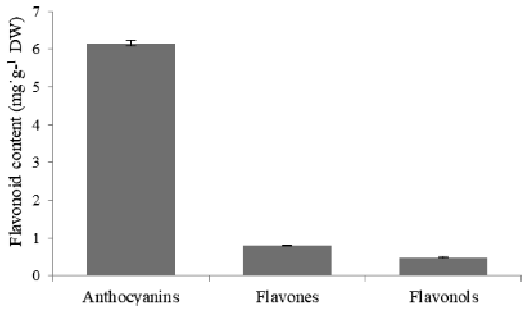

Figure 2. Flavonoid content in the leaves of *V. agnus-castus*. Note: UV-vis assay: Anthocyanidins at 520 nm (aqueous phase) and flavonic aglycones at 420 nm for flavonols and 340 nm for flavones (organic phase)

Qualitative profile of phenolic compounds by HPLC-DAD method

The phytochemical profiles of ethanolic leaf extracts obtained by HPLC-DAD at 280 nm revealed 40 phenolic compounds in total (fig. 3), 29 of which were detected in the 10 µL injected during the first test and 38 in the 30 µL injected during the second test; 27 compounds were common between the two tests (table 2). Five major peaks (1, 20, 23, 32 and 38) were obtained for the two volumes injected (10 and 30µL), they represent 23.13 / 15.63%, 5.47 / 5.99%, 6.27 / 4.23%, 13.64 / 13.92%, and 6.38 / 6.84% of the chromatogram area; four peaks (1, 23, 32 and 38) of them could not be identified. UV spectra analysis and retention time of the major peaks indicated that the substances were from phenolic compound group. Phenolic acids and derivatives (peaks 3, 6, 10, 11 and 19) represented, respectively, by gallic acid (0.32%), chlorogenic acid (1.90 / 3.02%), caffeic acid (4.18 / 2.84%), vanillin (1.36 / 1.51%) and *trans*-cinnamic acid (1.74%). In addition, compounds of the flavonoid family identified in the two analyzed ethanolic extracts, two flavones (peaks 16 and 20): luteolin (3.39 / 3.47%) and apigenin (5.47 / 5.99%) and two flavonols (peaks 8 and 21): isorhamnetin (2.15 / 2.12%) and casticin (2.85 / 2.88%).

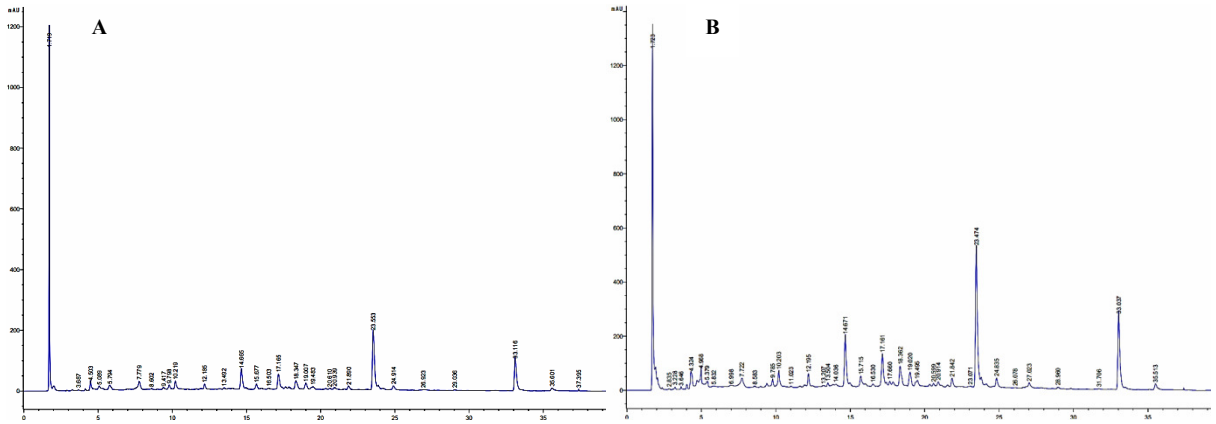

Figure 3. Chromatographic profiles at 280 nm of the leaf ethanolic extract of *V. agnus-castus* by HPLC-DAD method. Note: Volume injected 10 µL (A) and 30 µL (B); Mobile phase: Water-acetic acid (0.2%) pH=3.1 and acetonitrile in linear elution gradient (30 min/1 mL·min^{-1}) 95% water to 100% acetonitrile

