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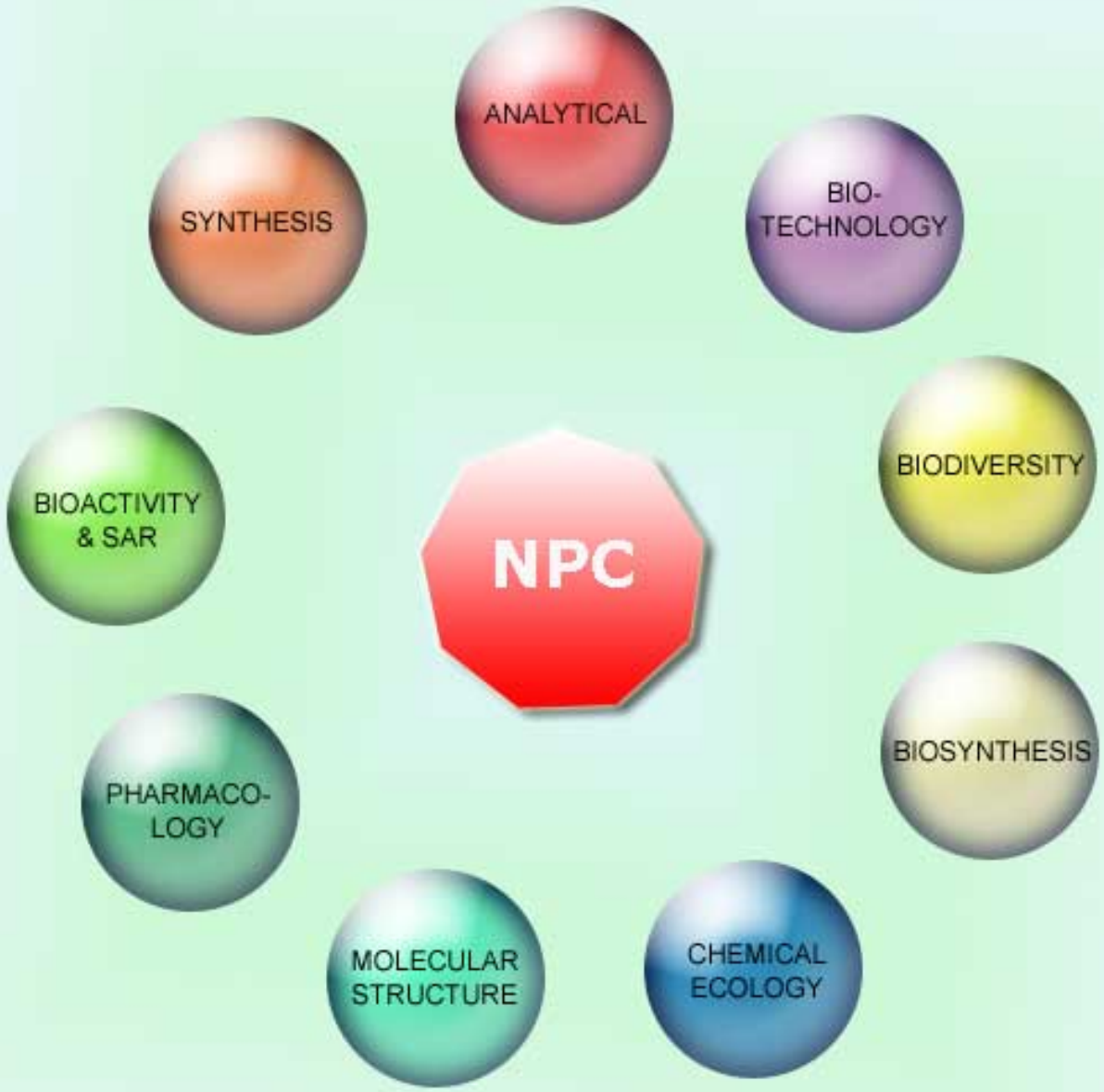

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Acetylcholinesterase Inhibition and Antioxidant Activity of Essential Oils from *Schinus areira* L. and *Schinus longifolia* (Lindl.) Speg.

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The essential oils of *Schinus areira* L. and *S. longifolia* (Lindl.) Speg. (Anacardiaceae) have been studied for their *in vitro* anti-acetylcholinesterase and antioxidant activities. The chemical composition of the oils obtained by hydrodistillation was determined by GC-MS. Fruit and leaf oils of *S. areira* were analyzed separately. The essential oil from *S. longifolia* elicited marked enzymatic inhibition (IC_{50} = 20.0 ± 1.0 μg/mL) and showed radical scavenger activity (IC_{50} = 25.2 ± 2.4 μg/mL). The essential oil from *S. areira* leaves was more active than that of the fruits in both bioassays.

Keywords: *Schinus areira*, *Schinus longifolia*, Anacardiaceae, acetylcholinesterase inhibition, antioxidants, essential oils.

