Composition and Biological Properties of the Volatile Oil of *Artemisia gorgonum* Webb

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The chemical composition of *Artemisia gorgonum* Webb essential oil from Cape Verde was analyzed by GC and GC/MS. A total of 111 volatile compounds, accounting for 94.9% of the essential oil, were identified by GC and GC/MS. The major compounds were camphor (28.7%), chrysanthenone (10.8%), lavandulyl 2-methylbutanoate (9.5%), α-phellandrene (5.5%), lavandulyl propanoate (4.2%), camphene (4.0%), and *p*-cymene (3.4%). The volatile oil of this endemic plant, which is used in Cape Verdean folk medicine against several ailments, was tested for its antioxidant and antimalarial properties, and was found to exhibit free-radical scavenging on 1,1-diphenyl-2-picrylhydrazyl (DPPH), prevention of lipid peroxidation–*in vitro* by TBARS (thiobarbituric acid reactive species) assay, and antiplasmodial activity.

1. Introduction. – Aromatic plants are frequently used in traditional medicine, and their essential oils, mixtures of volatile compounds normally isolated by steam distillation, have been known since antiquity to possess many biological properties [1]. Consequently, they have some therapeutic potential. The major constituents of many of these oils are phenolic compounds (terpenoids and phenylpropanoids) such as thymol, carvacrol, or eugenol, of which antimicrobial and antioxidant activities are well-documented. Nevertheless, aromatic plants producing non-phenolic essential oils, like some *Artemisia* species, are also used as spices, and in folk remedies as antiseptics [1].

The large Asteraceae family contains 25,000–30,000 species belonging to over 1000 genera. The chemistry of members of this family has been studied intensively, and more than 28,000 substances have been identified so far [2]. The genus *Artemisia*, one of the largest and most widely distributed genera of the Asteraceae, is usually represented by small herbs and shrubs. Among them, the endemic Cape Verdean *Artemisia gorgonum* Webb known as '*losna*' by Cape Verdean people is an aromatic plant used in local folk medicine for treatment of many ailments, such as fever, headache, *etc.* [3].

A. gorgonum has been the subject of a recent phytochemical study, which revealed the presence of 13 sesquiterpene lactones that exhibited antiplasmodial activities [4]. Many species of the genus *Artemisia* have proved to be rich in substances with several medicinal uses, including antimalarial, antiviral, antitumor, spasmolytic, and others [5]; among them, artemisinin is undoubtedly a lead compound as a potent antimalarial

agent [6]. The essential oils of several species of this genus are also well-known to have important biological activities [7][8]. However, to the best of our knowledge, there is no report in the literature regarding the chemical analyses and biological activity of the volatile-oil constituents of this species.

In this context, here we describe the chemical composition and biological properties of the volatile-oil constituents isolated from the aerial parts of *Artemisia gorgonum* Webb.

2. Results and Discussion. – 2.1. *Chemical Composition*. The steam distillation of air-dried aerial parts of *A. gorgonum* gave a volatile oil with 0.4% (*w*/*w*) yield. This value indicates the low content of volatiles in this species. To identify the chemical constituents, the volatile oil from *A. gorgonum* was subjected to GC/MS analysis. The compounds identified in the oil are listed in the *Table* according to their order of elution on a *HP-1* column.

One hundred and eleven volatile compounds, representing 94.9% of the total composition, were identified; all of them are reported for the first time in *A. gorgonum*, with the exception of arborescin, which was found previously in an EtOH extract of the aerial parts [4]. Among all molecules identified and quantified, the major components included camphor (**1**; 28.7%), chrysanthenone (**2**; 10.8%), lavandulyl 2-methylbutanoate (**3**; 9.5%), α-phellandrene (**4**; 5.5%), lavandulyl propanoate (**5**; 4.2%), camphene (**6**; 4.0%), and *p*-cymene (**7**; 3.4%); some other important components such as lavandulyl butyrate (**8**; 2.2%), arborescin (**9**; 2.0%), and geranyl valerate (**10**; 1.7%) were also identified (*Fig. 1*).