Table 2. Phenolic compounds detected by HPLC-DAD (280 nm) in *V. agnus-castus* ethanolic leaf extracts

Peak	RT (min)	A (area %)	B (area %)	Identification
1	1.719–1.723	23.1301	15.6297	Major peak NI
2	2.835	-	0.1953	NI
3	3.228	-	0.3178	Gallic acid
4	3.687–3.646	0.6240	0.2846	NI
5	4.503–4.324	2.3665	2.3061	NI
6	5.089–4.968	1.8968	3.0231	Chlorogenic acid
7	5.379	-	1.2775	NI
8	5.794–5.832	2.1514	2.1153	Isorhamnetin
9	6.998	-	1.1731	NI
10	7.779–7.722	4.1780	2.8416	Caffeic acid
11	8.602–8.583	1.3596	1.5131	Vanillin
12	9.417	1.6346	-	NI
13	9.798–9.785	1.4853	2.1591	NI
14	10.219–10.203	2.5107	2.8223	NI
15	11.023	-	0.5741	NI
16	12.185–12.195	3.3919	3.4726	Luteolin
17	13.207	-	1.1880	NI
18	13.492–13.504	3.6003	0.7750	NI
19	14.036	-	1.7348	*trans*-Cinnamic acid
20	14.665–14.671	5.4667	5.9894	Apigenin
21	15.677–15.715	2.8501	2.8772	Casticin
22	16.503–16.530	1.1097	1.1000	NI
23	17.165–17.161	6.2713	4.2275	Major peak NI
24	17.660	-	1.8826	NI
25	18.347–18.362	3.1376	3.1643	NI
26	19.007–19.020	2.0095	2.1093	NI
27	19.483–19.495	2.0934	2.2175	NI
28	20.610–20.599	1.3712	1.5178	NI
29	20.939–20.914	1.2612	1.4520	NI
30	21.890–21.842	2.1871	2.8562	NI
31	23.071	-	0.7009	NI
32	23.553–23.474	13.6435	13.9177	Major peak NI
33	24.914–24.835	1.5854	2.0776	NI
34	26.078	-	0.5444	NI
35	26.923–27.023	1.0351	1.8489	NI
36	29.036–28.960	0.3392	0.4448	NI
37	31.706	-	0.1605	NI
38	33.116–33.037	6.3773	6.8425	Major peak NI
39	35.601–35.513	0.7875	0.6659	NI
40	37.395	0.1451	-	NI
The number of compounds		29	38	
Total area (%)		100	100	

Note: Injected volume 10 µL (A) and 30 µL (B); RT: Retention time (min); Mobile phase: Water-acetic acid (0.2%) and Acetonitrile in linear elution gradient (30 min/1 mL˙min^{-1}) 95% water to 100% acetonitrile; NI: Unidentified

Biochemical results of essential oils

Essential oil yield

The leaves essential oil obtained by hydrodistillation (3 h) has a yield of 0.37±0.04%, is viscous in nature, and is golden yellow in color, and its smell is intense and pleasant.

Quantitative characterization of essential oils by the GC-MS method

In *V. agnus-castus*, chemical profiling of leaves essential oil is much diversified (fig. 4); 167 compounds were detected, in all accounting for of 98.97% of the total oil; among them, 99 molecules were in the form of traces (total 2.14%) and are not considered in this study. Of the 68 selected substances, 52 are identified totaling 89.72%; the others are unidentified (7.11%). Total monoterpenes (46.5%) and total sesquiterpenes (42.37%) were more or less uniformly distributed in the leaves. These two groups are marked by the abundance of oxygenated compounds (63.96%); they represent an area of 32.12% in oxygenated monoterpenes and 31.84% in oxygenated sesquiterpenes. Oxygenated compounds are more abundant (64.69%) than their hydrocarbon counterparts (25.03%); 32 volatile compounds distributed as follows represent them: 25 monoterpenes (32.12%), 3 sesquiterpenes (31.84%), 1 diterpene (0.16%), 2 norisoprenoids (0.29%) and a 9-carbon non-terpene oxygen compound named cryptone (0.28%) (table 3).

