# Côa Valley's medicinal plants as potential cosmetic ingredients: cytotoxic and antioxidant assessment

**Mário Pedro Marques**[1,2], Euclides Landim[1,2,3], Carla Varela[1,2,4], Joana Marques[5], Ricardo M.F. da Costa[5], Luís A.E. Batista de Carvalho[5], Aida Carvalho[6,7,8], Paulo J. Oliveira[2,9], Célia Cabral[1,2,3*]

[1]University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Clinic Academic Center of Coimbra (CACC), Faculty of Medicine, 3000-548 Coimbra, Portugal
[2]University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), 3000-548 Coimbra, Portugal
[3]Center for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[4]University of Coimbra, CIEPQPF, Rua Sílvio Lima, 3030-790 Coimbra, Portugal
[5]University of Coimbra, Molecular Physical-Chemistry R&D Unit, Department of Chemistry, Rua Larga, 3004-535 Coimbra, Portugal
[6]Instituto Politécnico de Bragança, Campus Santa Apolónia, Bragança, Portugal
[7]Centro de Investigação, Desenvolvimento e Inovação em Turismo (CiTUR), Pólo Guarda, Av. Dr. Francisco Sá Carneiro 50, 6300-559 Guarda
[8]Fundação Côa Parque, Rua do Museu, 5150-620 Vila Nova de Foz Côa
[9]CNC-Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, 3004-504 Coimbra, Portugal

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## CONTEXTUALIZATION

**Where is located and what is the importance of river Côa Valley?**

- The river Côa Valley is part of the Guarda District, in the Northeast of Portugal. The Valley comprises an Archeological Park, which is considered "the most important open-air Paleolithic rock art site" in the world, being classified as UNESCO World Heritage Site since 1998.

**What are the natural resources' relevance of this region?**

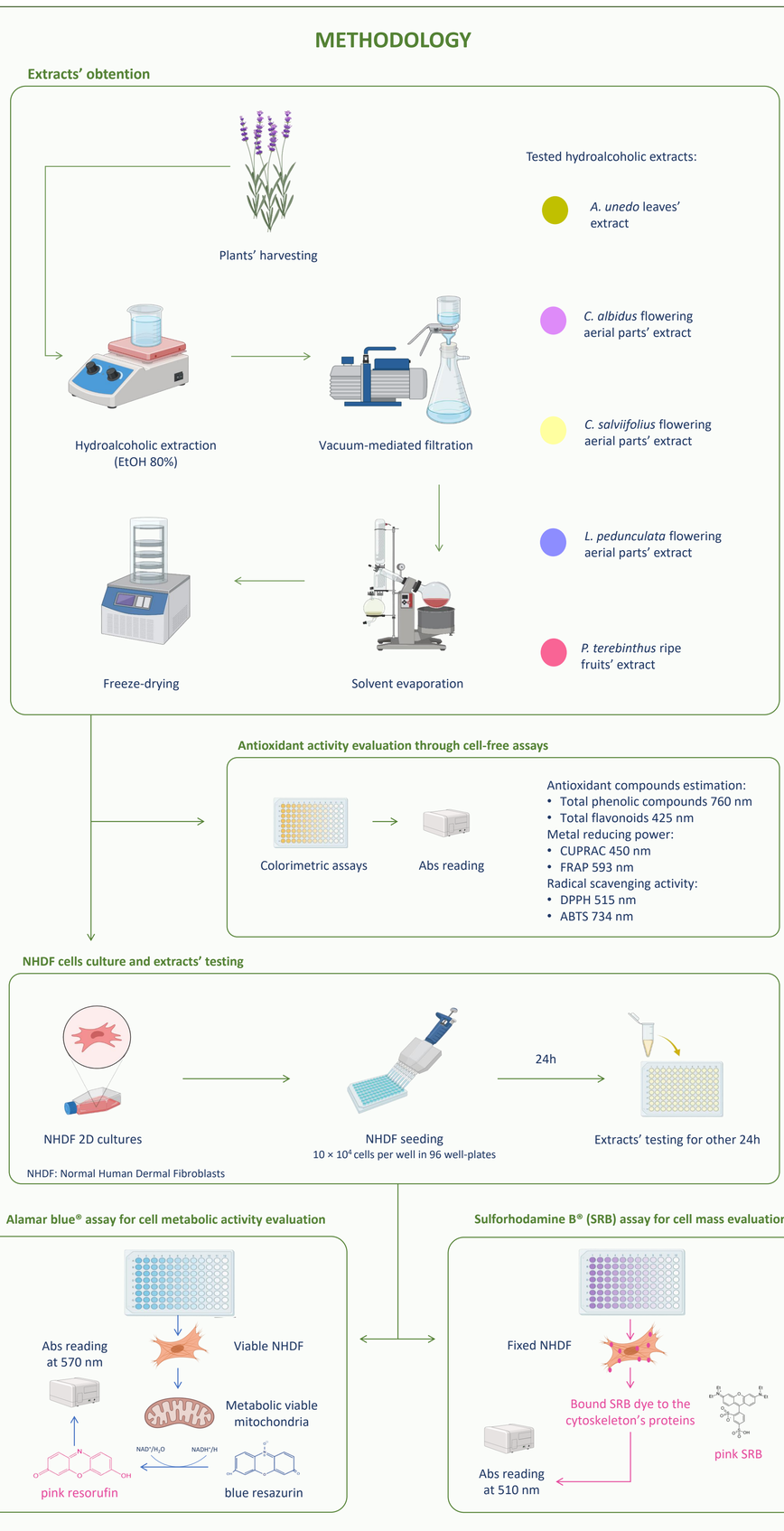
- The flora of the Archeologic Park in river Côa Valley comprises approximately 500 to 600 different plant species, some Portuguese and/or Iberian endemic species, according to a botanical survey recently carried out by us. Even though, so far, little is known about the natural endogenous resources of this territory, namely concerning medicinal plants.

