Cytotoxic, antibacterial, antioxidant, and anticholinesterasic activity of *Juniperus brevifolia* extracts

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Introduction

Juniperus brevifolia, the unique conifer trees endemic to the Azorean archipelago, is locally known as "cedro-do-mato", and is classified as belong to Cupressaceae family. This species is a typical component of the primitive *laurisilva* forest and occurs in almost all of the islands, except S. Maria and Graciosa. Old stands of some size are now rare, since *J. brevifolia* has been widely used in shipbuilding and carved work due to the high quality of its wood. Our interest on the study of *J. brevifolia* was the search for new bioactive compounds. In this context, the cytotoxic, antibacterial, antioxidant and anticholinesterasic (anti-AChE) activities of the methanol and acetone extracts of *J. brevifolia* wood and bark were determined.

Materials and Methods

In vitro Artemia salina toxicity assay

The methodology described by Solis *et al.*[1] with some modifications was used. Briefly, 100 µL of sample with different concentrations and 100 µL of seawater containing 15-20 *nauplii* were placed in each well of a 96-well microplate. Two types of control were used, one with ethanol and another with potassium dichromate. The microplates were incubated at 25 °C for 24 hrs, then examined under the stereoscopic microscope (12.5 X), where the dead individuals (*i.e.*, without movement) were numbered. After 15 min, 50 µL of acetone were added to each well and total number of *nauplii* was counted. Each extract was tested in triplicate.

Antibacterial activity

From a culture of Gram(+) *Bacillus subtilis* (DSM 10), *B. cereus* (DSM31), *Micrococcus luteus* (DS 420030) and Gram(-) *Escherichia coli* (DSM 498) and *Enterobacter cloacae* (DSM 30850), the inoculums were prepared in 0.85% sterile saline (NaCl). The suspension was compared, using qualitative methods, with a suspension of barium sulphate Tube 0.5 McFarland Scale (bioMerieux®, France).
The antibacterial assay was performed by a modification of Bauer's diffusion technique[2] on solid medium using 6 mm filter paper discs. The diameter of inhibition caused by the addition of 10 µL of each extract was recorded after 24 h. Gentamicin and chloramphenicol were used as positive control.

DPPH radical antioxidant activity

Antioxidant activity was assessed by the DPPH radical scavenging activity assay using a modification of the Blois method[3]. Different concentrations of plant extracts were added to the assay medium containing DPPH solution, allowed to stand in the dark for 30 min and the absorbance at 515 nm measured and compared with controls absorbance without the extract samples. Antioxidant activity percentage (AA%) was calculated as AA% = 1 – (Ac–As)/Ac x100, where Ac is the absorbance of control and As is the absorbance of each sample. Quercetin was used for comparison.

Acetylcholinesterase inhibition

The AChE activity determination was assessed following the assay described by Arruda *et al.*[4] at pH 8.0 and using acetylthiocholine iodide as substrate, in the presence of buffer containing DTNB and extract in different concentrations DMSO never exceeded 2.5%. The reaction was followed for 7.5 min at 415 nm, using e Bio-Rad Model 680 Microplate reader. Every experiment was done in triplicate.

Conclusion

The present study show that bark extracts of *J. brevifolia* are a plant source with interesting biological properties, justifying further detailed investigations of its potential applications.

Acknowledgement
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Results

Cytotoxicity on the brine shrimp *Artemia salina*

The preliminary lethality bioassay showed that only bark extract present considerable activity (> 50% mortality) at 2.5 mg/mL.

LC values of bark methanolic and acetone extracts were found to be 2.03 and 2.12 mg/mL, respectively (Table 1).

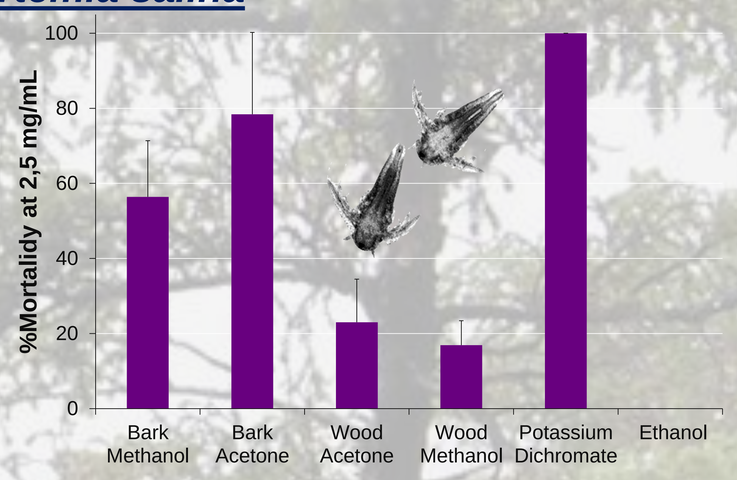

Table 1. LC values for cytotoxicity on *Artemia salina*

Bark extract	Concentration (mg/mL)	*Artemia salina* LC_{50} (95% CL)	LC_{90} (95% CL)	Slope ±SEM (95% CL)
Methanol	(1, 1.25, 1.5, 2.0, 2.5)	2.03 a (1.87 - 2.25)	4.53 a (3.69 – 6.27)	3.67 ± 0.44 (2.80 – 4.53)
Acetone	(1, 1.25, 1.5, 2.0, 2.5)	2.12 a (2.02 - 2.25)	3.55 a (3.18– 4.19)	5.72 ± 0.60 (3.69 – 6.27)

LC values and 95% confidence limits (CL) expressed in mg/mL. LC values and slopes within a column followed by the same letter are not significantly different based on nonoverlapping 95% CL.

Table 2. *In vitro* antibacterial activity of methanol and acetone extract of *J. brevifolia*

Test organism	Diameter of inhibition (mm) (mean ± SD) Methanol extract (200 µg/disc) bark	Methanol extract (200 µg/disc) wood	Acetone extract (200 µg/disc) bark	Acetone extract (200 µg/disc) wood	Gentamicin (10 µg/disc)	Cloramphenicol (10 µg/disc)
B. subtilis	0	0	9 ± 1	0	-	31 ± 0.6
M. luteus	0	0	10 ± 0	0	-	32 ± 1.5
B. cereus	10 ± 0.6	0	11 ± 0.6	10 ± 1	24 ± 0.6	-
E. coli	0	0	0	0	-	17 ± 0.6
E. cloacae	0	0	0	0	20 ± 0	-

Antibacterial activity

The bark acetone extract showed activity against *Bacillus cereus*, *B. subtilis* and *Micrococus luteus*, while the wood acetone extract showed activity only against *B. cereus*.
No activity was observed against Gram (-) bacteria.

Table 3. Anti-AChE and antioxidant activity of metanol and acetone extract of *J. brevifolia*

Plant extract (parts used)	*AChE inhibitory activity* IC_{50} (mg/mL)	*DPPH scavenging activity* EC_{50} (µg/mL)
Methanol		
bark	0.499 ± 0.021	33.0 ± 6.81
wood	0.742 ± 0.023	49.6 ± 0.14
Acetone		
bark	0.193 ± 0.042	7.0 ± 0.68
wood	0.825 ± 0.294	15.0 ± 0.74
Quercetin	-	3.24 ± 0.07

Antioxidant activity

The acetone extracts showed good activity when compared to quercetin (Table 3).

AChE inhibition

The methanol and acetone extracts from bark and wood presented IC_{50} values between 0.193 and 0.825 mg/mL (Table 3).

References
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