Qualitative profile of essential oils by the GC-MS method

Twenty-five oxygenated monoterpenes (32.12%) were detected, the best represented among these compounds are terpinene-4-ol (7.23%), linalool (4.97%), myrtenyl acetate (3.24%), carvacrol (2.93%), 1,8-cineole (2.3%), δ-terpineol (1.69%), geraniol (1.52%), δ-terpinyl acetate (1.5%) and *cis*-sabinene hydrate (1.35%). Nine components are hydrocarbon monoterpenes (14.38%), dominated by α-pinene (5.61%), limonene (3.62%) and α-thujene (2.14%). Oxygenated sesquiterpenes are abundantly represented by a single compound, caryophyllene oxide (CAS# number 1139-30-6), which represents one-third (31.5%) of the total area. *Cis*- and *trans*-β-farnesene

(5.12%), viridiflorene (2.44%) and γ-cadinene (1.28%) define hydrocarbon sesquiterpenes (10.53%). We also report that this essential oil contains traces of diterpenes (manool oxide and 13-epi-dolabradiene) and norisoprenoids (dihydroedulan I and *trans*-β-damascenone) (table 4).

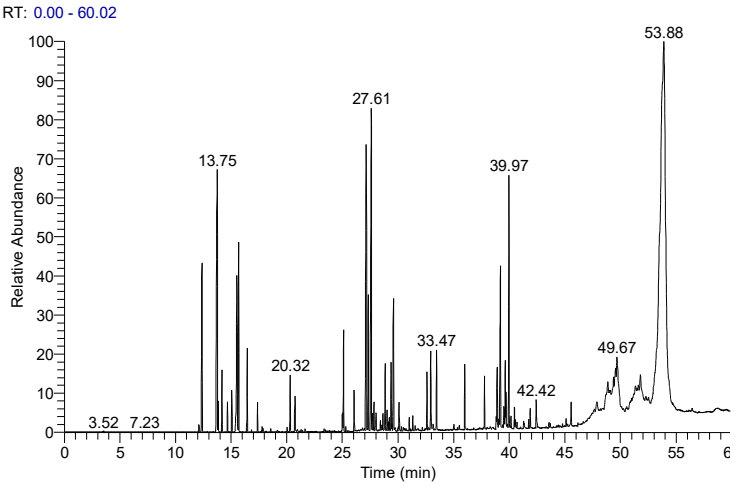

Figure 4. Chromatographic profile of the leaf essential oil of *V. agnus-castus*, by GC-MS method

Table 3. Quantitative characterization of essential oils by the GC-MS method

Nature	Compounds	Number	Total area (%)
Total terpenes	Compounds detected by GC-MS	167	98.97
	Compounds unselected (trace < 0.09%)	99	2.14
	Compounds identified (compound ≥ 0.1%)	52	89.72
	Unidentified compounds (NI)	16	7.11
	Total oxygenated compound	32	64.69
	Total compounds hydrocarbons	20	25.03
Monoterpenes	Total monoterpenes	34	46.5
	Oxygenated monoterpenes	25	32.12
	Monoterpenes hydrocarbons	9	14.38
Sesquiterpenes	Total sesquiterpenes	13	42.37
	Oxygenated sesquiterpenes	3	31.84
	Sesquiterpenes hydrocarbons	10	10.53
Diterpenes	Total diterpenes	2	0.28
	Oxygenated diterpene	1	0.16
	Diterpenes hydrocarbons	1	0.12
Norisoprenoids	Oxygenated norisoprenoid	2	0.29
Other	Non-terpene oxygenated compound	1	0.28

Table 4. The chemical composition of essential oil of *V. agnus-castus* L. leaves from the Algerian Sahara

