Chemical characterization of olive mill waste and its bioactivity on human and mammalian cancer cell lines.

Karen Attard | Dr. Frederick Lia | Institute of Applied Science | Contact info: karen.attard@mcast.edu.mt

Introduction

Over the last few years, the beneficial effect on human and mammalian health and anticancer effect of many plant-derived polyphenols have been subject to numerous studies. Synergistic effect of polyphenol combinations has been found to exhibit biological activities different from those detected with individual phenolic compounds

Research Aim and Objectives

The main aim of this proposal is to develop innovative solutions significantly contributing the use of olive mill waste as a potential source of bioactive constituents to be used for nutraceuticals application and potential use in the treatment of cancers.

In the project the following specific objectives are proposed:

1. Collection of raw materials from 10 or more different cultivars.
2. Develop and optimize an extraction and purification method for the recovery of bioactive constituents from olive mill waste solids.
3. Physicochemical characterization of the olive mill waste extracts using a profiling-based approach for the quantification of the major bioactive classes present in the extracts.
4. Invitro bioactivity testing on 3 non-adherent cell lines.
5. Assessment of mode of cell death by histological preparations.

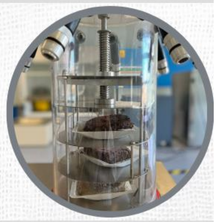
Methodology

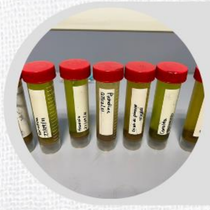
1. Filtration

Filtration of sample in order to separate pomace and olive mill wastewater

2. Lyophilizer

Lyophilizer is used in order to remove any water from the sample



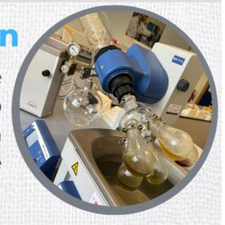


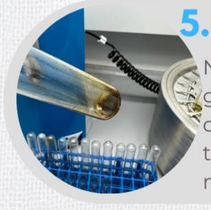
3. Extraction

Optimizing an extraction method for the recovery of bioactive constituents from olive mill waste solid

4. Rotary Evaporation

The extract is passed through the rotary evaporator in order to remove the volatile solvent from interest. This will lead to a more pure extract



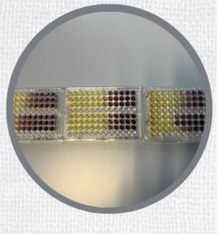


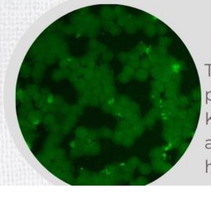
5. Nitrogen Evaporation

Nitrogen evaporation produce a gentle stream of nitrogen gas which continuously blow on the surface of the extract. This will dissolve the methanol and leave a pure extract

6. Assays

Various assays were done such as total phenolic, flavonoid and *ortho*-diphenolic content, metal ion reducing activity including in FRAP and CUPRAC, ABTS, DPPH, hydrogen peroxide and superoxide scavenging activity

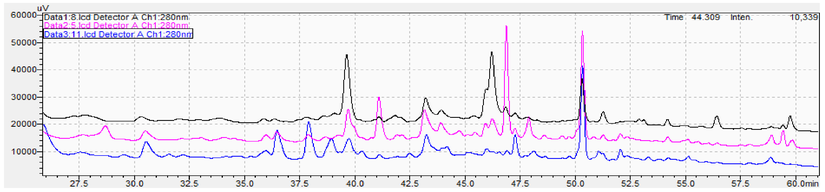




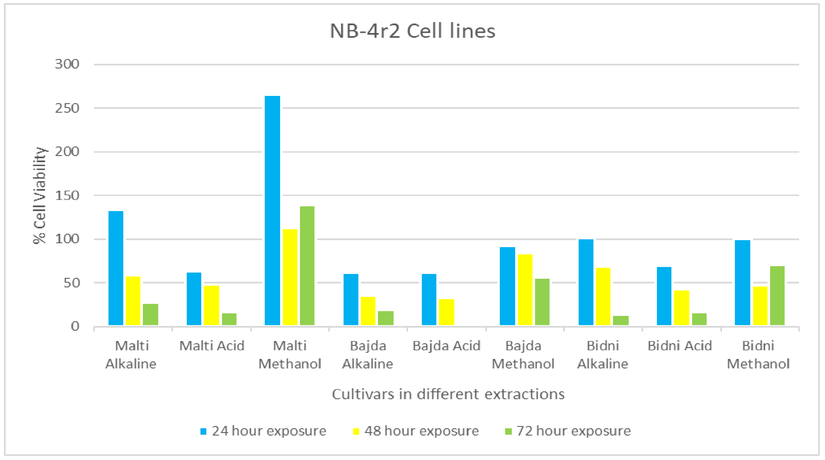
7. Non-adherent cell lines and cell death

Three non-adherent cell lines of human promyelocytic leukaemia was done (HL-60, KG-1a and Nb4R2 cell lines). For cell death an assessment of mode of cell death by histological preparations was done

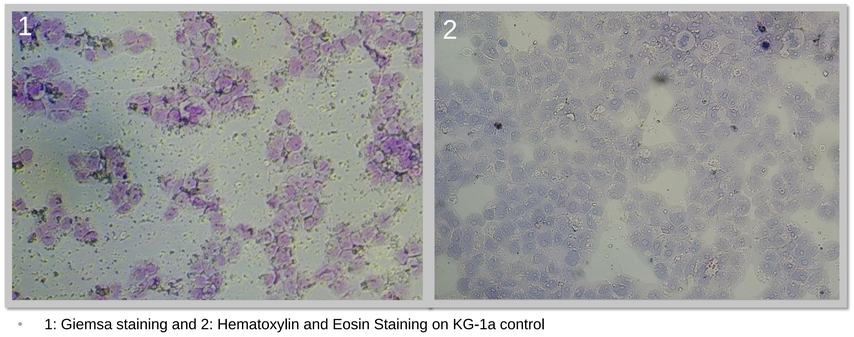
Results



- HPLC chromatogram of olive mill waste methanolic fraction observed at 280 nm. Different colours represent different cultivars. Black = 'Bidni' methanol, Blue = 'Malti' methanol and Pink = 'Bajda' methanol.



- The % cell viability of NB-4r2 cell lines after 24-, 48- and 72-hour exposure.



- 1: Giemsa staining and 2: Hematoxylin and Eosin Staining on KG-1a control

Conclusion

- Alkaline hydrolysed derived extracts showed the highest antioxidant activity with respect to TPC, DPPH, FRAP and CUPRAC assays whereas acid hydrolysis had the highest ABTS scavenging activity.
- Majority of extracts showed that the cell viability was the highest during the 24-hour exposure whilst at 48- and 72-hour exposure it starts to decrease.