The genus *Schinus* L. (Anacardiaceae) is represented in Argentina by 22 species. *S. areira* L. [syn. *S. molle* L. var. *areira* (L.) DC.], commonly known as 'aguaribay', 'árbol de la pimienta' or 'pimientero', is a native species from the north-west region of Argentina. The trees are 2 to 5 metres high with evergreen leaves made up of 2-25 pairs of leaflets, and the fruit is a purplish drupe, 5-6 mm in diameter [1a-c]. *S. longifolia* (Lindl.) Speg. is also a native species, which is very common throughout the centre of the country. Its common name is 'Molle', 'Incienso' or 'Trementina'. It is a bush or tree up to 5 metres in height, frequently parasitized by different insects that produce round galls [1b], with persistent leaves and a fruit that is a lilac colored drupe, 5-6 mm in diameter [2].

S. areira is used as a purgative, diuretic, parasiticide, insecticide, vulnerary, and topical disinfectant, and for the treatment of rheumatism, stomach upsets, menstrual disorders, bronchitis and conjunctivitis [3a,b]. The essential oil of *S. areira* has shown significant antibacterial and antifungal activity and has been reported as an allelopathic agent [3c-e]. *S. longifolia* is used in the treatment of colds, as an expectorant, laxative, emollient and for toothache [4a,b].

Acetylcholinesterase (AChE) inhibitors are used in medicine and in pest control. A few of them are in clinical use for the early stages of Alzheimer's disease (AD). Currently available approved drugs for the symptomatic treatment of AD patients inhibit AChE thus enhancing acetylcholine levels in the brain, which is associated with improvement of cognitive functions. Since these drugs are frequently associated with adverse effects, there is great interest in finding better AChE inhibitors.

A large body of evidence suggests that oxidative stress is closely related with the development and progression of a neurodegenerative disease like AD since free radical oxidative damage is extensive in the brains of AD patients [5]. Recent studies have

Table 1: Chemical composition of essential oils from *S. areira* and *S. longifolia*.

Compounds [a]	KI	Percentage		
		S. areira leaves	*S. areira* fruits	*S. longifolia*
Tricyclene	927	1.1	-	-
α-Pinene	939	5.4	3.1	0.2
Camphene	953	6.3	1.8	-
Sabinene	976	4.3	-	-
β-Pinene	981	2.7	0.8	-
β-Myrcene	991	3.4	19.3	-
2-Carene	1002	-	0.6	0.3
α-Phellandrene	1004	16.2	31.8	0.4
α-Terpinene	1017	0.3	-	-
p-Cymene	1026	5.1	3.0	-
β-Phellandrene	1031	17.6	19.9	2.3
α-Ocimene	1056	0.3	-	-
Terpinen-4-ol	1177	1.2	-	-
α-Terpineol	1188	-	-	1.7
Bornyl acetate	1285	1.4	-	-
α-Cubebene	1351	-	-	0.6
α-Copaene	1377	0.4	-	2.5
β-Caryophyllene	1418	2.0	2.0	9.0
β-Copaene	1432	-	-	0.7
Aromadendrene	1441	-	-	3.5
α-Humulene	1454	0.8	-	1.9
Alloaromadendrene	1461	0.5	-	1.1
β-Cadinene	1473	0.7	-	3.0
γ-Muurolene	1477	5.1	0.9	5.0
Germacrene D	1480	-	-	1.9
β-Selinene	1485	1.5	-	7.1
α-Muurolene	1499	1.3	0.9	1.9
α-Farnesene	1508	0.5	-	-
γ-Cadinene	1512	1.4	0.9	1.8
δ-Cadinene	1524	2.7	4.6	6.3
α-Elemol	1547	0.9	-	-
Germacrene B	1556	0.5	-	3.0
Palustrol	1560	0.6	1.2	-
Spathulenol	1565	0.6	-	5.1
Globulol	1575	0.5	1.1	14.2
Viridiflorol	1591	0.9	1.1	10.5
Cedrol	1595	-	-	4.0
Guaiol	1597	6.2	-	-
δ-Cadinol	1636	2.9	3.3	4.1
β-Eudesmol	1649	0.5	0.6	1.3
α-Cadinol	1653	1.0	-	-
Total		95.8	97.0	93.3

[a] Compounds are listed in order of elution from a DB-5 column

shown that antioxidant therapies are safe and produce no adverse, but beneficial effects in AD patients [6]. Treatment with antioxidants appears to be an alternative approach for slowing disease progression.

Essential oils have attracted attention as potential AChE inhibitors due to several recent reports on active oils and active terpenoids [7a-l]. Inhibition of AChE by different kinds of monoterpenoids has been evaluated and several studies have been conducted to identify structure-activity relationships [7b-g,7l].

As part of our ongoing research on bioactive natural compounds [8], essential oils from *S. areira* and *S. longifolia* were analyzed for AChE inhibitory and antioxidant capacity. The essential oils from the fruits and leaves of *S. areira* were separately studied. The oils, obtained from fresh plant material collected around Bahía Blanca city, were analyzed by gas chromatography and mass spectrometry. Their chemical composition is summarized in Table 1.