Peak	RT (min)	KI	Compound	The area peak (%)	MW (g/mol)	MF	CAS# number
0	1	2	3	4	5	6	7
1	12.38	922	α-Thujene	2.14	136.238	$C_{10}H_{16}$	2867-05-2
2	13.75	930	α-Pinene	5.61	136.238	$C_{10}H_{16}$	80-56-8
3	14.19	964	Sabinene	0.66	136.238	$C_{10}H_{16}$	3387-41-5
4	14.68	973	β-Pinene	0.29	136.238	$C_{10}H_{16}$	127-91-3
5	15.07	1010	Myrcene	0.41	136.238	$C_{10}H_{16}$	123-35-3
6	15.52	1028	Limonene	3.62	136.238	$C_{10}H_{16}$	5989-27-5
7	15.67	1033	1,8-Cineole	2.3	154.249	$C_{10}H_{18}O$	470-82-6
8	16.44	1047	β-(E)-Ocimene	0.82	136.238	$C_{10}H_{16}$	3779-61-1
9	17.38	-	NI	0.29	-	-	-
10	17.77	-	NI	0.11	-	-	-
11	20.32	-	NI	0.64	-	-	-
12	20.73	1063	γ-Terpinene	0.41	136.238	$C_{10}H_{16}$	99-85-4
13	25.10	1066	*cis*-Sabinene hydrate	1.35	154.253	$C_{10}H_{18}O$	15537-55-0
14	26.06	1085	α-Terpinolene	0.42	136.238	$C_{10}H_{16}$	586-62-9
15	27.15	1094	Linalool	4.97	154.249	$C_{10}H_{18}O$	78-70-6
16	27.33	1164	δ-Terpineol	1.69	154.249	$C_{10}H_{18}O$	7299-42-5
17	27.61	1172	Terpinen-4-ol	7.23	154.249	$C_{10}H_{18}O$	562-74-3
18	27.84	1183	Cryptone	0.28	138.21	$C_9H_{14}O$	500-02-7
19	28.01	1187	α-Terpineol	0.25	154.253	$C_{10}H_{18}O$	98-55-5
20	28.43	1198	Myrtenal	0.14	150.221	$C_{10}H_{14}O$	564-94-3
21	28.64	1212	Verbenone	0.24	150.221	$C_{10}H_{14}O$	80-57-9
22	28.84	1221	*trans*-Carveol	0.75	152.237	$C_{10}H_{16}O$	2102-58-1
23	29.03	1226	α-Fenchyl acetate	0.23	196.29	$C_{12}H_{20}O_2$	4057-31-2

