Evaluation of the antioxidant and antiproliferative potential properties of phytocomplexes extracted from Mediterranean area fruits

P1-34

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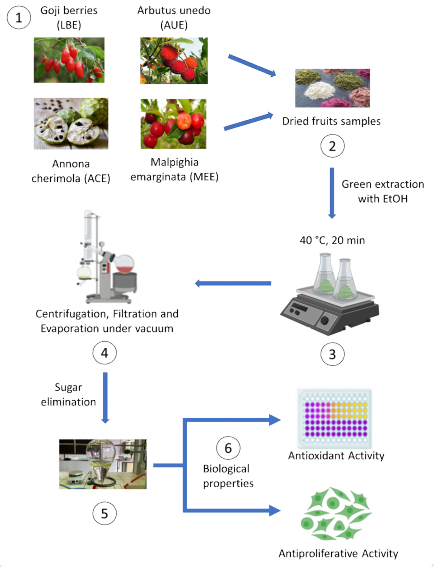
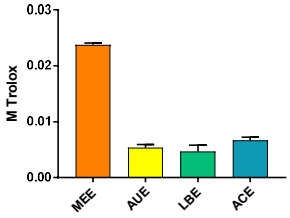
INTRODUCTION

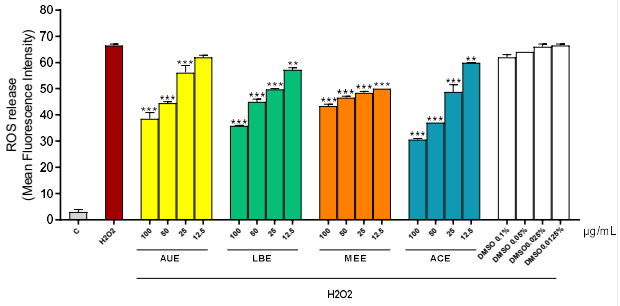
Breast and cervical cancer represents the first and the second cause of death for women worldwide[1,2]. Therefore, new advanced chemotherapies applications are very urgently needed this cancer. High attention has been paid to natural compounds in fruits and vegetables with potential nutraceutical properties. In this regard, dietary polyphenols have been widely demonstrated to be able to not only reduce oxidative and inflammatory stress, but also decrease proliferation of cancer cells. However, the biological activity of various food plants has not yet been studied. This work aims to characterize the nutraceutical potential of four fruits such as, *Malpighia emarginata* (MEE), *Arbutus unedo* (AUE), *Goji berries* (LBE), *Annona cherimola* (ACE). For this reason, our study focused on the evaluation of antioxidant potential and antiproliferative activity of polyphenol extracts on cervical cancer (HeLa) and breast cancer (MCF-7) cell line.



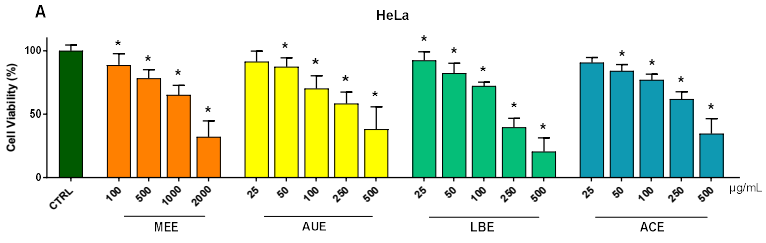
Antioxidant activity cell-free: ABTS was prepared and stored in the dark for 16 h. Thereafter, 2,5 µL of samples or reference compounds (trolox) were added to 96-well microplate followed by 250 µL ABTS solution. The plates were incubated in the dark for 20 min at room temperature and A734 was recorded using a microplate reader. The ABTS assay also highlighted an interesting antioxidant activity of the extracts with particular reference to the *Malpighia emarginata* extract.

