Cherry and cactus pear natural extracts for colon cancer therapy - *in vitro* evaluation of chemopreventive and chemosensitization effects

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BACKGROUND

Epidemiological data suggest that ingestion of bioactive compounds from fruits and vegetables, such as polyphenols and terpenes, may contribute to reduce the incidence of cancer in humans. The mechanisms by which these compounds inhibit tumourgenesis is widely described and include attenuation of tumour angiogenesis, induction of cell cycle arrest and promotion of apoptosis. Sweet cherries (*Prunus avium*) and cactus pears (*Opuntia spp.*) have been reported to be rich sources of perillyl alcohol, flavonoids, phenolic acids and betalains, which are already identified to exhibit *in vitro* and *in vivo* chemopreventive effect against several types of cancers.

AIM

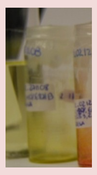
Development of natural chemotherapeutic agents derived from cherries and cactus pears and evaluation of their effectiveness in a colon cancer cell model

CHERRY EXTRACT

cherPOH

Raw Material: Cherry culls ("*Saco*" variety)

Process: Supercritical Fluid Extraction
(CO_2, P= 25MPa; T= 323K; t= 60 min)
+ Enhanced Solvent Extraction [1]
(90% CO_2: 10%EtOHv/v, P= 25MPa; T= 323K; t= 90 min)

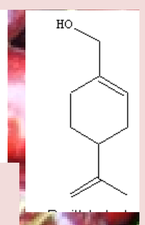


BIOACTIVE COMPOUNDS

Polyphenols

Total phenolic content= 2.5± 0.1 mg/g

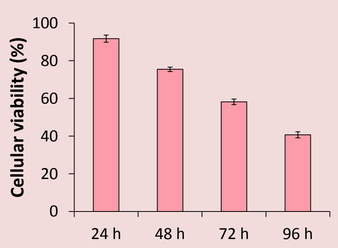
Perillyl alcohol (POH)



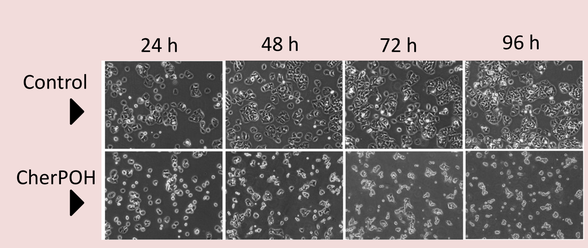
•The presence of POH in cherry extract was confirmed by TLC [1].

ANTIPROLIFERATIVE EFFECT ON HT29 CELLS

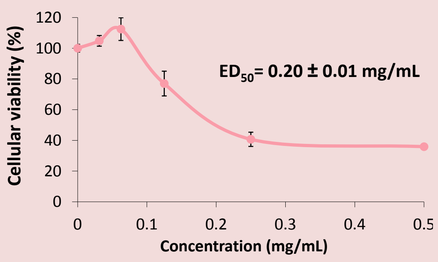
Time dependent effect



Note: Extract concentration – 0.5mg/mL



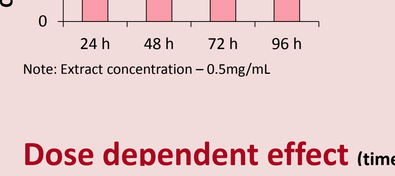
Dose dependent effect (time= 9

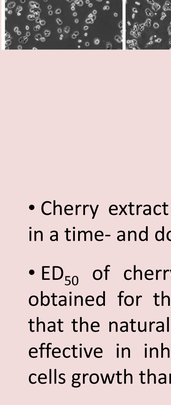


Note: Incubation time- 96h

- Cherry extract inhibited HT29 proliferation in a time- and dose-dependent effect.
- ED_{50} of cherry extract is less than that obtained for the whole fruit [2,3] indicating that the natural product is about 150x more effective in inhibiting human colon cancer cells growth than fresh "*Saco*" cherries.
- The extraction process was optimized[4] in order to obtain a natural ingredient with improved potency (32 fold; ED_{50}=0.2mg/mL at 24h of proliferation).

CELL CYCLE ARREST





- cherPOH induced cell cycle arrest in a different checkpoint (G1 phase) than doxorubicin (G2/M phase). This suggests that cherPOH can be used in combination with chemotherapeutic drugs to enhance the inhibition of tumor survival.

CACTUS PEAR EXTRACTS

oBET

Raw Material: *Opuntia rubusta* (fruit juice residues)

oFLAV

Opuntia ficus indica (fruit juice residues)

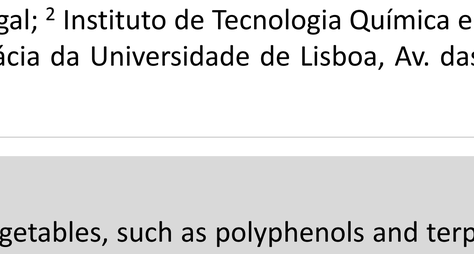
Process: Conventional Solvent Extraction [5] (50% EtOH: 50% H_2O)

Pressurized Liquid Extraction [5] (60% CO_2: 40%EtOHv/v, P= 20MPa; T= 313K)