0	1	2	3	4	5	6	7
24	29.23	1229	Nerol	0.25	154.253	$C_{10}H_{18}O$	106-25-2
25	29.37	1243	Carvone	0.77	150.221	$C_{10}H_{14}O$	99-49-0
26	29.59	1252	Geraniol	1.52	154.249	$C_{10}H_{18}O$	106-24-1
27	30.10	1268	*trans*-Ascaridol glycol	0.39	170.248	$C_{10}H_{18}O_2$	21473-37-0
28	30.47	1287	Bornyl acetate	0.12	196.286	$C_{12}H_{20}O_2$	76-49-3
29	31.00	1289	Dihydroedulan I	0.15	194.318	$C_{13}H_{22}O$	63335-66-0
30	31.33	1291	*para*-Cymen-7-ol	0.17	150.221	$C_{10}H_{14}O$	536-60-7
31	32.59	1293	Thymol	0.67	150.218	$C_{10}H_{14}O$	89-83-8
32	32.93	-	NI	1.21	-	-	-
33	33.15	-	NI	0.1	-	-	-
34	33.47	-	NI	0.91	-	-	-
35	35.01	-	NI	0.12	-	-	-
36	36.00	-	NI	0.69	-	-	-
37	37.77	-	NI	0.59	-	-	-
38	38.92	-	NI	1.36	-	-	-
39	39.22	1304	Carvacrol	2.93	150.218	$C_{10}H_{14}O$	499-75-2
40	39.63	1313	δ-Terpinyl acetate	1.5	196.286	$C_{12}H_{20}O_2$	93836-50-1
41	39.97	1326	Myrtenyl acetate	3.24	194.274	$C_{12}H_{18}O_2$	35670-93-0
42	40.15	1336	*cis*-Piperitol acetate	0.14	196.286	$C_{12}H_{20}O_2$	112028-22-5
43	40.46	1339	exo-2-Hydroxycineole acetate	0.39	212.289	$C_{12}H_{20}O_3$	72257-53-5
44	40.68	-	NI	0.11	-	-	-
45	41.29	-	NI	0.2	-	-	-
46	41.74	-	NI	0.12	-	-	-
47	41.89	1345	Piperitenone	0.26	150.221	$C_{10}H_{14}O$	491-09-8
48	42.42	1348	α-Terpinyl acetate	0.49	196.286	$C_{12}H_{20}O_2$	80-26-2
49	43.56	1350	Citronellyl acetate	0.13	198.306	$C_{12}H_{22}O_2$	150-84-5
50	44.39	1382	*trans*-β-Damascenone	0.14	190.286	$C_{13}H_{18}O$	23726-93-4
51	45.11	1390	7-epi-Sesquithujene	0.14	204.357	$C_{15}H_{24}$	159407-35-9
52	45.56	1409	α-Gurjunene	0.37	204.357	$C_{15}H_{24}$	489-40-7
53	47.66	1416	*trans*-β-Caryophyllene	0.39	204.357	$C_{15}H_{24}$	87-44-5
54	47.89	1425	β-Caryophyllene	0.53	204.357	$C_{15}H_{24}$	87-44-5
55	48.86	1438	*cis*-β-Farnesene	1.37	204.357	$C_{15}H_{24}$	28973-97-9
56	49.67	1452	*trans*-β-Farnesene	3.75	204.357	$C_{15}H_{24}$	18794-84-8
57	50.57	-	NI	0.11	-	-	-
58	51.34	1496	Viridiflorene	2.44	204.357	$C_{15}H_{24}$	21747-46-6
59	51.79	1513	γ-Cadinene	1.28	204.357	$C_{15}H_{24}$	1460-97-5
60	52.29	1525	β-Sesquiphellandrene	0.16	204.357	$C_{15}H_{24}$	20307-83-9
61	52.52	-	NI	0.16	-	-	-
62	53.05	1530	δ-Cadinene	0.1	204.357	$C_{15}H_{24}$	483-76-1
63	53.88	1577	Caryophyllene oxide	31.5	220.356	$C_{15}H_{24}O$	1139-30-6
64	56.15	1649	α-Cadinol	0.15	222.372	$C_{15}H_{26}O$	481-34-5
65	56.41	1679	epi-α-Bisabolol	0.19	222.372	$C_{15}H_{26}O$	515-69-5
66	57.92	1980	Manool oxide	0.16	290.491	$C_{20}H_{34}O$	596-84-9
67	58.74	-	NI	0.39	-	-	-
68	59.54	1992	13-epi-Dolabradiene	0.12	272.476	$C_{20}H_{32}$	NA

Note: RT (min): retention time (min); KI: Kovats index; MW (g/mol): molecular weight; MF: molecular formula; NI: unidentified compound; NA: Not available; CAS# number: Chemical Abstracts Service.

DISCUSSION

Quantitatively, the leaves of *V. agnus-castus* acclimatized in an arid desert region; represent a potential source of anthocyanidins (6.1±0.07 mg·g^{-1} DW). The results obtained in Tunisia on leaf samples from natural Mediterranean sites [42] expressed in malvidin equivalent are very low (0.38±0.01 mg·g^{-1} DW) compared to our results expressed in cyanidin equivalent. Anthocyanins can protect plants against damage caused by large amounts of ultraviolet radiation, and an immediate increase in their production is observed to compensate for this situation of abiotic stress. They can also absorb blue-green light to protect the plant against high light and drought.

