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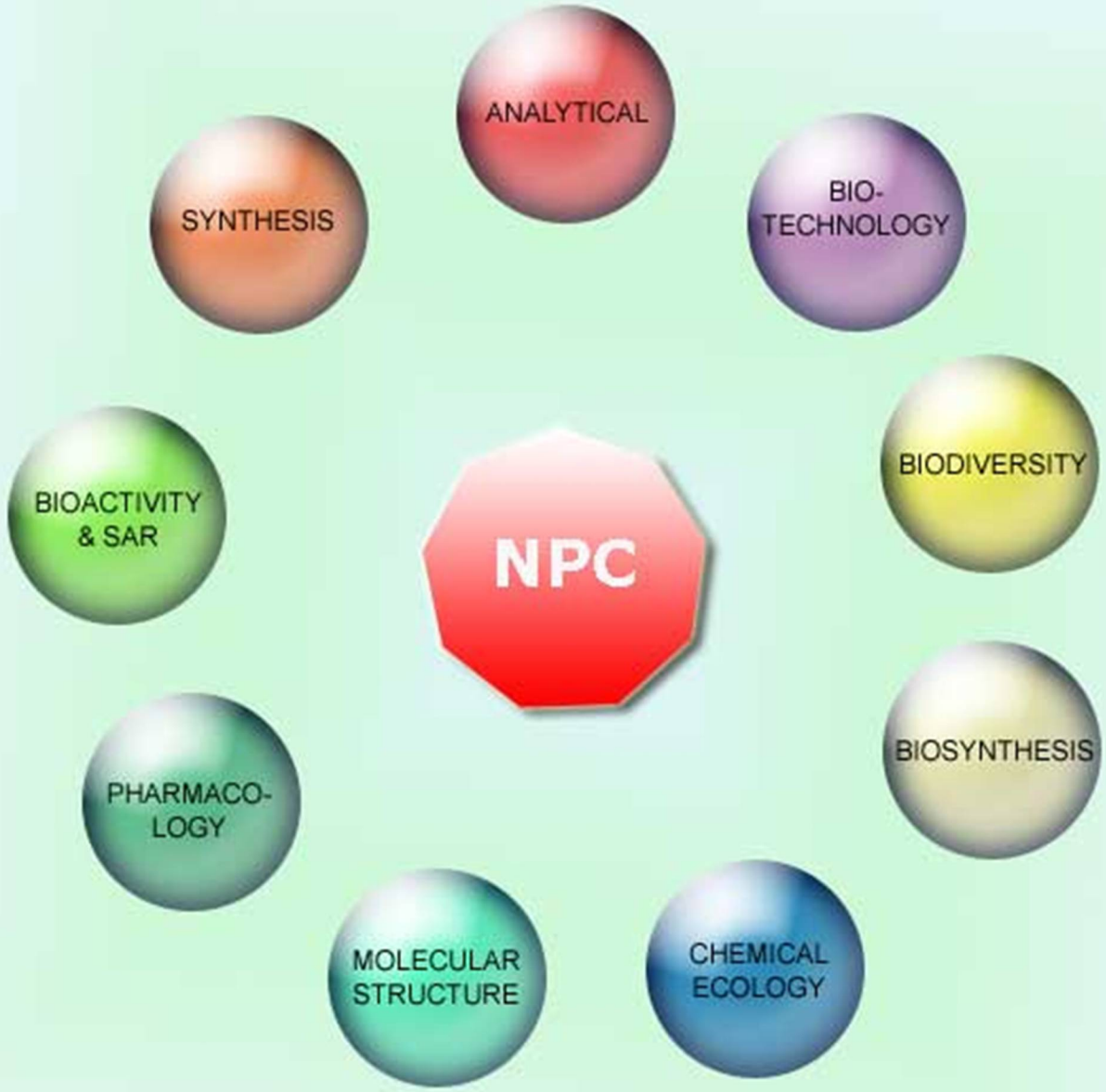


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# Antioxidant Activity and Chemical Composition of Essential Oil from *Atriplex undulata*

**Silvana A. Rodriguez[a] and Ana P. Murray[a,b*]**

[a]*Departamento de Química*, *Universidad Nacional del Sur*, *Av. Alem 1253*, *B8000CPB Bahía Blanca*, *Argentina*