**What is the aim of this investigation?**

- The main aim is to assess the cytotoxicity in skin fibroblasts (NHDF cell line) and the antioxidant activity through cell-free methods, of hydroalcoholic extracts obtained from selected plant species (Figure 1). These extracts are meant to be incorporated into scientific-validated plant-based cosmetic formulations, hence creating an exclusive cosmetic brand for Côa Valley.

**Figure 1.** Selected Côa Valley's medicinal plants from which hydroalcoholic extracts were tested. Ordered from the left to the right are *Arbutus unedo* L. (Ericaceae) in early fruiting stage, *Cistus albidus* L. (Cistaceae) flowering aerial parts, *Cistus salviifolius* L. (Cistaceae) flowering aerial parts, *Lavandula pedunculata* (Mill.) Cav. (Lamiaceae) flowering aerial parts, and *Pistacia terebinthus* L. (Anacardiaceae) with ripe fruits.

## METHODOLOGY




## CELL METABOLIC ACTIVITY RESULTS

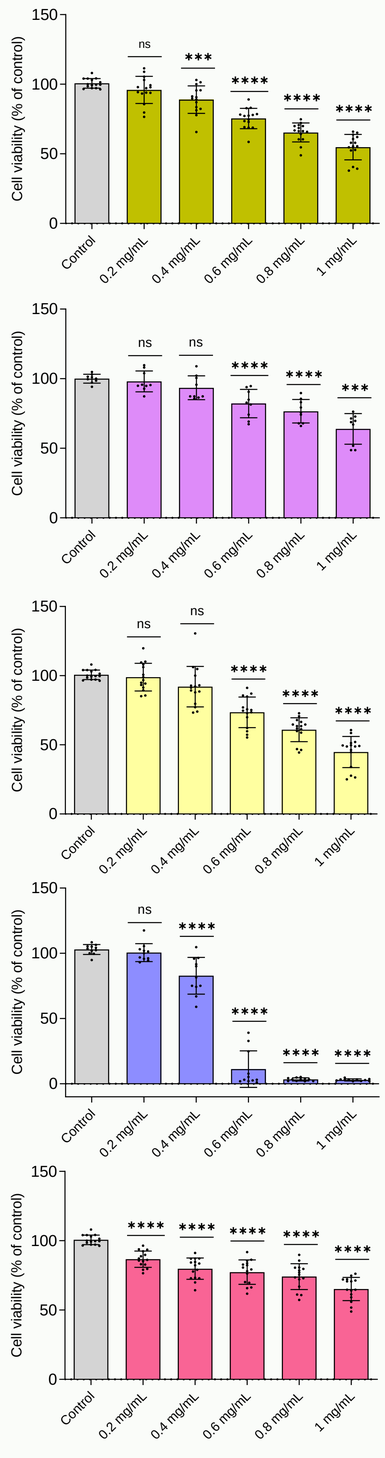



**Figure 2. Cell metabolic activity evaluated through the Alamar blue® assay**, after 24h of extract application. Data are represented as the mean ± standard deviation (SD), and the results were normalized to untreated NHDF cells (control set as 100%). Statistically significant differences between different tested concentrations were compared to the control using one-way ANOVA followed by Dunnett's test for multiple comparisons (ns means non-significant, ***$p < 0.0005$, ****$p < 0.0001$ *versus* control).