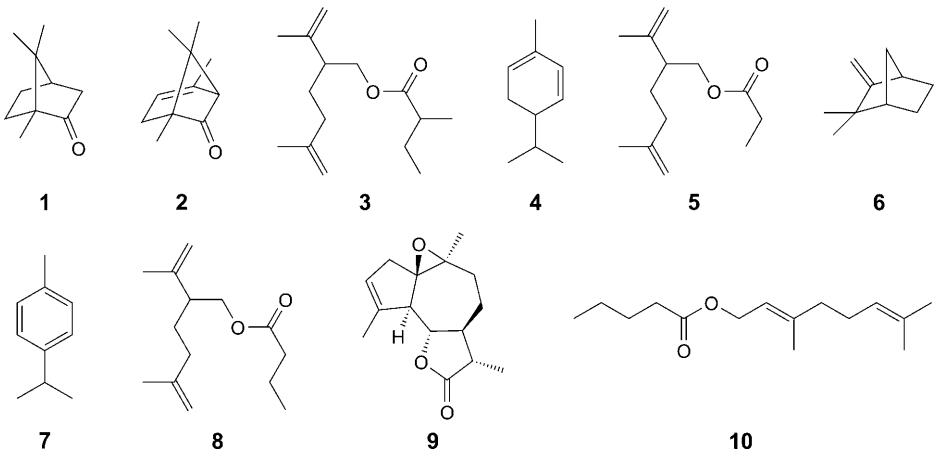

Fig. 1. *Representative volatile compounds of* Artemisia gorgonum *essential oil*

2.2. *Biological Studies.* Volatile oils can exhibit a large spectrum of biological activities such as antiviral, antibacterial, antimicrobiological, anti-inflammatory, antimalarial, and antioxidant properties [9]. The volatile oil of *A. gorgonum* from the aerial parts was assayed for antimalarial and antioxidant activities.

The antimalarial activity of *A. gorgonum* volatile oil, related to its ability to inhibit the growth of *Plasmodium falciparum* (FcB1) strain *in vitro*, was evaluated according

Table. *Chemical Composition of* A. gorgonum *Volatile Oil from Cape Verde*

Compound	$RI_{HP\text{-}1}$	$RI_{HP\text{-}20M}$	Area [%]
3-Methylbutan-1-ol	716	1184	tr[a])
2-Methylbutan-1-ol	720	1109	0.1
Methyl isovalerate	764	1008	tr
Isoamyl formate	773	1058	tr
Hexanal	780	1084	0.1
2-Furfural	815	1449	tr
2-Methylpentan-1-ol	826	1266	tr
(*E*)-Hex-2-enal	835	1268	tr
Ethyl 2-methylbutanoate	837	1207	tr
Ethyl isovalerate	840	1060	tr
Propyl isobutyrate	844	1049	tr
Hexan-1-ol	852	1059	0.1
Heptan-2-one	871	1044	tr
Heptan-2-ol	881	1284	tr
Isobutyl isobutanoate	902	1353	tr
Tricyclene	921	1010	0.2
α-Thujene	925	1036	0.1
Benzaldehyde	936	1502	tr
α-Pinene[b])	940	1037	1.6
Propyl isovalerate	943	1283	tr
Camphene[b]) (**6**)	947	1084	4.0
6-Methylhept-5-en-2-one	964	1335	0.1
Sabinene	969	1132	0.2
β-Pinene	972	1124	0.7
6-Methylhept-5-en-2-ol	974	1038	tr
2-Pentylfuran	978	1229	0.1
Myrcene	983	1338	0.8
Isobutyl 2-methylbutanoate	989	1124	tr
Isobutyl isovalerate	991	1171	tr
α-Phellandrene[b]) (**4**)	993	1184	5.5
Isoamyl isobutanoate	996	1187	0.2
2-Methylbutyl isobutanoate	1002	1174	0.1
α-Terpinene	1013	1189	tr
p-Cymene[b]) (**7**)	1015	1270	3.4
β-Isophorone	1016	1370	tr
1,8-Cineole	1018	1228	tr
β-Phellandrene	1023	1216	0.1
Limonene[b])	1025	1206	0.6
δ-3-Carene	1031	1186	tr
(*E*)-β-Ocimene	1038	1269	0.1
γ-Terpinene	1048	1250	0.1
cis-Sabinene hydrate	1079	1216	0.1
p-Cymenene	1081	1252	tr
Terpinolene	1083	1250	tr
Filifolone	1085	1546	2.0
2-Phenylethanol	1086	1859	tr
Isoamyl 2-methylbutanoate	1088	1273	0.4
Linalool	1090	1549	0.1
Isoamyl isovalerate	1092	1287	0.5
Amyl isovalerate	1094	1338	0.2

