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Phytochemical analysis and anti-aging activities of compounds from *juniperus brevifolia* bark

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Juniperus brevifolia (Seub.) Antoine (Fig. 1a) is an endemic Azorean taxon which is present in all islands of the Azores archipelago with the exception for Graciosa [1]. Although Juniperus brevifolia is not used in Azorean folk medicine, its interest comes from the wide range of biological activities reported for other Juniperus species and for their secondary metabolites [2]. In continuation of our study on the valorization of endemic plants of the Azores archipelago, we have examined this species.

Several secondary metabolites were isolated from hexane bark extract by chromatographic techniques and their structures were elucidated by spectroscopic methods. The anti-aging effects of some secondary metabolites identified in this extract were evaluated based on their elastase and tyrosinase inhibition ability, two enzymes whose activity causes loss of elasticity and skin hyperpigmentation, respectively. The compounds β-sitosterol (1), totarol (2), 12-methyl-sugiol ether (3), stigmast-4-en-3-one (4), abiet-8,11,13-trien-7-one (5), 5,6-dehydro-12-methyl-sugiol ether (6), 6α-hydroxy sugiol (7) were identified. Compounds 5 and 6 were here identified for the first time in J. brevifolia species while compound 7 has never been identified before in a Juniperus species. Furthermore, compound 7 (Fig. 1b) is the only one exhibiting anti-elastase activity whereas the communic acid (8), previously identified in this extract [3], was the only compound exhibiting anti-tyrosinase activity.

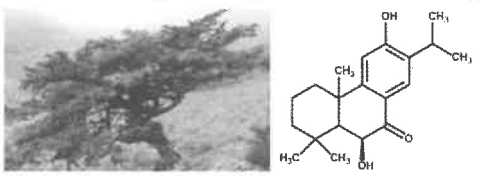

Figure 1. a) Juniperus.brevifolia; b)Compound 7.

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PHYTOCHEMICAL ANALYSIS AND ANTI-AGING ACTIVITIES OF COMPOUNDS FROM *Juniperus brevifolia* BARK

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Introduction

Juniperus brevifolia (Seub.) Antoine (Fig. 1) is an endemic Azorean taxon which is present in all islands of the Azores archipelago with the exception for Graciosa [1]. Although *J. brevifolia* is not used in Azorean folk medicine, its interest comes from the wide range of biological activities reported for other *Juniperus* species and for their secondary metabolites [2]. The hexane extract from *J. brevifolia* bark, was previously analyzed by GC-MS [3] and it exhibits cytotoxic activity against Hep-2 cell line [4].

Elastase is an enzyme primarily responsible for the breakdown of elastin, an important protein found in the extracellular matrix. Elastin, because of its elastic recoil properties, is vital to give elasticity to arteries, lungs, ligaments and skin. As far as anti-aging is concerned, finding inhibitors of this enzyme may be useful in preventing the loss of skin elasticity and, thus, in its flaccidity [5].

Tyrosinase is an enzyme that plays a critical role in melanin biosynthesis, whereas hyperpigmentation of the skin, which is common in aging, results from dysregulation of tyrosinase activity and causes aesthetic problems [6]. Thus, inhibition of tyrosinase is one of the main strategies to achieve skin whitening and depigmentation after sunburn [7].

In the continuation of our research on natural products with biological activity, we isolated and elucidated the structure of several secondary metabolites from the hexane extract of the bark of *J. brevifolia*.

The anti-aging effects of some secondary metabolites identified in this extract were evaluated based on their elastase and tyrosinase inhibition ability.

Fig. 1: *Juniperus brevifolia* tree in the Flores Island, Azores

Methods and Materials

1. Phytochemical analysis

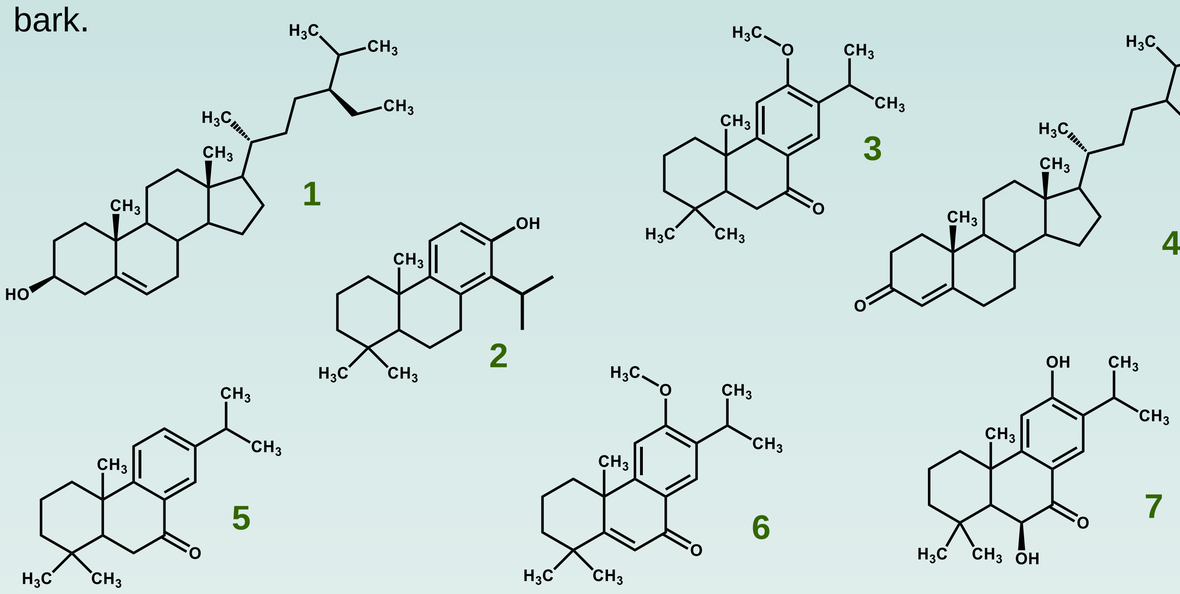
Plant was harvested in Pedreira, São Miguel, Azores → The air-dried bark (516.9 g) was ground and extracted with hexane using a soxhlet apparatus. → 6.08 g of extract were fractionated by preparative column chromatography, eluted with Hexane: AcOEt (0% -100%). → Several of this fractions were purified by preparative TLC eluted with mixture of solventes with different polarity. → Structural elucidation of the purified secondary metabolites by spectroscopic tecnhiques (1D and 2D NMR and MS).