The essential oils were preliminary evaluated over TLC plates with detection of AChE inhibition [9]; all the samples showed enzymatic inhibition. Then, a spectrophotometric assay was carried out in order to quantify the enzymatic activity [10]. The results, expressed as IC_{50} values, are summarized in Table 2. The essential oil from leaves of *S. areira* was more active (IC_{50} = 233.8 ± 1.0 μg/mL) than that from the fruits (IC_{50} = 487.9 ± 1.2 μg/mL), but *S. longifolia* oil exhibited the best AChE inhibition with an IC_{50} value = 20.0 ± 1.0 μg/mL. These results are comparable with, and in many cases better than those reported in the past for other essential oils [7c,7i,7k,7l,11,12]. The AChE inhibition observed for the *S. longifolia* oil could be attributed in part to the presence of viridiflorol (10.5%), a potent inhibitor [7i]. The higher levels of active monoterpenes, like α-pinene and β-pinene, in the leaves than in the fruits of *S. areira* could explain the differences observed in activity [7f, 7l].

The antioxidant activity of the essential oils was evaluated through their ability for free radical scavenging against the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). All the samples gave a positive result in the preliminary assay over TLC. Then, a spectrophotometric assay was carried out and the percentage DPPH reduction was calculated for each oil. *S. longifolia* essential oil was the most active (IC_{50} = 25.2 ± 2.4 μg/mL), with an antioxidant capacity comparable to that of the reference compound. On the other hand, the essential oil from the leaves (IC_{50} = 38.7 ± 2.5 μg/mL) elicited a higher activity than that from the fruits of *S. areira* (IC_{50} = 59.6 ± 3.1 μg/mL). The high percentages of oxygenated sesquiterpenes could explain the better results obtained for *S. longifolia,* even though a correlation between components and activity is not clear for these oils.

Taking into account the results obtained in our study, the essential oils from *S. areira* (leaves) and, mainly, *S. longifolia* appear as promising candidates for more detailed *in vitro* and *in vivo* studies of their AChE inhibition and antioxidant capacities. The seasonal variation of the chemical composition of these oils and their biological activities will be a matter of future studies.

Experimental

Equipment and chemicals: GC-MS were performed with a HP6890 chromatograph connected to a HP5972A 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 (1 mL/min). The GC oven temperature was held at 50ºC for 2 min, programmed at 5ºC/min to 200ºC, then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. GC analyses were performed on a Shimadzu G14B chromatograph with a fid detector on a DB-5 column (30 m x 0.25 mm, 0.25 μm film thickness) with the same analytical conditions used for the GC-MS analyses. UV spectra were recorded on a GBC Spectral UV-VIS spectrophotometer. Butylated hydroxytoluene (BHT), DPPH, AChE, 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATC) and eserine were purchased from Sigma.

Plant material: Aerial parts of *S. longifolia* and *S. areira* were collected in March 2006 (summer), from healthy plants from five different locations. The taxonomy of this material was determined by Dr M.G. Murray. Voucher specimens are kept in the "Herbario del Departamento de Biología, Bioquímica y Farmacia-UNS (BBB)" under the numbers MGM121 (*S. areira*) and MGM400 (*S. longifolia*).

Essential oils: Essential oils were obtained by hydrodistillation, as previously reported [13]. The chemical composition of each oil was determined by GC and GC-MS. The compounds were identified by their retention indices and by comparison of their mass spectra with those stored in MS databases (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas.

Acetylcholinesterase inhibition: AChE used in the assay was from electric eels. The lyophilized enzyme (425 units/mg solid) was prepared in buffer phosphate (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4) to obtain 5 U/mL stock solution. Further enzyme dilution was carried out with buffer phosphate (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4, 0.15 M NaCl, 0.05% Tween 20, pH 7.6) to produce 0.126 U/mL enzyme solution. Samples were dissolved in the same buffer with methanol as co-solvent (2.5%). AChE solution (300 μL) and 300 μL of sample solution were mixed in a test tube and incubated for 30 min at room temperature. The reaction was started by adding 600 μL of the substrate solution (0.1 M Na_2HPO_4, 0.5 mM DTNB, 0.6 mM ATCI, pH 7.5). The absorbance was read at 405 nm for 180 s. Enzyme activity was calculated by comparing reaction rates for the sample to the blank (containing 2.5% MeOH, n = 3). IC_{50} values were determined with probit analysis (EPA Probit 1.4). Eserine was used as the reference AChE inhibitor. The TLC assay with detection of AChE inhibition was carried out by a previously reported method [9].

Antioxidant activity: For the preliminary test, analytical TLC on silica gel plates were developed with 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 minutes later active compounds appeared as yellow spots against a purple background. The spectrophotometric assay was carried out at five different concentrations of the essential oils (10-100 μg/mL 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 DPPH 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). BHT was used as reference compound.

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