The flavonic aglycones extracted from leaf samples, expressed in luteolin and quercetin, have a total content of 1.29±0.015 mg·g^{-1} DW, which is lower than that (2.23±0.02 mg·g^{-1} DW) of total flavonoids expressed in orientine obtained in leaves from the National Botanical Garden of Kyiv (Ukraine), located in a temperate region [79]. Flavonoids are present in all parts of the plant and are often found in the glycosylated form. Glycosylation has the effect of making them more water-soluble, thus allowing their accumulation in the vacuoles of the cells of the epidermis and the mesophyll of the leaves [8]. Flavones and flavonols not usually found together in the same compartment of the plant [52]. Only aglycones (genins) are located in the cuticles of leaves or in the form of crystals in the cells of some Cactaceae and plants in arid regions. These act in the defense systems of plant cells in response to certain stresses such as ultraviolet radiation [35].

Qualitatively, five phenolic acids (gallic, chlorogenic, caffeic and *trans*-cinnamic) and derivative (vanillin), two flavones (luteolin and apigenin) and two flavonols (isorhamnetin and casticin) were identified in these samples. Previous phytochemical analysis revealed the presence of phenolic acids and flavonoids in the leaf extracts of *V. agnus-castus* [66]. Şarer and Gökbulut [67] reported that phenolic acids (caffeic and

chlorogenic) content in the leaves and fruits of *V. agnus-castus* collected from Turkey have been determined by HPLC. Küçükboyaci and Şener [38] identified flavonic aglycones (apigenin, casticin and luteolin) in leaf, fruit and seed extracts, while Hirobe et *al.* [31] were able to isolate luteolin and isorhamnetin at the root level. Phenolic compounds accumulate in different cellular compartments of the plant under the influence of several stimulating factors [34]. These compounds are involved in many interactions of the plant with the biotic and abiotic factors of its environment [22].

Hydrodistillation of *V. agnus-castus* leaves from the Algerian Sahara yielded a golden-yellow oil with a pleasant aroma characterizing the Lamiaceae family, which is similar to that of Neves and da Camara [56]. Yields obtained from leaves of *V. agnus-castus* collected around the world summarized in table 5. The yield (%) of the essential oil of our samples is proportional to that obtained in Italy (Calabria) [21], it is more or less close to those reported in Morocco (Tétouan) [41], Montenegro (Igalo) [71] and Albania (Tepelenë and Divjakë) [12]. The yields obtained in Nigeria (Ilorin) [29] and in Iran (Maraghe) [37] are higher, while those indicated in Benin (Godomey) [55] and Brazil (Recife) [56] lower compared to the yield of our samples. The results given by Habbab et *al.* [25] in Algeria (Fendi) and Ekundayo et *al*. [16] in Nigeria (Ife) do not corroborate our results; this would probably be under the effect of the hydrodistillation time, the nature (dry or fresh) of the sample analyzed, the harvest period and the location of the study station.

Compared to some studies, the essential oil of *V. agnus-castus* leaves is marked by a large quantitative and qualitative variability, with several chemotypes described according to the sample geographic origin (table 6). We note that our samples and those of Habbab et *al.* [25] collected in Algeria in regions with a subtropical desert climate (BWh) show a proportionality between total monoterpenes (TMT) and total sesquiterpenes (TST). GC-MS analysis of the essential oil of leaves harvested from several localities around the world dominated by TMT according to the Köppen classification. These regions have either a tropical savanna climate (Aw) [16, 55], a hot-summer Mediterranean climate (Csa) [71], a warm temperate climate (Csa) [12], a cold semi-arid climate (BSk) [37] or a tropical monsoon climate (Am) [56].

In addition, the oxygenated compounds proportionally distributed in our samples, the oxygenated monoterpenes (OMT) and oxygenated sesquiterpenes (OST) represent respectively 32.12% and 31.84% of the total area of the essential oil. Oxygenated monoterpenes are mostly dominant in the different essential oils obtained in other regions of the world.

This is valid for regions with a subtropical desert climate (BWh) [25], tropical savanna climate (BWh) [16, 29, 55], hot-summer Mediterranean climate (Csa) [71] and warn temperate climate (Csa) [12].

Four to six major molecules (area > 7% for each compound) characterize the essential oil of samples taken from low altitude regions (0 to 28 m above sea level) [12, 56, 69, 71]. While samples taken from regions at approximately high altitude contain two to three major substances (area > 7%) [12, 16, 21, 25, 29, 37] as is the case for our essential oil.