Table (cont.)

Compound	$RI_{HP\text{-}1}$	$RI_{HP\text{-}20M}$	Area [%]
Chrysanthenone[b]) (**2**)	1100	1546	10.8
trans-*p*-Menth-2-en-1-ol	1105	1565	0.1
Camphor[b]) (**1**)	1128	1519	28.7
trans-Verbenol	1131	1549	0.2
(*E*)-Non-2-enal	1135	1287	0.1
Borneol	1152	1697	0.1
Lavandulol[b])	1155	1517	0.9
p-Cymen-8-ol	1165	1846	tr
Terpinen-4-ol	1169	1626	0.2
Methyl salicylate	1177	1752	tr
α-Terpineol	1184	1694	0.2
Verbenone	1194	1730	0.1
α-Phellandrene oxide[b])	1196	1848	0.9
Decanal	1198	1485	0.1
trans-Piperitol	1202	1770	tr
Nerol	1216	1757	0.1
trans-Chrysanthenyl acetate	1218	1547	0.2
Hexyl 2-methylbutanoate	1222	1418	tr
Hexyl isovalerate	1228	1433	tr
Geraniol	1235	1776	tr
Isopiperitenone	1239	1722	0.4
Geranial	1245	1731	0.1
Thymol	1270	2105	0.1
Lavandulyl acetate[b])	1274	1433	1.7
Carvacrol	1297	2159	0.1
Hexyl tiglate	1310	1621	0.1
Eugenol	1351	2103	0.2
Lavandulyl propanoate[b]) (**5**)	1371	1534	4.2
Hexyl hexanoate	1374	1621	0.1
α-Copaene	1398	1519	tr
α-Cedrene	1410	1602	tr
Lavandulyl isobutyrate	1412	1625	0.7
β-Caryophyllene	1431	1617	0.5
γ-Decalactone	1437	2101	tr
trans-β-Farnesene	1448	1600	0.2
Lavandulyl butanoate[b]) (**8**)	1469	1656	2.2
Dehydrosesquicineole	1471	1780	0.3
2-Phenylethyl isovalerate	1473	1596	0.2
ar-Curcumene	1475	1786	0.7
γ-Curcumene	1476	2101	0.5
Germacrene D	1478	1714	0.3
β-Selinene	1480	1764	0.3
β-Himachalene	1495	1718	0.8
Lavandulyl 2-methylbutanoate[b]) (**3**)	1497	1779	9.5
β-Curcumene	1510	1756	0.1
δ-Cadinene	1524	1776	tr
(*E*)-Nerolidol	1552	1727	0.4
Neryl isovalerate	1574	1574	0.1
Geranyl isovalerate	1593	1895	0.3
Caryophyllene oxide	1595	2000	0.3

Table (cont.)