[b]*Consejo Nacional de Investigaciones Científicas y Técnicas*, *Argentina*

*apmurray@uns.edu.ar*

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The essential oil from aerial parts (stems and leaves) of *Atriplex undulata* (Moq) D. Dietr. (Chenopodiaceae) has been studied for its *in vitro* antioxidant activity. The chemical composition of the oil obtained by hydrodistillation was determined by GC and GC-MS. The major constituents were *p*-acetanisole (28.1%), β-damascenone (9.3%), β-ionone (5.1%), viridiflorene (4.7%) and 3-oxo-α-ionol (2.2%). The antioxidant activity of the oil was determined by two methods: Crocin bleaching inhibition (Krel= 0.72 ± 0.15) and scavenging of the DPPH radical ($IC_{50}$ = 36.2 ± 1.6 μg/mL). The presence of active compounds like *p*-acetanisole, carvone, vanillin, 4-vinylguaiacol, guaiacol, terpinen-4-ol and α- terpineol could explain the antioxidant activity observed for this oil.

**Keywords:** *Atriplex undulata*, Chenopodiaceae, antioxidant activity, essential oil.

The genus *Atriplex* (family Chenopodiaceae) is represented in Argentina by 34 species. It is one of the most widespread plant genera, having colonized many arid and semi-arid regions [1]. *A. undulata*, commonly known as Zampa crespa or Cachiyuyo, is an endemic species that is found in Patagonia and the south of Buenos Aires province and which is used as an astringent and antiequimotic [2]. As part of our ongoing investigations of bioactive essential oils from native plants [3a-3d], we have studied the volatile oil composition of the aerial parts of *A. undulata,* growing wild in Bahía Blanca, Argentina.

Since oxygen reactive species, in particular free radicals, are involved in a variety of pathological conditions, such as cancer, cardiovascular disease, arteriosclerosis and neurodegenerative diseases, the study of new sources of natural antioxidants has received increasing attention in the last decades. Essential oils, which are complex mixtures of natural compounds, are a rich source of bioactive metabolites, in many cases with strong antioxidant capacity [4a-4g]. In the present work we are reporting the antioxidant activity of the essential oil of *A. undulata* being evaluated by two complementary test systems: as free radical scavenging activity by the reduction of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) [5], and by the Crocin bleaching inhibition method [6]. This is the first report of the chemical composition and biological activity of the essential oil from *A. undulata*.

The essential oil, obtained from fresh plant material, was analyzed by gas chromatography and mass spectrometry, leading to the identification of 34 components, which are listed in Table 1. The identification of each volatile compound was achieved by comparing its retention index with those cited in the literature, its mass spectrum with those of the database, and its retention time with authentic samples, when available.

The major components were *p*-acetanisole (28.1%), β-damascenone (9.3%), β-ionone (5.1%), viridiflorene (4.7%) and 3-oxo-α-ionol (2.2%). According to their functional groups, the most abundant types were ketones (53.3%), alcohols (21.6%) and hydrocarbons (10.2%). Aldehydes and other compounds, including sulfur and nitrogen containing compounds, were found in very low percentages. The essential oil possesses a high percentage of volatile phenols and other benzene derivatives (43.7%), followed by norisoprenoids (23.6%), sesquiterpene hydrocarbons (10.2%), oxygenated sesquiterpenes (7.8%) and oxygenated monoterpenes (6.6%).

