Evaluation of bioactive extracts derived from cherries (*Prunus avium*) and cactus pear (*Opuntia ficus indica*) as promising natural chemotherapeutic agents

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AIM

- Extract a natural ingredient from cherries to be applied in new nutraceutical formulations with chemotherapeutic effect
- Explore cactus pear fruit as raw material of a functional beverage to be used to reinforce conventional colon cancer therapy

METHODS

Characterization of bioactive compounds

Total phenolic compounds (TPC): Determined according to the modified Folin Ciocalteau colorimetric method[1]. Results are expressed as gallic acid equivalents (GAE) in mg per gram or liter of extract and are a mean of 6 replicates.

HPLC analysis: Was carried out using a Surveyor apparatus with a diode array detector and an electrochemical detector[2]. Identification of compounds was done by comparing retention time, spectra and spiking samples with known amounts of pure standards.

TLC analysis: Perillyl alcohol (POH) identification in cherry extracts was performed using silica gel plates with 254nm fluorescent indicator and chloroform as the mobile phase[3]. Detection was done with iodine and results were compared with pure standard.

Antioxidant activity

ORAC assay: The antioxidant capacity of the extracts towards peroxyl radicals was carried out by following the method described elsewhere [4]. All data were expressed as micromoles of trolox equivalent antioxidant capacity (TEAC) per L of extract. Results are a mean of 6 replicates.

HORAC assay: The assay was performed as described elsewhere [5] using caffeic acid as the standard. Data were expressed as micromoles of caffeic acid equivalents (CAEAC) per L of extract. Results are a mean of 6 replicates.

Cellular assays

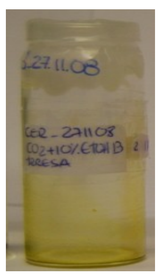
Cell culture: Human colon cancer cell lines, HT29 and Caco2, were obtained from ATCC and DSMZ, respectively**.** Both cell lines were grown in RPMI 1640 medium supplemented with 10% of FBS and 2mM of glutamine. Stock cells were maintained as monolayers in 175cm2 culture flasks and incubated at 37 ◦C with 5% CO_2 in a humidified atmosphere.

Cytotoxicity assay: Toxicity assays were performed using completely differentiated Caco2 cells as described previously[6]. Cell viability was accessed after 24, 48 and 72h of incubation with extracts using Cell Titer assay. Results were expressed in terms of percentage (%) of cellular viability relative to control. Assays were performed in triplicate.

Antiproliferative assay: Antiproliferative activity of natural extracts was evaluated in HT29 and Caco2 cells as described elsewhere[6]. 24h after seeding cells were allowed to proliferate for 24, 48 and 72 h with and without (control) natural extracts. Cell viability was determined with Cell Titer kit assay and results were expressed in terms of percentage (%) of cellular viability relative to control. Assays were performed in triplicate. For some extracts, the amount of sample necessary to decrease 50% of the cellular viability, ED50 (effective dose), was calculated.

Cell cycle analysis: The induction of cell cycle arrest was evaluated in HT29 cells as previously described using a flow cytometer CyFlow Space [6]. Samples tested were cherry extract and doxorubicin.

NATURAL INGREDIENT



CHERRY EXTRACT

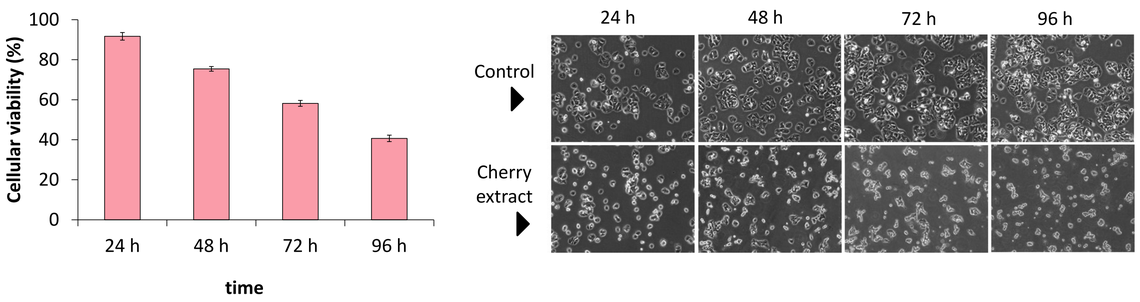
Raw material: Saco cherry culls

Extraction solvent: CO_2 and EtOH[3]

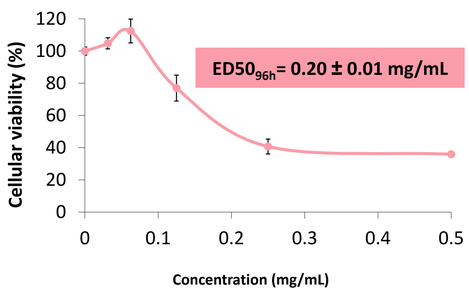
Bioactive compounds: Perillyl alcohol and polyphenols (neochlorogenic acid, catechin, epicatechin, rutin, quercetin-3-glucoside, luteolin, sakuranetin, sakuranin)

ANTIPROLIFERATIVE EFFECT ON HT29 CELLS

- Time dependent effect (Incubation time= 96h)