Compound	$RI_{HP\text{-}1}$	$RI_{HP\text{-}20M}$	Area [%]
Neryl tiglate	1601	1982	tr
Neryl valerate	1610	1930	0.5
Geranyl valerate[b]) (**10**)	1632	1960	1.7
β-Eudesmol	1643	2248	0.1
β-Bisabolol	1666	2014	0.1
α-Bisabolol	1672	2212	1.1
Chamazulene	1715	1991	0.1
β-Costol	1759	2421	0.5
Nonadecane	1900	1900	0.1
Arborescin (**9**)	1922	2740	2.0
Heneicosane	2100	2100	0.1
Monoterpene hydrocarbons			17.4
O-Containing monoterpenes			64.4
Sesquiterpene hydrocarbons			3.5
O-Containing sesquiterpenes			4.8
Aliphatic compounds			4.4
Phenols			0.4
Total			**94.9**

[a]) tr = Trace ($< 0.1\%$). [b]) Identification by injection of an authentic sample and mass spectrometry.

to the method described in [10]. The results showed a mild antiplasmodial activity for *A. gorgonum* volatile oil (IC_{50} 5.2 ± 0.7 μg/ml and IC_{90} 8.7 ± 0.9 μg/ml). Chloroquine was used as positive control (IC_{50} 0.02 ± 0.01 μg/ml and IC_{90} 0.05 ± 0.02 μg/ml).

To establish the antioxidant activity of this volatile oil, we used two well-established *in vitro* assays. The first is based on the free-radical-scavenging capacity of the stable DPPH (=1,1-diphenyl-2-picrylhydrazyl) radical, and the second concerns the spectrophotometric detection of TBARS (=thiobarbituric acid reactive species), namely of malonaldehyde (MDA), one of the secondary lipid peroxidation products, whose quantification gives a measure of the extent of lipid degradation.

For the first assay, solutions with volatile-oil concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.2 mg/ml, and different doses of ascorbic acid (positive control) were prepared to evaluate the DPPH radical-scavenging capacity. The respective scavenging capacities ranged from $17.6 \pm 1.2\%$ to $80.0 \pm 1.3\%$ for the volatile-oil (*Fig. 2*) with an EC_{50} value of 0.48 ± 0.02 mg/ml. For the second test, different concentration of volatile oil, 20, 50, 100, 150, 200, 250, and 500 μg/ml, and BHT (butylated hydroxytoluene = 2,6-di(*tert*-butyl)-4-methylphenol) as positive control also showed antioxidant activities in a dose-dependent manner (*Fig. 3*) and had $42.60 \pm 0.09\%$ to $81.35 \pm 0.07\%$ inhibition on lipid peroxidation. The IC_{50} value was found to be 0.06 ± 0.04 mg/ml.

These results demonstrated that *A. gorgonum* essential oil has an effective activity as a hydrogen donor and as a primary antioxidant by reacting with the free radicals. This may be responsible for the suppression of autooxidation, both in DPPH and thiobarbituric acid assays. It is known that most natural antioxidants often work synergistically to produce a broad spectrum of antioxidative activity that creates an

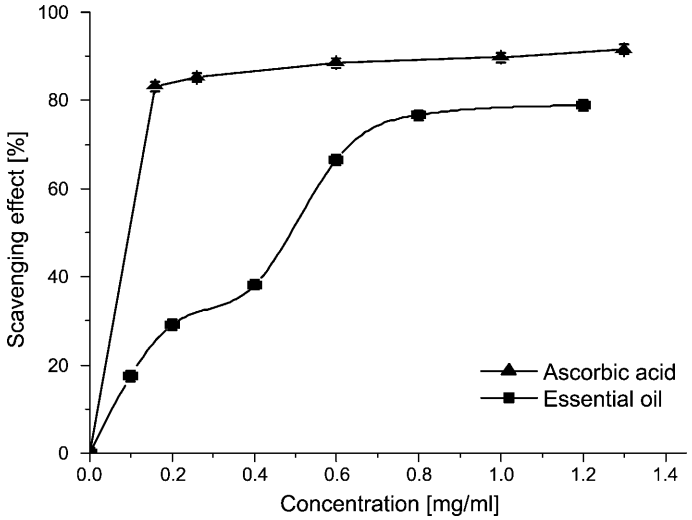

Fig. 2. *Scavenging effect of different doses of ascorbic acid and* A. gorgonum *volatile oil on DPPH radicals.* Values are expressed as mean ± standard deviation ($n=3$). Ascorbic acid was used as the standard. Coefficients of variance (CV) are less than 12%.

