



## V\_OP\_Chemical characterization, cytotoxic evaluation and anti-SARS-CoV2 activity of plant extracts rich in hydrolysable tannins

Adma N.F. Melo<sup>1</sup>, Tiago B. Afonso<sup>1</sup>, Marta Carvalho<sup>1</sup>, Cláudia Rodrigues<sup>4</sup>, Tânia Ribeiro<sup>1</sup>, Márcio Carocho<sup>2,3</sup>, Miguel Marques Pinto<sup>5</sup>, Freni Tavaría<sup>1</sup>, Paula Teixeira<sup>1</sup>, J. Pedro Simas<sup>4</sup>, Lillian Barros<sup>2,3</sup>, Manuela Pintado<sup>1</sup>

<sup>1</sup>*Centro de Biotecnologia e Química Fina (CBQF), Universidade Católica Portuguesa, Portugal; e-mail: [mcarocho@ipb.pt](mailto:mcarocho@ipb.pt)*

<sup>2</sup>*Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal*

<sup>3</sup>*Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*

<sup>4</sup>*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal*

<sup>5</sup>*Next Generation Chemistry, Unipessoal Lda, Porto, Portugal*

Acorn husks, chestnut flowers, and leaves of eucalyptus, rockrose and laurel, extracted with water or water/ethanol were screened for their polyphenolic profile, antioxidant, antimicrobial and anti-SARS-CoV2 activity, as well cytotoxicity and mutagenicity assays. The highest amounts of hydrolysable tannins, analysed by HPLD-DAD/MS were found in the eucalyptus extracts, followed by rockrose, while laurel only showed condensed tannins. In terms of their antioxidant analysis, the most antioxidant extract depended on the assay, still, the highest total phenols and DPPH values were found for acorn husks, while rockrose and the chestnut flower showed highest ABTS values. Considering ORAC, laurel showed the highest values (3703±87 µMol trolox/mg extract). Considering the antimicrobial activity, the extract that had highest activity against the tested 43 bacterial strains were rockrose and acorn husks. The antifungal activity was quite low, with the extracts only showing mild inhibition in three of the 12 tested species. For the anti-SARS-CoV2 activity, all extracts showed strong inhibition (over 90%) of this coronavirus, although acorn husk and eucalyptus achieved a growth inhibition of 99 and 97%, respectively. To prove the lack of toxicity of these extracts, extracts were screened for cytotoxic and mutagenic properties. In terms of prevention and degradation of DNA, most of the extracts showed preventive action with eucalyptus, rockrose and laurel protecting 100% of the DNA. In the mutagenic evaluation using *Salmonella typhimurium*, none of the extracts showed mutagens that could cause frameshift mutations. Finally, in the human keratinocyte cell line, none of the extracts showed inhibition values, since no inhibition was higher the 30% threshold. Overall, the extracts proved to have interesting antioxidant activity while only average antibacterial effects and very low antifungal capacity. Still, the very high inactivation of SARS-CoV2 activity makes them have promising applicability as antivirals, both in surface cleaning agents and woven into linen for health care workers. Acorn husks and the eucalyptus leaves seem to be the most promising of all extracts.

### Acknowledgment

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). M. Carocho thanks FCT for his individual employment contract (CEEC-IND/00831/2018), and L. Barros also thanks the national funding by FCT through the institutional scientific employment program–contract for her contract.