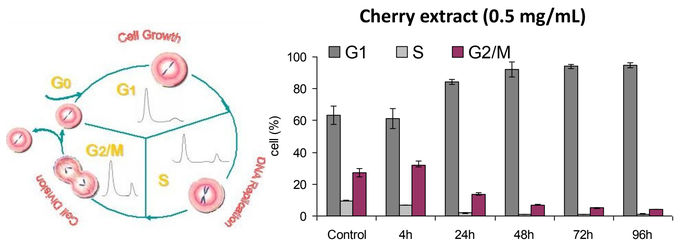
- Dose dependent effect (Incubation time= 96h)



▸Cherry extract inhibits HT29 proliferation in a time- and dose-dependent effect

▸ ED50$_{96h}$ of cherry extract is less than that obtained for the whole fruit (5.4mg dw/mL)[7] indicating that the natural product is about 150 times more effective in inhibiting human colon cancer cells growth than fresh *Saco* cherries (%water = 82.5%)

- Cell cycle arrest

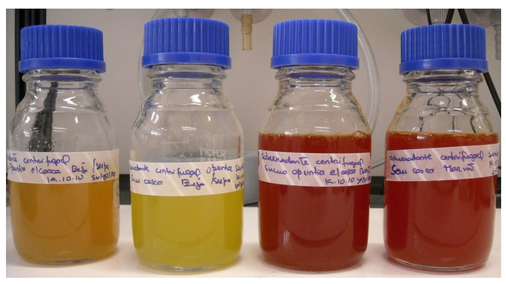


▸ Cherry extract induces cell cycle arrest in the G1 phase

CONCLUSION

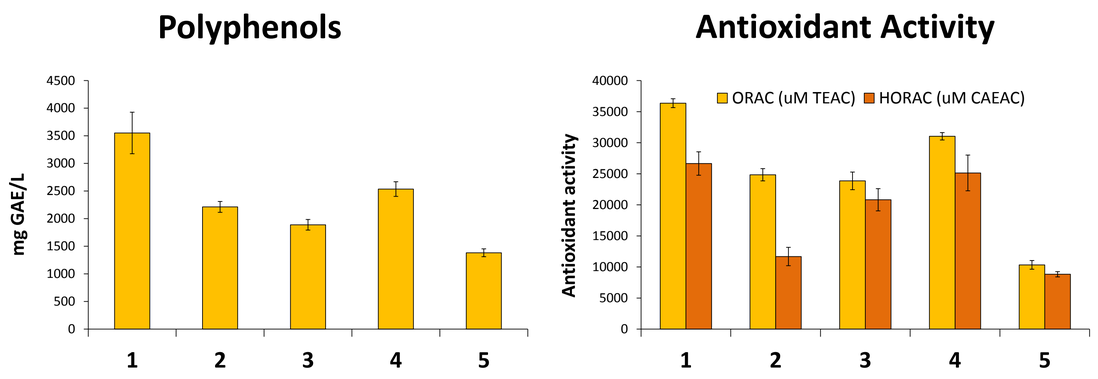
Cherry extract contains perillyl alcohol (a powerful anticancer compound) and induces cell cycle arrest in a different checkpoint than doxorubicin[6] suggesting that it could be used in combination with the drug in colon cancer therapy

FUNCTIONAL BEVERAGE



Juices	Fruit harvest site
1	Tramagal
2	Serpa
3	Marvão
4	Sines
5	Sesimbra

POLYPHENOLS AND ANTIOXIDANT ACTIVITY

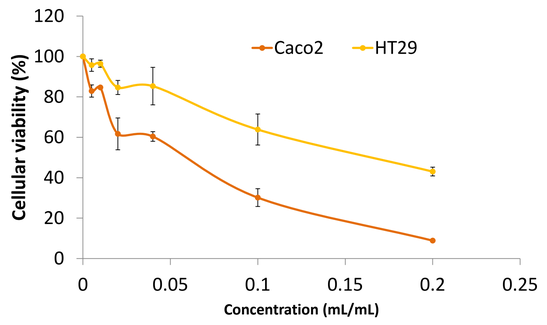


▸ *Opuntia ficus indica* juices from "Tramagal" has the highest total polyphenolic concentration and ORAC and HORAC values whereas the juices from "Sesimbra" fruits have the lowest phenolic concentration and antioxidant effect.

▸ Higher correlations were obtained between the total phenolic content of fruit juices and ORAC values (r= 0.93) than with HORAC results (r= 0.78)

ANTIPROLIFERATIVE EFFECT ON HT29 AND CACO2 CELLS

Sample 4- "Sines"



ED50$_{72h}$values of cactus pear juices in HT29 and Caco2 cells

Samples	HT29	Caco2
1- Tramagal	>0.2	0.055
2- Serpa	0.178	0.139
3- Marvão	>0.2	0.102
4- Sines	0.162	0.057
5- Sesimbra	>0.2	0.127

▸ Cactus pear juices inhibit HT29 and Caco2 cell growth in a dose-dependent effect.

▸ Cactus pear juices exhibit higher antiproliferative effect in Caco2 cells than HT29 cells.

▸ Juice of "Tramagal" fruits has the highest antiproliferative effect in Caco2 cells whereas the juice from "Sines" fruits is the most effective in inhibiting HT29 cell growth.

CONCLUSION

Cactus pear juices are rich sources of bioactive compounds with antioxidant and antiproliferative properties, representing a promising functional beverage to reinforce conventional colon cancer therapy

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