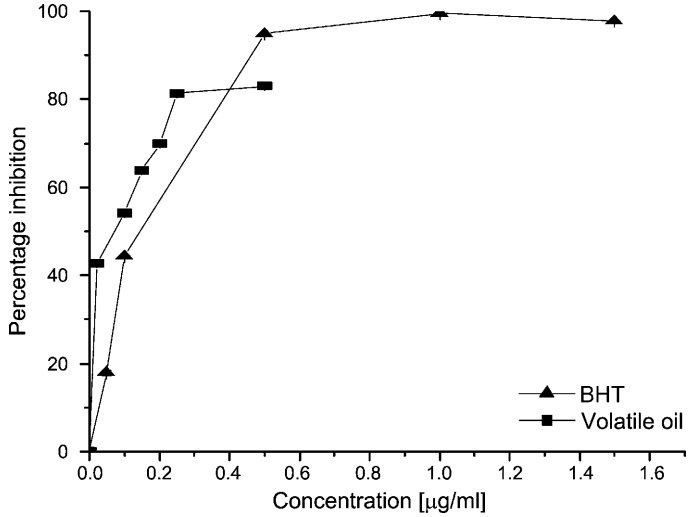

Fig. 3. *Percentage inhibition of lipid peroxidation by different doses of butylated hydroxytoluene* (BHT) *and of* A. gorgonum *volatile oil.* Values are expressed as mean ± standard deviation ($n=3$). BHT was used as the standard. Coefficients of variance (CV) are less than 12%.

effective defense system against free-radical attack. This volatile oil could, thus, be used as a natural antioxidant in place of synthetic ones.

Oxygenated monoterpenes such as camphor, which is a representative component in *A. gorgonum* and some *Artemisia* essential oils [11–13], was reported to exhibit

antimicrobial activity [11][14] and antioxidant effects [15]. Arborescin was also identified and quantified (2.0%) in the volatile oil; this sesquiterpene lactone has been previously reported to inhibit the growth of *Plasmodium falciparum* strain *in vitro* [4]. However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the biological activities exhibited.

3. Conclusions. – The present results demonstrate that the *A. gorgonum* volatile oil has several biological properties, including a remarkable antioxidant activity, and it is a radical scavenger, which points out its effectiveness against diseases caused by over-production of radicals. Further studies are needed to evaluate the *in vivo* potential of this oil in animal models.

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Experimental Part

Plant Material. The aerial parts of *A. gorgonum* were collected in Santiago, Cape Verde islands, in March 2006. Taxonomical identification was performed by Dr. *Izildo Gomes* (Instituto Nacional de Investigação e Desenvolvimento Agrário, Cape Verde). A voucher specimen was deposited with the Herbarium of INIDA, Santiago, Cape Verde.

Isolation of Essential Oil. Aerial parts of *A. gorgonum* were harvested, washed with dist. water, and air-dried for *ca.* 3 d. The oil was obtained from 45.6 g of material by simultaneous distillation-solvent extraction with 50 ml of CH_2Cl_2 for 2 h (previously distilled). The extract was dried (anh. Na_2SO_4) and concentrated with a *Kuderna–Danish* apparatus to 1 ml, and then, with a gentle stream of N_2 until total elimination of the solvent. The yield was calculated according to the weights of oil and plant material before distillation.