The essential oil of *V. agnus-castus* from this study station, growing in severe climatic conditions, characterized by the presence of a major tricyclic oxygenated sesquiterpene (31.5%), named caryophyllene oxide or caryophyllene epoxide. This molecule is absent or very rare in all other essential oils indicated in the literature (table 6).

These are rather marked by acyclic oxygenated monoterpene (1,8-cineole or eucalyptol) [12, 16, 21, 25, 55, 56, 69, 71], a bicyclic hydrocarbon sesquiterpene (β-caryophyllene) [12] or by a bicyclic hydrocarbon monoterpene (α- and β-pinene) [29, 37].

The oxidation of β-caryophyllene leads to caryophyllene oxide [19], which would be a response of the plant to the severe conditions of the desert environment. Caryophyllene oxide has been found to exhibit antioxidant and antiviral [30], anti-inflammatory [72], anti-carcinogenic [81] and analgesic [70] properties.

The quantitative and qualitative variability of foliar essential oil exposed to the severe climatic conditions of the Algerian Sahara would be the consequence of several pedoclimatic factors such as geographical position, temperature and precipitation [11], soil nutrient content [48], saline or thermal water deficit [82], harvest season, physiological state and genotype of the plant [18, 33, 47]. Also, the extraction method, time of treatment with the solvent, and conditions of drying and storage of the samples [5]. These constraints could induce the preferential synthesis of precise metabolites [7, 23], which would be specific indicators that can differentiate between provenances of the plant; this inevitably leads to the existence of different chemotypes for the same species from different localities.

In conclusion, the phytochemical analysis of our *Vitex agnus-castus* population acclimatized to the severe environmental conditions of the Algerian Sahara made it possible to quantify three classes of flavonoids (anthocyanidins, flavones and flavonols) and to detect 40 constituents in phenolic extracts. The foliar essential oil is much diversified in volatile compounds (68); its chromatographic profile is mainly terpene, dominated by caryophyllene oxide (31.5%). Comparative analysis between the aromatic components of our population and those reported throughout the world showed that the biotope would be partly involved in the phytochemical polymorphism of *Vitex agnus-castus* species. In perspective, a complete identification of the phenolic compounds of the species is desirable in order to better understand the strategies of adaptation to environmental conditions, which could enrich the current study.

Table 5. Yields of essential oils from *V. agnus-castus* leaves collected from different regions in the world

Characteristics	Our study	[25]	[41]	[55]	[16]	[29]	[21]	[71]	[56]	[12]	[12]	[37]
Country	Algeria	Algeria	Marocco	Benin	Nigeria	Nigeria	Italy	Monténégro	Brazil	Albania	Albania	Iran
Study area	Ouled Aïssa	Fendi	Tétouan	Godomey	Ife	Ilorin	Calabria	Igalo	Recife	Tepelenë	Divjakë	Maraghe
Leaves	Dried	Dried	Dried	Fresh	Fresh	Fresh	-	Dried	Fresh	Dried	Dried	Dried
The extraction method	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr	Hydr
Extraction time (h)	3	6	4	4	-	3	-	3	2	-	-	2.5
Apparatus type	Clev	-	Clev	Clev	-	Clev	-	Clev	Clev	Clev	Clev	Clev
Yield (%)	0.37±0.04	5.5	0.49	0.2	0.023	0.8	0.35	0.56	0.22	0.61	0.69	1.3

Note: Clev: Clevenger; Hydr: Hydrodistillation; -: not indicated

Table 6. Geographical, climatic and analytical characteristics of the essential oil of *V. agnus-castus* leaves collected in some regions of the world

