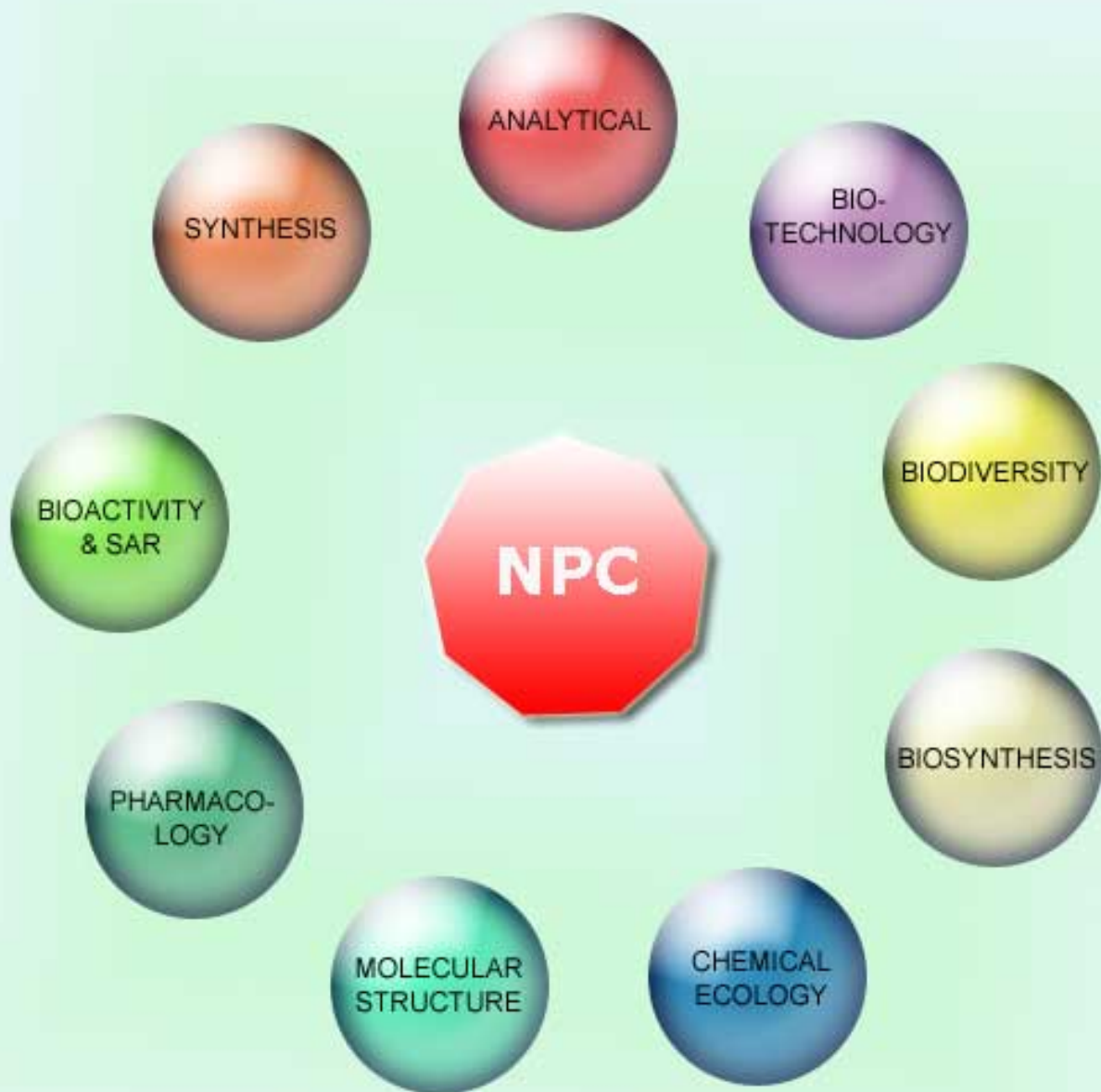


NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all
Aspects of Natural Products Research



Volume 4. Issue 9. Pages 1171-1318. 2009
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

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Composition and Antioxidant Properties of the Essential Oil of the Endemic Cape Verdean *Satureja forbesii*

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Received: January 26th, 2009; Accepted: March 31st, 2009

The chemical composition of essential oil from the air-dried aerial parts of *Satureja forbesii* (Benth.) Briq. from Cape Verde was studied by GC and GC/MS. Thirty-nine volatile compounds were identified of which geranial (42.0%) and neral (31.2%) were the major constituents. Using the 2,2-diphenyl-2-picrylhydrazyl free-radical scavenging method and the *in vitro* assay for prevention of lipid peroxidation by thiobarbituric reactive species, significant activities were evidenced.

Keywords: Essential oil, *Satureja forbesii*, Lamiaceae, antioxidant activity, DPPH, lipid peroxidation, geranial, neral.