GC and GC/MS Analyses. The GC and GC/MS analyses on a non-polar column were carried out using an *Agilent 6890 N* gas chromatograph equipped with a FID and a quadrupole *Agilent 5973* network mass-selective detector (EI mode at 70 eV, mass range of 35–400 amu). The gas chromatograph was equipped with *HP-1* fused silica column (50 m × 0.2 mm, 0.33 μm film thickness). The anal. parameters (identical for GC and GC/MS analyses unless specified) were: carrier gas He, 0.9 ml/min; the oven temp. was 50–250° at 2°/min and held isothermal for 40 min; injection mode, split ratio 1:100; injector temp. 250°. The FID temp. was set at 250°, and in the GC/MS analyses, the temps. of the ion source and transfer line were 170 and 280°, resp.

The GC and GC/MS analyses on a polar column were performed with a *Hewlett-Packard 5890* chromatograph equipped with a FID and a *HP 5970A* mass-selective detector (EI mode at 70 eV, mass range of 35–400 amu), under the following conditions: *HP-20M* fused silica column (50 m × 0.2 mm, 0.1-μm film thickness); injection mode, split ratio 1:100; oven temp., 60–220° at 2°/min and then held isothermal for 30 min; carrier gas, He (0.8 ml/min); injector and transfer line temps., 230°.

Compound Identification. The linear retention indices (*RI*s) of the compounds were determined relative to the retention times of a series of *n*-alkanes (C_7–C_{28} on the *HP-1* and *HP-20M* columns), and the percent compositions were obtained from electronic integration measurements without taking relative response factors into account. Peak identification was carried out by comparison of the mass spectra obtained on both polar and non-polar columns with mass spectra available on database of NIST, NBS, Adams 2001, *Wiley* libraries, and in-house Flavorlib library. The compound identification was finally confirmed by comparison of the relative *RI*s in both columns with those of relative standards or with published data [16–19].

Antioxidant Activity. 1,1-Diphenyl-2-picrylhydrazyl (*DPPH*) *Radical-Scavenging Assay.* The anti-oxidant activity of *A. gorgonum* essential oil was determined in terms of free-radical scavenging ability

according the method described in [20] with minor modifications. Basically, a 60 μM MeOH soln. of DPPH (980 μl; *Sigma-Aldrich Co.*, St. Louis, MO, USA), prepared daily, was placed in a spectrophotometer cuvette, and different concentrations of the essential oil (0.10–1.20 mg/ml) or ascorbic acid (standard; 0.16–1.30 mg/ml) in MeOH (*v*/*v*) soln. (20 μl) were added. The decrease in absorbance at 515 nm was determined in a *UV-1201* spectrophotometer (*Shimadzu*, Japan), until the reaction plateau step was reached. MeOH was used to zero the spectrophotometer. The EC_{50} values were determined from the plotted graph of scavenging activity *vs.* the concentration of samples, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out, and their scavenging effect was calculated based on the percentage of DPPH scavenged.

TBARS (= Thiobarbituric Acid Reactive Species) *Assay.* The lipid peroxidation assay as TBARS was carried out according to a modified method [21]. The reaction mixture containing, in a final volume of 1.1 ml, 100 μl of cerebral tissue (whole brain) and 1 ml (0.05M) of KH_2PO_4/K_2HPO_4 buffer, pH 7.4 in NaCl (0.9%), and seven concentrations of *A. gorgonum* essential oil (20–500 μg/ml) was incubated at 37° for 1 h. Then, 1 ml of thiobarbituric acid (TBA; 0.5%) and 1 ml of Cl_3CCOOH (20%) were added to the test tubes and were incubated at 100° for 60 min. After cooling, absorbance was measured at 532 nm against control and buffer, BHT (= butylated hydroxytoluene = 2,6-di(*tert*-butyl)-4-methylphenol) being used as reference compound. All the experiments were performed in triplicate, and the results were averaged. The inhibition percentage was determined by comparison of the results between the samples and control.

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