**Table 1**: Chemical composition of essential oils from *A. undulata*.

| Compounds [a] | KI [b] | Percentage | Identification [c] |
|---|---|---|---|
| Benzyl alcohol | 1045 | 0.9 | $R_i$, MS |
| 2-Phenyl acetaldehyde | 1043 | 1.2 | $R_i$, MS |
| 1-Octanol | 1075 | 2.7 | $R_i$, MS |
| Guaiacol | 1088 | 1.1 | $R_i$, MS |
| 6-Methyl-3,5-heptadien-2-one | 1092 | 1.3 | $R_i$, MS |
| 2-Phenylethanol | 1120 | 1.3 | $R_i$, MS |
| 4 H-1,3 Oxazine 5,6-dihydro 2,4,4,6-tetramethyl | 1134 | 1.4 | MS |
| 1-Nonanol | 1157 | 2.4 | $R_i$, MS |
| Terpinen-4-ol | 1179 | 1.2 | $R_i$, MS |
| α-Terpineol | 1190 | 1.5 | $R_i$, MS |
| Coumaran | 1219 | 1.1 | $R_i$, MS |
| Carvone | 1245 | 1.9 | $R_i$, MS, S |
| 2-Cyclohexen-1-one,5-methyl-2-isopropyl | 1249 | 1.9 | $R_i$, MS |
| 4-Vinylguaiacol | 1320 | 1.2 | $R_i$, MS |
| *p*-Acetanisole | 1332 | 28.4 | $R_i$, MS |
| α-Cubebene | 1353 | 1.3 | $R_i$, MS |
| β-Damascenone | 1396 | 9.3 | $R_i$, MS |
| 1-(6,6-di-Methyl-2-methylenecyclohex-3-enyl)-buten-3-one | 1423 | 1.6 | $R_i$, MS |
| Vanillin | 1426 | 2.0 | $R_i$, MS, S |
| γ-Elemene | 1428 | 1.5 | $R_i$, MS |
| α-Ionone | 1430 | 1.8 | $R_i$, MS |
| Dihydro-β-ionone | 1435 | 1.5 | $R_i$, MS |
| Geranyl acetone | 1456 | 2.0 | $R_i$, MS |
| Alloaromadendrene | 1467 | 1.2 | $R_i$, MS |
| β-Ionone | 1475 | 5.1 | $R_i$, MS |
| Bicyclogermacrene | 1498 | 1.6 | $R_i$, MS |
| Viridiflorene | 1528 | 4.7 | $R_i$, MS |
| 1-Methyl-3[2-methylpropyl)thio]benzene | 1535 | 3.1 | $R_i$, MS |
| Spathulenol | 1568 | 1.2 | $R_i$, MS |
| Globulol | 1583 | 1.8 | $R_i$, MS |
| Guaiol | 1591 | 1.6 | $R_i$, MS |
| Viridiflorol | 1597 | 1.6 | $R_i$, MS |
| α-Cadinol | 1609 | 1.6 | $R_i$, MS |
| 3-Oxo-α-ionol | 1664 | 2.2 | $R_i$, MS |
| Total | | 96.4 | |

[a] Components are listed in order of elution on HP-5 column. [b] Ki retention index on HP-5column. [c] Ri = retention index identical to bibliography, MS = identification based comparison of mass spectra , S = retention time identical to authentic compound

C13-norisoprenoids are terpenoids commonly found in the flowers, fruits, and leaves of many plants that have interesting flavor aroma properties together with low aroma thresholds. Some of them, like β-damascenone, have a high sensorial impact on wine aroma [7a-7c]. This compound is the most intensive C13-norisoprenoid volatile aroma constituent of rose essential oil and shows antispasmodic activity [8a,8b].

Like other low-molecular weight phenolic compounds, *p*-acetanisole has antioxidant and antimicrobial activities and can be used as a food preservative [9]. *p*-Acetanisole is also used as an intermediate for pharmaceuticals, flavorings agents, agrochemicals and other organic compounds [10].

The ‘‘crocin bleaching assay” (CBA) has been applied for the evaluation of the antioxidant capacity of individual compounds, plant extracts, and plasma [11a-11d]. This method is interesting because it can be used in both lipophilic and hydrophilic environments [12], and is based on the bleaching of crocin as a result of its oxidation by a source of radicals, AAPH [2,2´-azo-bis(2-aminopropane) dihydrochloride]. In the present work, peroxyl radical scavenging was evaluated according to the protocol of Tubaro *et al.* [6]. The bleaching rate of crocin in the presence of the sample was monitored at 40ºC, by means of an UV-VIS spectrophotometer equipped with a thermostable cell block. The reaction was started by the addition of increasing amounts of sample (10-100 μM) to 1 mL of crocin aqueous solution. The bleaching rate of crocin by peroxyl radicals in the absence ($V_0$) and presence ($V_a$) of antioxidants was recorded for 10 min. Trolox was used as reference compound under the same experimental conditions. The interaction of peroxyl radical with *A. undulata* essential oil and its ability to scavenge peroxyl radical were further analyzed by the competition kinetics of crocin bleaching. These studies demonstrated that the essential oil of *A. undulata* possesses a moderate peroxyl radical scavenger activity with a relative constant ($TEV_{Krel}$) of 0.72 ± 0.15.

The antioxidant activity of the essential oil was also evaluated through its ability as free radical scavenger against DPPH. The preliminary test was performed using a rapid TLC screening method in order to detect the antioxidant activity. Then, a spectrophotometric assay was carried out at different concentrations of the essential oil and the percentage of DPPH reduction was calculated taking into account the absorbance of the blank solutions and the negative control. Trolox was used as reference compound under the same experimental conditions. Essential oil from *A. undulata* elicited a marked radical scavenger activity ($IC_{50}$ = 34.8 ± 0.08 μg/mL), with an antioxidant capacity comparable to that of Trolox ($IC_{50}$ = 22.9 ± 1.0 μg/mL).