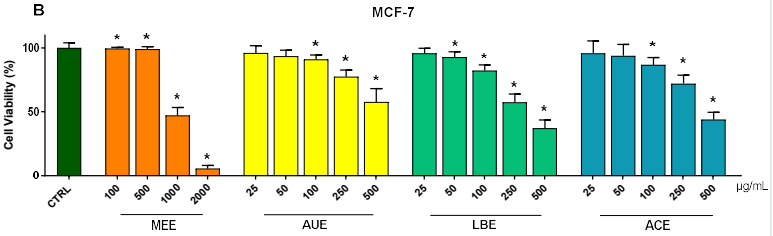


***In vitro* antioxidant assay**: To evaluate antioxidant potential, the ethanolic extracts (12,5–100 µg/mL) were tested in IEC-6 cells treated with the pro-oxidant stimulus H_2O_2 (1 mM). The IEC-6 cells were seeded in 24-well plates and allowed to adhere for 24 h. After adhesion, cells were incubated with ethanolic extracts and H_2O_2 for 24 h. Cells were then washed twice with PBS and then incubated in PBS containing H_2DCF-DA. After 15 min, cells fluorescence was evaluated using a fluorescence-activated cell sorting and elaborated with Cell Quest software. Our results indicate that the extracts significantly reduce ROS release. ***, ** and * denote respectively P<0.001, P<0.01 and P<0.05 vs H_2O_2.

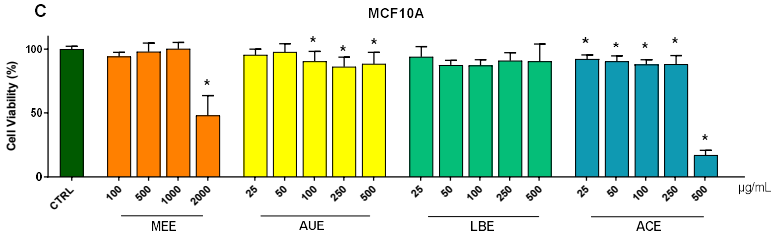


***In vitro cytotoxicity assay*:** The cytotoxic effects of the compounds were assayed by means of the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega), which is based on the reduction of an MTS tetrazolium compound. Cells were seeded in 96-well plates at a density of 12×10³ cells/well for HeLa and 15×10³ cells/well for MCF-7 and MCF10A. After treating cultures for 24 h, assays were performed by adding 10 µl of the CellTiter 96® AQueous One Solution Reagent directly to culture wells, incubating cells for 15 min (HeLa and MCF-7 cells) and 1 h (MCF10A) at 37 ºC, and then recording absorbance on a microplate reader (Infinite M200, Tecan).





Dose-response curve of the ethanolic extracts on HeLa and MCF-7 cell viability. HeLa (**Figure A**) and MCF-7 (**Figure B**) cells were treated for 24 hours with increasing concentrations between 100 and 2000 µg/ml for MEE and between 25 and 500 µg/ml for the other extracts. *Goji Berry* extract showed the highest cytotoxicity with an IC_{50} of 191.5 µg/ml and 324,9 µg/ml on HeLa and MCF-7 cells, respectively. Values are presented as the mean ± SD from 5 separate experiments and expressed as a percentage of the control values. * P <0.05 compared to control values.



Dose-response curve of the ethanolic extracts on MCF10A cell viability. MCF10A cells (non-malignant breast epithelial cells, **Figure C**) were treated for 24 hours with increasing concentrations between 100 and 2000 µg/ml for MEE and between 25 and 500 µg/ml for the other extracts. The extracts have an IC_{50} much higher than 1000 µg/ml . Values are presented as the mean ± SD from 5 separate experiments and expressed as a percentage of the control values. * P <0.05 compared to control values.

CONCLUSION

Our results showed that the extracts (12.5-100 µg/mL) significantly inhibited ROS production in IEC-6 cells, with the greatest capacity shown by *Annona cherimola* extract. The ABTS assay also highlighted an interesting antioxidant activity of the extracts with particular reference to the *Malpighia emarginata* extract. Additionally, our data showed that the extracts (100-2000 µg/mL and 25-500µg/mL) exhibit a dose-dependent effect on cell viability of HeLa and MCF-7 cell lines, with the greatest reduction obtained from *Goji berries* extract. An important result is the absence of mortality in non-malignant cells (MCF10A), with *Goji berries* and *Arbutus unedo* extracts having the best profile on both cancer and non-cancer cells. In conclusion, the results obtained suggest a potential use of extracts tested in the onconutraceutical field.

References

1. Catarino, R., Petignat, P., Dongui, G., & Vassilakos, P. (2015). *World journal of clinical oncology*, 6(6), 281.
2. Ke, Y., Al Aboody, M. S., Alturaiki, W., Alsagaby, S. A., Alfaiz, F. A., Veeraraghavan, V. P., & Mickymaray, S. (2019). *Artificial cells, nanomedicine, and biotechnology*, 47(1), 1938-1946.

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