Characteristics	Our study	[25]	[55]	[16]	[29]	[71]	[21]	[69]	[12]	[12]	[37]	[56]
Continental location	North Africa	North Africa	West Africa	West Africa	West Africa	Southern Europe	Southern Europe	Southern Europe	Southeast Europe	Southeast Europe	West Asia	South America
Country	Algeria	Algeria	Benin	Nigeria	Nigeria	Montenegro	Italy	Italy	Albania	Albania	Iran	Brazil
Locality	Timimoun	Bechar	Atlantique	Osun	Kwara	Herceg Novi	Catanzaro	Salerne	Gjirokastër	Fier	East Azerbaijan	Pernambuco
Study area	Ouled Aïssa	Fendi	Godomey	Ife	Ilorin	Igalo	Calabria	Cilento	Tepelenë	Divjakë	Maraghe	Recife
Altitude (m)	356	792	13	280	320	0	110	28	336	10	1 464	4
Latitude	29°25'06"N	31°50'23"N	6°22'00"N	7°28'00"N	8°29'48"N	42°27'19"N	38°06'37"N	40°15'37"N	40°18'00"N	40°59'47"N	37°23'31"N	8°03'14"S
Longitude	0°05'15"W	1°28'34"W	2°21'00"E	4°34'00"E	4°32'32"E	18°30'26"E	15°39'40"E	15°04'32"E	20°01'00"E	19°31'58"E	46°14'21"E	34°52'51"W
Köppen climate classification	BWh Subtropical desert	BWh Subtropical desert	Aw Tropical savane	Aw Tropical savane	Aw Tropical savane	Csa Hot-summer Mediterranean	Csa Hot-summer Mediterranean	Csa Warm temperate	Csa warm temperate	Csa warm temperate	BSk Cold semi-arid	Am Monsoon tropical
Analysis method	GC-MS	GC-MS	GC/GC-MS	GC/GC-MS	GC/GC-MS	GC/GC-MS	GC-MS	GC-MS	GC/GC-MS	GC/GC-MS	GC-MS	GC-MS
Main constituent (%)	- Caryophyllene oxide (31.5) - Terpinen-4-ol (7.23)	- 1,8-Cineole (18.27) - β-Caryophyllene (8.6)	- 1,8-Cineole (22.6) - Sabinene (19.4) - β-Farnesene (7.7)	- 1,8-Cineole (50.9) - Sabinene (10.8) - α- and β-Pinene (9)	- α- and β-Pinene (29.1) - Viridiflorol (9.8) - *cis*-Ocimene (8.4)	- 1,8-Cineole (22) - α-Pinene (9.4) - β-Farnesene (9.4) - β-Caryophyllene (8.2) - Terpinen-4-ol (7.8)	- 1,8-Cineole (35.2) - α- and β-Pinene (7.6)	- 1,8-Cineole (15.6) - β-Caryophyllene (8.9) - β-Farnesene (8.6) - α-Terpineol (8.5)	- 1,8-Cineole (31.84) - Sabinene (16.98)	- β-Caryophyllene (20.33) - Sabinene (18.65) - 1,8-Cineole (15.97) - α- and β-Pinene (8.01)	- α-Pinene (19.48) - Limonene (13.37) - β-Caryophyllene (8.55)	- 1,8-Cineole (17.6) - β-Farnesene (13.6) - β-Caryophyllene (9.4) - Isodaucene (8.9) - α-terpenyl acetate (8.3) - α-Terpineol (7.4)
Chemical diversity	68	20	21	31	34	46	38	-	39	43	32	47
TMT	46.5	32.55	66.2	90	77.7	63.1	-	-	72.73	57.14	64.68	55.3
TST	42.37	26.69	17.6	4.8	18.2	26.7	-	-	15.66	25.73	21.97	41.6
HMT	14.38	4.46	37.2	30.3	53.2	23	-	-	26.6	33.85	54.95	-
HST	10.53	19.91	15.9	2.4	8.3	19.4	-	-	-	-	16.8	-
OMT	32.12	28.09	29	59.7	24.5	40.1	-	-	46.13	23.29	9.73	-
OST	31.84	6.78	1.7	2.4	9.9	7.3	-	-	-	-	5.17	-
DT	0.28	1.72	-	-	-	7.5	-	-	3.93	7.02	-	1.8
Total area (%)	98.97	66.38	88.9	94.8	98.5	98.4	95.8	-	93.05	89.89	99.36	98.8

Note: TMT: Total monoterpenes; TST: Total sesquiterpenes; HMT: Hydrocarbon monoterpenes; HST: Hydrocarbon sesquiterpenes; OMT: Oxygenated monoterpenes; OST: Oxygenated sesquiterpenes; DT: Diterpenes; (-): No indicate

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