## CELL MASS RESULTS

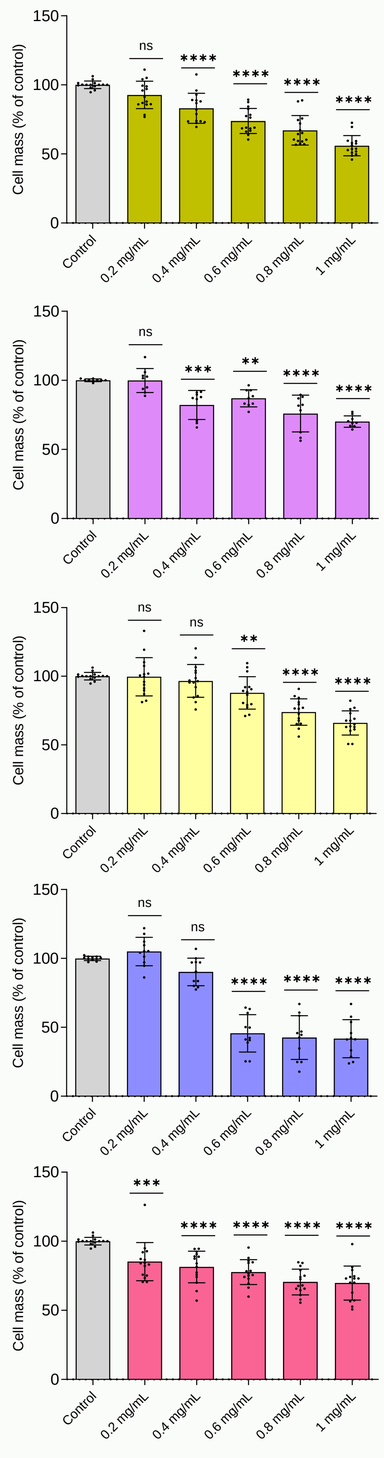



**Figure 3. Cell mass evaluated through the SRB® assay**, after 24h of extract application. Data are represented as the mean ± standard deviation (SD), and the results were normalized to control untreated NHDF cells (cell viability set as 100%). Statistically significant differences between different tested concentrations were compared to the control using one-way ANOVA followed by Dunnett's test for multiple comparisons (ns means non-significant, **$p < 0.01$, ***$p < 0.0005$, ****$p < 0.0001$ *versus* control).

## ANTIOXIDANT ACTIVITY RESULTS

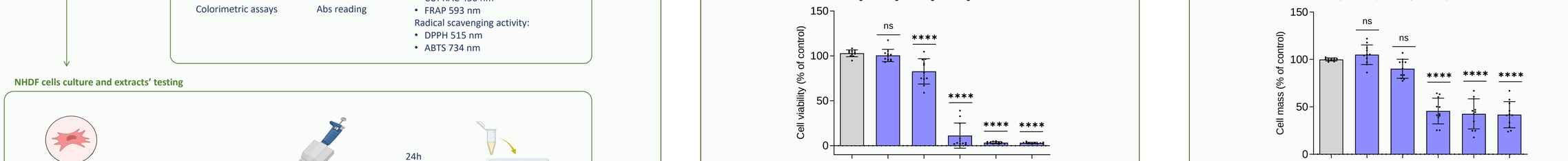



**Figure 4. (A) total phenolic compounds**, expressed in mg gallic acid equivalents (GAE) $g^{-1}$ extract dry weight (DW) and **(B) total flavonoids** content expressed in mg quercetin equivalents (QE) $g^{-1}$ extract dry weight (DW), **(C) cupric (CUPRAC assay)** and **(D) ferric (FRAP assay) reducing capacity** expressed in mg Trolox equivalents (TE) $g^{-1}$ extract dry weight; and **scavenging activity of the radicals (E) 2,2-diphenyl-1-picrylhydrazyl (DPPH)** and **(F) 20-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)** presented as $IC_{50}$ values (mg/mL), and using butylated hydroxytoluene (BHT) as positive control (commercialized reference antioxidant). Plants' extracts are abbreviated as AU for *A. unedo*, CA for *C. albidus,* CS for *C. salviifolius,* LP for *L. pedunculata* and PT for *P. terebinthus*. Statistically significant differences between different plants extracts in (A), (B), (C) and (D) assays were compared using one-way ANOVA followed by Tukey's post hoc test (ns means non-significant, *$p < 0.05$ **$p < 0.01$, ***$p < 0.0005$, ****$p < 0.0001$). For the assays (E) and (D) statistically significant differences between different plants extracts were compared to the positive control BHT using one-way ANOVA followed by Dunnett's test for multiple comparisons (ns means non-significant, *$p < 0.05$ , ****$p < 0.0001$ *versus* control).

## CONCLUDING REMARKS

- In general, the extracts revealed to be non-toxic at 0.4 and 0.2 mg/mL. However, *P. terebinthus* fruits' extract was toxic for the tested concentrations.
- In comparision to the reference antioxidant BHT, all the extracts stood out by inhibiting the DPPH radical ($p < 0.0001$). On the other hand, diferences were found in the inhibition of the ABTS radical, despite the *C. salviifolius* and *P. terebinthus* extracts presented a similar antioxidant activity to BHT.
- The extracts presented higher cupric than ferric reducing capacity, with *C. salviifolius* and *L. pedunculata* presenting higher TE $g^{-1}$ extract dry weight.
- The observed metal reducing power as well as radicals' inhibition are probably related with the presence of phenolic compounds and flavonoids in these extracts.

## FUTURE PERSPECTIVES

- A similar screening assay considering the non-toxic concentrations herein determined will be performed in a 3D human skin model.
- The antioxidant evidences found in cell-free assays will be now further explored in-depth, considering *in vitro* models of oxidative stress.
- Crucial properties of the extracts for cosmetics development will be afforded, namely senolytic, photoprotective, anti-irritant, and anti-enzymatic activities.
- Chemical characterization of these extracts is an ongoing work, that will allow to identify bioactive compounds responsible for the extract-cells interactions herein observed.

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