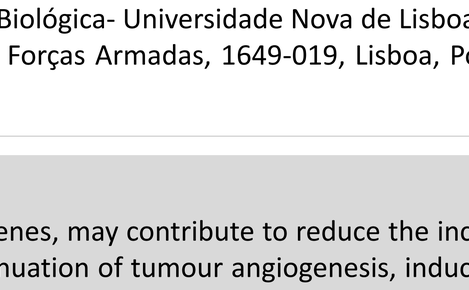
BIOACTIVE COMPOUNDS

Polyphenols

- oFLAV contains flavonoid compounds whereas oBet presents betalains pigments

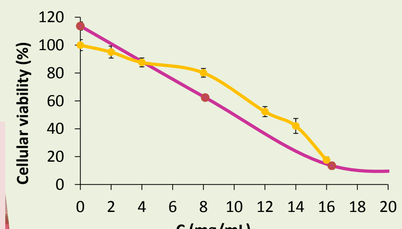


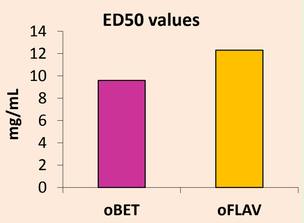
Total phenolic content= 17.5± 0.4 mg/g



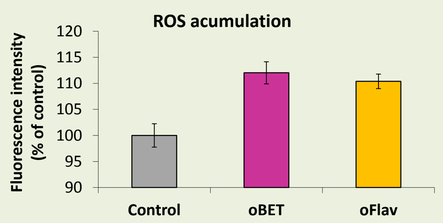
Total phenolic content= 7.3± 0.2 mg/g

ANTIPROLIFERATIVE EFFECT ON HT29 CELLS





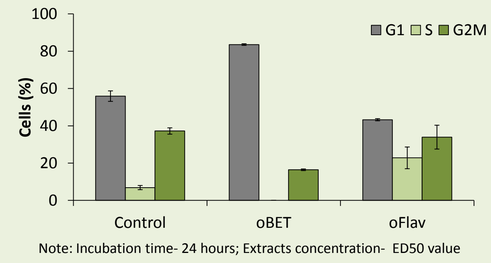
Note: Incubation time- 24 hours



Note: Incubation time- 24 hours; Extracts concentration- 0.5mg/mL

- Both extracts inhibited HT29 cell growth in a dose dependent manner probably by generating ROS at a cellular level.
- Both extracts exhibited different responses on cell cycle arrest: oBET induced cell cycle arrest into G1 phase whereas oFLAV promoted similar distribution in all cell cycle phases. This could be related with the distinct composition of samples.

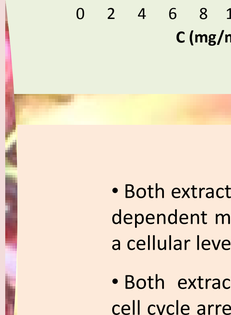
CELL CYCLE ARREST

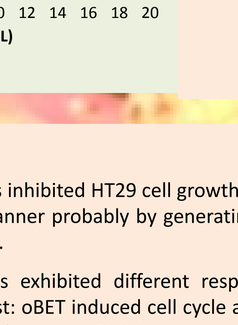


Note: Incubation time- 24 hours; Extracts concentration- ED50 value

CHEMOSENSITIZATION EFFECT

Characterization of HT29dx population



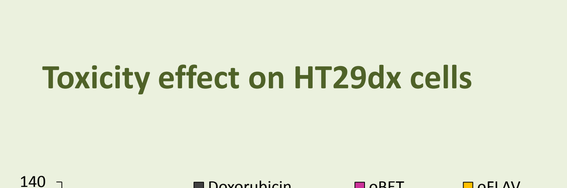


- HT29 normal cell line accumulated significantly more doxorubicin (almost 50%) than the resistant cell line (HT29 dx).
- ED50 value of drug was higher in HT29 dx.

Toxicity effect on HT29dx cells

Note: Extracts were incubated (ED50 value) for 24h followed by 1h of drug incubation. Cell viability was accessed 72 h after cell proliferation .

- oFLAV had a strong effect on HT29 dx viability (below 50%) whereas Q1 had no effect



Note: Extracts were incubated (ED50 value) for 24h followed by 1h of drug incubation. Cell viability was accessed 72 h after cell proliferation .

- HT29dx cells pre treated with oFLAV showed high sensitivity to the drug. This effect was closer than those verified for doxorubicin alone on HT29 normal cell population.

CONCLUSION

phytochemical- rich extracts from cherries and cactus pears are promising natural chemotherapeutic and chemosensitization ingredients for colon cancer therapy

ACKNOWLEDGEMENTS:

The authors acknowledge the financial support received from Fundação para a Ciência e Tecnologia (FCT) through PTDC/AGR-AAM/099645/2008 project, and also through grant PEst-OE/EQB/LA0004/2011and for the doctoral (SFRH/BD/19238/2004)fellowship.

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[1] Serra *et al.* (2010) *J Supercr Fluids* 55, 184-191; [2] Serra et al. (2011) *Food Chem* 125, 318-325; [3] Gonçalves et al. (2007*) Revista da APH* 90, 14–19; [4] Serra *et al.* (2011) *J Supercr Fluids* 55, 1007-1013; [5] Poejo, J (2011), Master thesis, FCT-UNL