EVALUATION OF THE ANTICANCER POTENTIAL AND INDUCTION OF THE APOPTOSIS DEATH PATHWAY BY OCOTEA INDECORA ESSENTIAL OIL EXTRACT IN DIFFERENT CANCER CELL LINES

Diniz, A. C. S. [1*]; Machado, F. P.[2]; Queiroz, L. N.[2]; Rocha, L. M.[2]; Robbs, B. K.[1,3]

[1]*Laboratório Multiusuário de Pesquisa Biomédica, Instituto de Saúde de Nova Friburgo, Universidade Federal Fluminense, Nova Friburgo, Brasil*
[2] *Laboratório de Tecnologia de Produtos Naturais, Universidade Federal Fluminense, Rua Doutor Mario Viana 523, Santa Rosa, CEP 24241-000, Niterói, RJ, Brasil.*
[3] *Programa de Pós-Graduação em Ciências Aplicadas a Produtos para Saúde, Faculdade de Farmácia, Universidade Federal Fluminense, Niterói Brasil*
ana_diniz@id.uff.br

Introduction

Cancer is the second cause of death in the world. Essential oils extracted from plants of the genus Ocotea spp have activity against cancer cells and contain a variety of phytochemicals with cytotoxic potential. A species that has not yet been studied in the literature is Ocotea Indecora in relation to its antitumor activity. Analysis of the cytotoxic potential and selectivity index of Ocotea indecora essential oil diluted in DMSO used in different cancer cell lines, namely B16/F10 (melanoma), Hela (cervical cancer), HepG2 (human hepatocarcinoma), HT29 (colon cancer) and SCC9 (squamous cell carcinoma of the tongue) and normal fibroblasts not processed and BEAS.

Material and Methods

The essential oil of fresh leaves of *Ocotea indecora* was obtained using the hydrodistillation method in a modified Clevenger-type apparatus for 4 h. After that, the essential oil was dried with anhydrous sodium sulfate and stored in an amber bottle at -4 °C. The chemical identification of the essential oil was performed using a gas chromatograph (GC-MS QP2010, Shimadzu) coupled to a mass spectrometer (MS) and quantified in a gas chromatograph (GC) coupled to a flame ionization detector (FID). Cytotoxicity and selective index determination were performed by MTT. The IC^{50} was calculated using a non-linear regression curve in the GraphPad Prism 8 program. For morphological analysis, HepG-2 cells were treated with essential oil ($2xIC^{50}$) and a video was taken in time lapse format. Determination of the formation of reactive oxygen species was performed using the Ros-GloTM H^2O^2 Assay (Promega) and effector caspase 3/7 was determined by CellEvent Caspase-3_7 Green Ready Probes Reagent (ThermoFisher). The hemolytic potential was also performed using human erythrocytes incubated with hybrids in a hemolysis assay approved by the research ethics committee of the Universidade Federal Fluminense (CAAE: 43134721.4.0000.5626).

Results and Discussion

Cell viability assays showed that the essential oil was cytotoxic in the following tumor cell lines SCC9 (IC^{50}=124.6 µg/ml), HT29 (IC^{50}=45.62 µg/mL), HepG2 (IC^{50}=44.91µg/mL), B16 (IC50=51µg/mL) and in normal cells, human tongue fibroblasts in primary culture (IC^{50}=84.5 µg/ml) and also in the Beas strain (IC^{50}=67.62 µg/mL). However, it obtained the highest selectivity index in HepG2, being (IS=1.9) in fibroblasts and (IS=1.50) in Beas, being selected to continue the tests. HepG2 treated with the essential oil showed characteristics of apoptosis such as formation of membrane bubbles, cell retraction and later vacuoles in the membrane of small amounts of cells, which could be late apoptosis. Furthermore, HEPG2 cells exhibited low ROS production at 6-hour and caspase 3/7 activation. The oil was not hemolytic in tested concentrations (500 and 1000 µg/mL) demonstrating promising properties

to proceed to animal tests - Ethics Committee on the Use of Animals at Universidade Federal Fluminense (#2699110419). The characterization of the compounds present in the essential oil is under evaluation.

Conclusion

After analysis, it can be concluded that the essential oil shows activity against all the cell lines tested. The result of a higher selectivity index against HepG-2 strongly points to apoptosis induction and moving towards animal testing models.

Acknowledgments:
The authors thank CNPq, PROPPI/UFF, FAPERJ (E-26/210.068/2021 and E-26/210.915/2021) and CAPES (Finance Code 001).

Bibliographic References:

[1] Siegel, R.L., Miller, K.D., Wagle, N.S., Jemal, A. “Cancer statistics, 2023”. CA Cancer J Clin 2023 73, 17–48.
[2] Machado, F.P., Folly, D., Salas Enriquez, J.J., *et al*;. Nanoemulsion of *Ocotea indecora (Shott) Mez* essential oil: Larvicidal effects against Aedes aegypti. 2023 Ind Crops Prod 192, 116031.
[3] Pinto, L. de A., Machado, F.P., Esteves, R., *et al*. Characterization and Inhibitory Effects of Essential Oil and Nanoemulsion from *Ocotea indecora (Shott) Mez* in Aspergillus Species. Molecules. 2023 28, 3437.
[4] Wright, B.S., Bansal, A., Moriarity, D.M., *et al*,. Cytotoxic Leaf Essential Oils from Neotropical *Lauraceae*: Synergistic Effects of Essential Oil Components. Nat Prod Commun 2, 2007, 2(12).
[5] Nichol, R.J.O., Khalaf, A.I., Sooda, K.. *et al*,. Selective in vitro anti-cancer activity of non-alkylating minor groove binders. Medchemcomm. 2019 10, 1620–1634.