The genus *Satureja* {family Lamiaceae (Labiatae), subfamily Nepetoideae, tribe Mentheae} [1] contains more than 200 species of aromatic herbs and shrubs, largely distributed from the Mediterranean region to Europe, West Asia, North Africa, the Canary Islands, and South America, with chemotypes of carvacrol, β -caryophyllene, citral, isomenthone, linalool and pulegone [2,3a-3e]. Eugenol, *p*-cymene, menthone, γ -terpinene and thymol have also been reported as other major constituents [4a-4d].

During recent years, antioxidant [2,3b,5,6], antibacterial, antifungal [3a,3b,7a-7e], antiviral [3d], antinociceptive, antiinflammatory [7d], antispasmodic and antidiarrhea effects have been reported for different species of *Satureja* growing in different parts of the world [8a,8b].

S. forbesii (Benth.) Briq., syn. *Micromeria forbesii* Benth., is an aromatic and endemic plant known as 'erva-cidreira' and used in folk medicine by Cape Verdeans, mainly as an infusion for the treatment of ailments, such as coughs, indigestion and diarrhea [8c]. However, to the best of the authors' knowledge,

detailed investigation of the volatile compounds of this species has not been undertaken. Therefore, the aim of the present work was to determine the antioxidant properties and chemical profile of the oil obtained from aerial parts of this species from Cape Verde.

Hydrodistillation of air-dried aerial parts of *S. forbesii* (Benth.) Briq. gave an essential oil with a yield of 0.4% (w/w). The compounds identified in the oil are listed in Table 1 according to their order of elution from a HP-1 column. Thirty-nine volatile compounds, representing 90% of the total composition, were identified in the essential oil. The most abundant components were geranial (42.0%) and neral (31.2%).

Compounds present in the essential oil of *S. forbesii* were able to scavenge DPPH radicals and also to prevent lipid peroxidation with IC₅₀ values of 0.60 ± 0.02 mg/mL and 0.17 ± 0.04 mg/ml, respectively. Geranial and neral are not efficient antioxidants [9a] and phenols were not detected in the essential oil. However, investigations have demonstrated that

Table 1: Chemical composition of *Satureja forbesii* from Cape Verde.

Compound	RI _{HP-1}	RI _{HP-20}	%
(<i>E</i>)-2-Hexenal ^a	832	1207	0.1
Benzaldehyde ^a	936	1502	0.1
6-Methyl-5-hepten-2-one ^a	964	1335	t
3-Octanone ^a	966	1236	1.3
1-Octen-3-ol ^a	968	1426	0.4
Sabinene ^a	970	1132	0.4
Dehydro-1,8-cineole	975	1198	0.7
3-Octanol ^a	986	1389	0.1
Phenylacetaldehyde	1004	1646	0.2
<i>p</i> -Cymene ^a	1015	1270	0.3
<i>cis</i> -Linalool oxide (furanoid)	1068	1423	0.2
<i>trans</i> -Linalool oxide (furanoid)	1078	1451	0.2
Linalool ^a	1090	1545	1.9
1-Octen-3-yl acetate	1094	1374	0.2
<i>trans</i> -Chrysanthemal	1109	1489	0.2
<i>cis</i> -Verbenol	1113	1547	t
<i>trans</i> -Verbenol	1131	1549	0.1
Borneol ^a	1152	1697	0.1
<i>p</i> -Cymen-8-ol	1165	1846	0.3
α -Terpineol	1184	1694	0.1
<i>trans</i> -Carveol ^a	1209	1790	t
β -Citronellol ^a	1214	1722	0.5
<i>cis</i> -Carveol ^a	1216	1820	t
Nerol ^a	1218	1757	t
Neral ^a	1227	1680	31.2
Piperitone	1242	1739	0.2
Geraniol ^a	1247	1797	0.2
Geranial ^a	1252	1731	42.0
Citronellyl formate	1256	1603	0.1
<i>p</i> -1-Menthen-3-one-8-ol ^b	1264	1949	2.8
Neric acid	1316	2322	t
Geranic acid	1361	2433	t
Geranyl acetate ^a	1364	1735	2.4
β -Bourbonene	1406	1546	0.2
α -Bergamotene	1436	1590	0.1
Myristicin	1488	2314	t
Caryophyllene oxide ^a	1595	2000	3.1
Humulene epoxide II	1625	2011	0.7
α -Cadinol	1628	2224	0.1

t = trace (<0.1%)

^aidentification by injection of an authentic sample and mass spectra.^btentative identification. Mass spectral data, m/z (%) = 82(100), 59(60), 95(32), 81(30), 97(28), 67(25), 69(25), 79(20), 110(13), 135(5), 153(3), 168[M⁺](4).

some essential oils rich in non-phenolic compounds also exhibit antioxidant potential [9b,9c]. In this context, the antioxidant effects of *S. forbesii* essential oil can be attributed to non-phenolic constituents. Nevertheless, it is difficult to assign the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to bioactivity. Possible synergistic and antagonistic effects of compounds in the oil should also be taken into consideration.

