Essential oil of Azorean *Cryptomeria japonica* seed cones: chemical composition, antibacterial activity and *Artemia salina* toxicity

Filipe Arruda[1,2*], Ana Lima[1,4], Alexandre Pavão[2], José Baptista[1,4], José S. Rosa[2,3], A. Cristina Figueiredo[5], Elisabete Lima[1,4]

[1] Institute of Agricultural and Environmental Research and Technology, Azores University, Angra do Heroísmo, PORTUGAL; [2] Department of Biology, Faculty of Science and Technology, Azores University, Ponta Delgada, PORTUGAL
[3] Biotechnology Centre of Azores, Azores University, Ponta Delgada, PORTUGAL; [4] Department of Physics, Chemistry and Engineering, Azores University, Ponta Delgada, PORTUGAL
[5] Centro de Estudos do Ambiente e do Mar (CESAM Lisboa), Faculdade de Ciências da Universidade de Lisboa, CBV, DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, PORTUGAL

*filipe.mp.arruda@uac.pt

Introduction

The emergence of antibiotic-resistant bacterial strains has become a major global concern. This ongoing threat requires often new agents in order to keep pace against this fast-adapting organisms.

Essential oils (EOs) are plant-based products regarded as new possible weapons to fight antimicrobial resistance due to their inherent antimicrobial properties [1].

The interest in EOs from *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae) wastes, by the scientific community and EO markets, is rapidly increasing [2]. However, some of these biomass wastes, such as seed cones, have been less exploited.

Thus, this study aimed to determine the chemical composition of dried seed cones EO from Azorean *C. japonica*, and to evaluate its antibacterial activity and toxicity against *Artemia salina*.

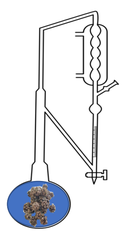
Materials and methods

I. Essential oil extraction of dried seed cones (DSC)

- DSC were subjected to hydrodistillation (HD) over 3 h, according to Arruda *et al*. [3]. The HD was performed in a Clevenger-type apparatus and the ratio of plant material to water was 1:10 g/mL.

***C. japonica* foliage with seed cones**

Azorean *C. japonica* dried seed cones



Hydrodistillation in Clevenger apparatus

Essential oil of *C. japonica* dried seed cones (DSC-EO)

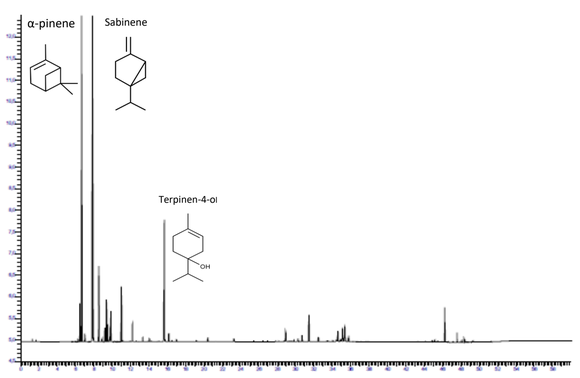
II. Essential oil analysis

- **Quantification by GC**

- Perkin Elmer Clarus 400 with 2 FID
- DB-1 + DB-17HT columns
- Carrier gas: hydrogen at 30 cm/s
- Split ratio: 1:40
- Oven temp: 45-175 °C, 3 ° C/min
  175-300 °C, 15 °C/min

- **Identification by GC-MS**

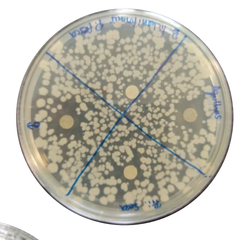
- Perkin Elmer Clarus 600T-MS
- DB-1 (30m x 0.25mm ID, 0.25 µm thickness)
- Carrier gas: helium at 30 cm/s
- Split ratio: 1:40
- Ionization energy: 70 eV
- Oven temp: 45-175 °C, 3 ° C/min
  175-300 °C, 15 °C/min



GC profile of essential oil from *C. japonica* dried seed cones

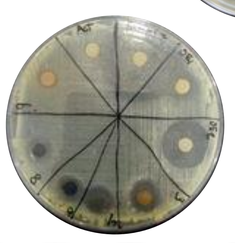
III. Antibacterial activity

- Antibacterial activity of DSC-EO was carried out by using the agar disk diffusion assay, according to the method described by Iseppi *et al*., with some modifications [1].



Disk-difusion in agar method against:

- **Gram-positive**
  - *Bacillus subtilis* (DSM 10**)**
  - *Micrococus luteus* (DSM 20030)
- **Gram-negative**
  - *Enterobacter cloacae* (DSM 30054)
  - *Escherichia coli* (DSM 498)

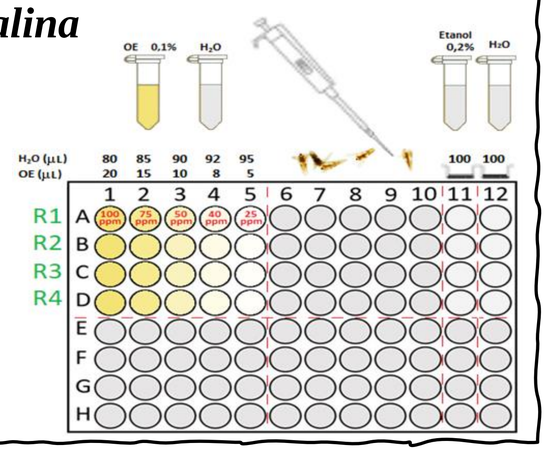


IV. Toxicity against *Artemia salina*

- The toxicity of DSC-EO was perfomed by an *in vivo* assay, using nauplii of *A. salina*, according to Meyer *et al*. with some modifications [4].
- Determination of LD_{50} and LD_{90} against *A. salina* nauplii (Probit analysis – software IBM SPSS 22.0)