2. Anti-aging activities

Anti-tyrosinase and anti-elastase activities were performed by adapting the protocols described by Manosroi *et al.* [8] and Ndlovu *et al.* [9], and by Thring *et al.* [5] respectively, based on spectrophotometric methods.

Results and Discussion

The compounds β-sitosterol (**1**), totarol (**2**), 12-methyl-sugiol ether (**3**), stigmast-4-en-3-one (**4**), abiet-8,11,13-trien-7-one (**5**), 5,6-dehydro-12-methyl-sugiol ether (**6**), 6α-hydroxy sugiol (**7**) were identified in hexane extract of *J. brevifolia* bark.



- Compound **1** was previously identified in acetone extract from *J. brevifolia leaves* [10] while compounds **2**-**4** were identified previously by GC-MS in the hexane extract of *J. brevifolia* bark [3].
- Compounds **5** and **6** were here identified for the first time in *J. brevifolia* although they have already been identified in other *Juniperus* species [2].
- Compound **7** was previously isolated in the species *Thuja standishii* and *Sequoia sempervirens* [11] but has never been identified before in a *Juniperus* species.
- Among the compounds identified, only the compounds **1** and **7** were isolated in sufficient quantity to be tested as tyrosinase and elastase inhibitor.
- Several of these compounds are described in the literature as exhibiting cytotoxic and/or antibacterial activities [2].

The isolated compounds sitosterol (**1**) and 6α-hydroxy sugiol (**7**) together with communic acid (**8**), sandarocopimaric acid (**9**) and dehydroabietic acid (**10**), previously identified in the hexane extract of *J. brevifolia* bark by GC-MS [3], were, for the first time, evaluated as anti-aging agents. The compounds *N*-methoxysuccinyl-Ala-Ala-Pro-cloromethyl-S (**A**) and kojic acid (**B**) were used as the anti-elastase and anti-tyrosinase reference compound, respectively.

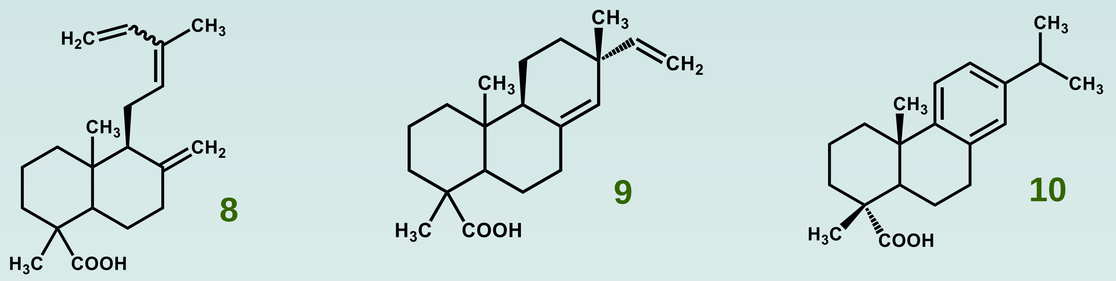


Table 1. The anti-elastase and anti-tyrosinase activities of the compounds **1** and **7**-**10** identified in *J. brevifolia* bark. (Maximum concentration tested 250 µg/mL).

Bioactivity / Compounds	Anti-elastase		Anti-tyrosinase	
	% of inhibition	IC_{50}	% of inhibition	IC_{50}
A	95.51 ± 8.96*	0.150 ± 0.002		
B			99.22 ± 0.36**	1.82± 0.13
1	0	>250	11.99 ± 0.67	>250
7	83.81 ± 1.10	82.23 ± 6.02	33.21 ± 1.68	>250
8	0	>250	65.50 ± 2.96	156.34 ± 4.63
9	0	>250	0	>250
10	0	>250	21.96 ± 0.76	>250

*At 10 µg/mL; ** At 150 µg/mL

- Compound 6α-hydroxy sugiol (**7**) is the only one exhibiting anti-elastase activity while the communic acid (**8**) was the only one exhibiting anti-tyrosinase activity. However, any of them is more active than the reference compounds.

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