Satureja essential oils vary greatly but show a large prevalence of the two isomeric phenol monoterpenes (carvacrol and thymol) and their biosynthetic monoterpene precursors *p*-cymene and γ -terpinene. For example, the main components of *S. boissieri* oil from Turkey were reported to be carvacrol (40.8%) and γ -terpinene (26.4%) [10]. The main constituents of *S. brownei* oil from Venezuela were found to be pulegone (64.3%) and menthone (20.2%) [11], whereas the major component of *S. parvifolia* oil from Argentina was piperitone oxide [12a]. The predominant constituents of *S. boliviana* oil were γ -terpinene, β -caryophyllene and germacrene D [12a]. In the essential oil of *S. cuneifolia* [12b], thymol and carvacrol were the main components, while menthone and isomenthone were the main constituents of the oils of *S. boliviana* and *S. breviculix* from Peru [12c]. The composition of *S. forbesii* oil was similar to that of *S. punctata* from Zimbabwe, being dominated by either geranial + neral (61.1%) and α -farnesene (12.4%) [12d] or by geranial (52.5%) and neral (33.4%) [12e].

Experimental

Plant materials: *Satureja forbesii* (Benth.) Briq. samples were collected in Santiago, Cape Verde islands in March 2006 and identified by Dr Izildo Gomes. A voucher specimen was deposited in the Herbarium of INIDA, Santiago, Cape Verde.

Distillation: Aerial parts of the plants were washed with distilled water, and air-dried for 3 days. The oil was obtained from 45.6 g of material by simultaneous distillation-solvent extraction with 50 mL of dichloromethane (previously distilled) for 2 h. The extract was dried over anhydrous sodium sulfate and concentrated with a Kuderna-Danish apparatus to 1 mL and then, with a gentle stream of nitrogen 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: 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 a HP-1 fused silica column (50 m x 0.2 mm, 0.33 μ m film thickness). The analytical parameters (identical for GC and GC-MS analyses unless specified) were the following: carrier gas helium,

0.9 mL/min; the oven temperature was 50-250°C at 2°C/min and held isothermal for 40 min; injection mode, split ratio 1:100; injector temperature 250°C. The FID temperature was set at 250°C, and in the GC-MS analyses, the temperatures of the ion source and transfer line were 170 and 280°C, respectively.

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), using the following conditions: HP-20M fused silica column (50 m x 0.2 mm, 0.1 µm film thickness); injection mode, split ratio 1:100; oven temperature, 60-220°C at 2°C/min and then held isothermal for 30 min; carrier gas, helium (0.8 mL/min); injector and transfer line temperatures, 230°C.

The linear retention indices of the compounds were determined relative to the retention times of a series of *n*-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. Peak identification was carried out by comparison of the mass spectra obtained on both polar and non-polar columns with mass spectra available on the databases of NIST, NBS, Adams 2001, Wiley libraries and in-house Flavorlib library. The compound identification was finally confirmed by comparison of the relative retention indices in both columns with those of either relative standards or with published data [13a-13d].

DPPH radical scavenging activity assay: The DPPH radical scavenging activity was measured according to a reported method with minor modifications [14].

Basically, a 60µM methanolic solution of DPPH (980 µL) was placed in a spectrophotometer cell, and different concentrations of samples in MeOH solution (20 µL) were added (v/v). The decrease in absorbance at 515 nm was determined in a UV-1201 (Shimadzu, Japan) spectrophotometer until the reaction plateau step was reached. Triplicate measurements were carried out and EC₅₀ values were determined from the plotted graph of scavenging activity against the concentration of samples.

Lipid peroxidation assay: The lipid peroxidation assay as TBARS was carried out by a modified method [15]. The reaction mixture contained in a final volume of 1.1 mL, 100 µL cerebral tissue (whole brain), 1 mL (0.05 M) of KH₂PO₄-K₂HPO₄ buffer, pH 7.4 in NaCl (0.9%) and 6 concentrations of extract (20-250 µg/mL). The reaction mixture was incubated at 37°C for 1 h before 1 mL of thiobarbituric acid (0.5%) and 1 mL of trichloroacetic acid (20%) were added to the tubes, which were then incubated at 100°C for 60 min. After cooling, absorbance was measured at 532 nm against control and buffer, with BHT 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.

Acknowledgments - We are grateful to EGIDE for financial support (R.O). We also want to express thanks to UNESCO and Boehringer Ingelheim Fonds for financial support (E.L.R). We thank Dr Izildo Gomes for collection and careful taxonomical plant identification.

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Natural Product Communications

2009

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