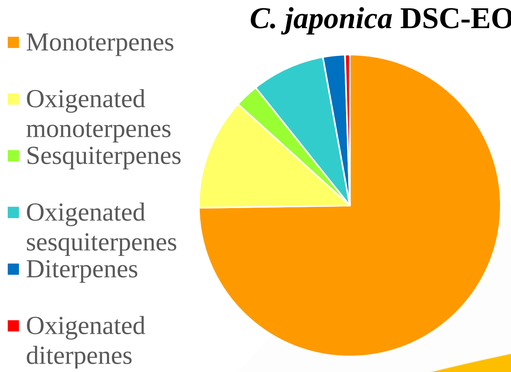
Adult

Nauplii



Results

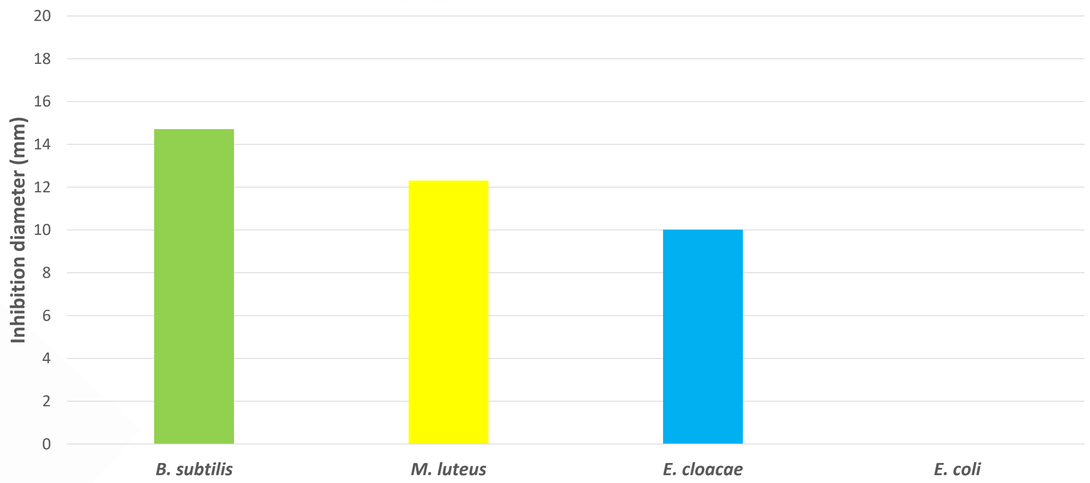
Chemical compostion of *C. japonica* DSC-EO



***C. japonica* DSC-EO presented:**

- 1.33% (v/w) yield
- 42 components were identified (97.9% total)
- Major components: α-pinene (32.6%), sabinene (23.7%) and terpinen-4-ol (9.8%)

Antibacterial activity (disk-difusion in agar) of *C. japonica* DSC-EO



Toxicity of *C. japonica* DSC-EO against *Artemia salina*

EO	LC_{50} (95% CI) [a]	LC_{90} (95% CI) [a]	Intercept ± SEM	Slope ± SEM	H^b
DSC	81.1 (76.05 - 85.56)	120.21 (108.43 - 146.83)	-9.30 ± 2.53	7.49 ± 1.32	0.45

[a] LC_{50}, LC_{90} values and 95% confidence interval (CI) expressed in µg/mL of EO required to cause death to 50 and 90% of *A. salina* nauplii.
[b] *H* (heterogenity factor), χ^2:df

Conclusion

***C. japonica* DSC-EO is regarded as an abundant source that possess high toxicity against *A. salina* and significant inhibition against common food-borne microbes.**

Potencial for development of food/cosmetic preservatives

Further investigation is still needed within DSC-EO bioactivities, in order to accumulate knowledge that will help to find new applications for this biomass waste.

Aknowledgements

This work is supported by funds from Direção Regional Ciência e Tecnologia (DRCT), under the project PotBioCJap - Determinação do potencial biotecnológico dos subprodutos de Cryptomeria japonica Açoriana.
Filipe Arruda thanks FRCT for Doctoral Research Scholarships (ref[a] M3.1.a/F/008/2021).

References

[1] Iseppi, R., Mariani, M., Condò, C., Sabia, C., & Messi, P. (2021). Essential oils: A natural weapon against antibiotic-resistant bacteria responsible for nosocomial infections. *Antibiotics*, 10, 417.
[2] Lima, A., Arruda, F., Medeiros, J., Baptista, J., Madruga, J., Lima, E. (2021). Variations in Essential Oil Chemical Composition and Biological Activities of *Cryptomeria japonica* (Thunb. ex L.f.) D. Don from Different Geographical Origins - A Critical Review. *Appl. Sci*. 11, 11097.
[3] Arruda, F.; Rosa, J.S.; Rodrigues, A.; Oliveira, L.; Lima, A.; Barroso, J.G.; Lima, E. (2022). Essential oil variability of Azorean *Cryptomeria japonica* leaves under different distillation methods, part 1: Color, yield and chemical composition analysis. *Appl. Sci*. 12, 452.
[4] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B.; Nichols, D.E. McLaughlin, J.L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 45, 31–34.