The results obtained in both the DPPH and Crocin assays demonstrate that the essential oil of this plant has marked antioxidant activity that could be explained by the presence of components like *p*-acetanisole (28.4%) [13a,13b] and β-ionone (5.1%) [14]. The presence of other antioxidant compounds, even though at low concentrations, like terpinen-4-ol (1.2%), α-terpineol (1.5%), 4-vinylguaiacol (1.2%), guaiacol (1.1%) and the potent antioxidant carvone (1.9%), may also contribute to the radical scavenging activity of this oil [15-18].

## Experimental

***General:*** Gas chromatography-mass spectrometry analyses were performed with a Hewlett-Packard 6890 chromatograph connected to a Hewlett-Packard 5972A mass spectrometer equipped with a capillary column

(HP-5, 25 m x 0.25 mm, 0.25 μm film thickness). The carrier gas was helium with a flow rate of 1 mL/min. The GC oven temperature was held at 50ºC for 2 min, programmed at 5ºC/min to 200ºC, and then held at this temperature for 15 min. MS were recorded at 70 eV. Mass range was from *m/z* 35 - 350 amu. The temperature of the injection block was 250ºC. GC analyses were performed on a Shimadzu G14B chromatograph with a flame ionization detector on a DB-5 column (30 m x 0.25 mm, 0.25 μm film thickness) using the same analytical conditions as those for the GC-MS analyses. UV spectra were recorded on a GBC Spectral UV-VIS spectrophotometer. Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2`-azobis(2-aminopropane) dihydrochloride (AAPH), and Crocin were purchased from Sigma-Aldrich. Silicagel 60 F254 plates (0.2 mm thickness) were purchased from Merck.

***Plant material:*** Aerial parts of *A. undulata* (stems and leaves) were collected around Bahía Blanca city, Buenos Aires province, Argentina, in May 2007. The taxonomy of this material was determined by Dr María G. Murray. A voucher specimen (MGM 450) is kept in the "Herbario del Departamento de Biología, Bioquímica y Farmacia-Universidad Nacional del Sur (BBB)".

***Essential oil:*** Essential oil was obtained by hydrodistillation in a Clevenger type apparatus (3-4 h). The chemical composition of the oil was determined by GC and GC-MS. The compounds were identified by comparison of their retention indices (Kovats Indices) with those of known compounds [19] and also by comparison of their MS with those stored in the MS database (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas. Results are summarized in Table 1

***Crocin bleaching inhibition assay:*** The concentration of crocin to 10 μM was based on an extinction coefficient reported in the literature ($\varepsilon_{MeOH}$) = 1.33 × $10^5$ $M^{-1}$ $cm^{-1}$). In brief, the reaction mixture contained 10 μM crocin and increasing amounts of samples (from 10 to 100 μM). The reaction was started by the addition of 10 mM AAPH to the reaction mixture pre-equilibrated at 40°C. The bleaching rate of crocin ($V_0$), that is, the rate of its reaction with peroxyl radicals, was calculated by measuring the decrease of its absorption at 443 nm in the first 10 min of reaction, using a spectrophotometer equipped with a thermostable cell block, against a blank. In the presence of various concentrations of essential oil, the corresponding bleaching rates were termed $V_a$. Each kinetic analysis was compared with a kinetic crocin bleaching.

The slopes, calculated by linear regression analysis of the plot [antioxidant]/[crocin] versus $V_a/V_o$, indicate the relative capacities of the different molecules to interact with $ROO^{\bullet}$. The antioxidant capacity of the essential oil, relative to the activity of trolox, was calculated by dividing the slope of each compound by the slope of trolox. Three replicates were made for each sample.

***DPPH assay:*** Analytical TLC on silica gel plates were developed under appropriate conditions after application of 5 μL of oil solution (5 mg/mL, ethyl ether), dried and sprayed with DPPH solution (0.2%, MeOH). Five mins later, active compounds appeared as yellow spots against a purple background. BHT (1 mg/mL) was used as positive control. The spectrophotometric assay was carried out at 6 different concentrations of the essential oils, ranging from 10 to 100 μg/mL, prepared in MeOH: $CH_2Cl_2$ (95:5). A sample of 300 μL was mixed with 2.5 mL of 0.004% DPPH methanolic solution. The absorbance was measured at 517 nm after 30 min of incubation. The percentage of reduction was calculated taking into account the absorbance of the blank solutions (2.5 mL MeOH plus 300 μL oil solution) and the negative control (2.5 mL DPPH solution plus 300 μL of MeOH:$CH_2Cl_2$ 95:5). Trolox was used as the reference compound using the same experimental conditions. Each assay was run in triplicate. $IC_{50}$ values were determined with probit analysis (EPA Probit 1.4)

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