Characterization of *Opuntia ficus spp.* fractions obtained using Sephadex LH-20

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AIM ▶ Comparison of Phenolic compounds (total phenolic content and chromatographic profiles), Betalains and Antioxidant activity of fractions obtained with different % of ethanol.

Methods

Sample preparation

Cactus fruits (cactus pear) were collected in Beja /Serpa and Quarteira region. Fruits were washed, cut and were centrifuged . The residues were extracted with a water:ethanol solution (50:50).

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-DAD analysis: Was carried out using a Surveyor apparatus from Thermo, with a diode array detector and an electrochemical detector[2] from Dionex.
HPLC-MS/MS: Was carried out in an HPLC in tandem with a triple quadrupole mass spectrometer (Micromass® Quatro MicroTM, Waters®) using an electrospray source (ESI) operating in negative mode.

Antioxidant activity

ORAC: The antioxidant capacity of the extracts towards peroxyl radicals was carried out by following the method described elsewhere [3]. 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 [4] using caffeic acid as the standard. Data were expressed as micromoles of caffeic acid equivalents (CAE) per L of extract. Results are a mean of 6 replicates.

Antiproliferative effect

Antiproliferative activity of natural extracts was evaluated in HT29 as described elsewhere[5]. 24h after seeding cells were allowed to proliferate for 24with and without (control) extracts and fractions. 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..

FRACTIONS USING SEPHADEX LH-20

Residues	Origin (Opuntia from)	color
A	Beja/Serpa	brownish
B	Quarteira	Red/pink

• **Opuntia B**

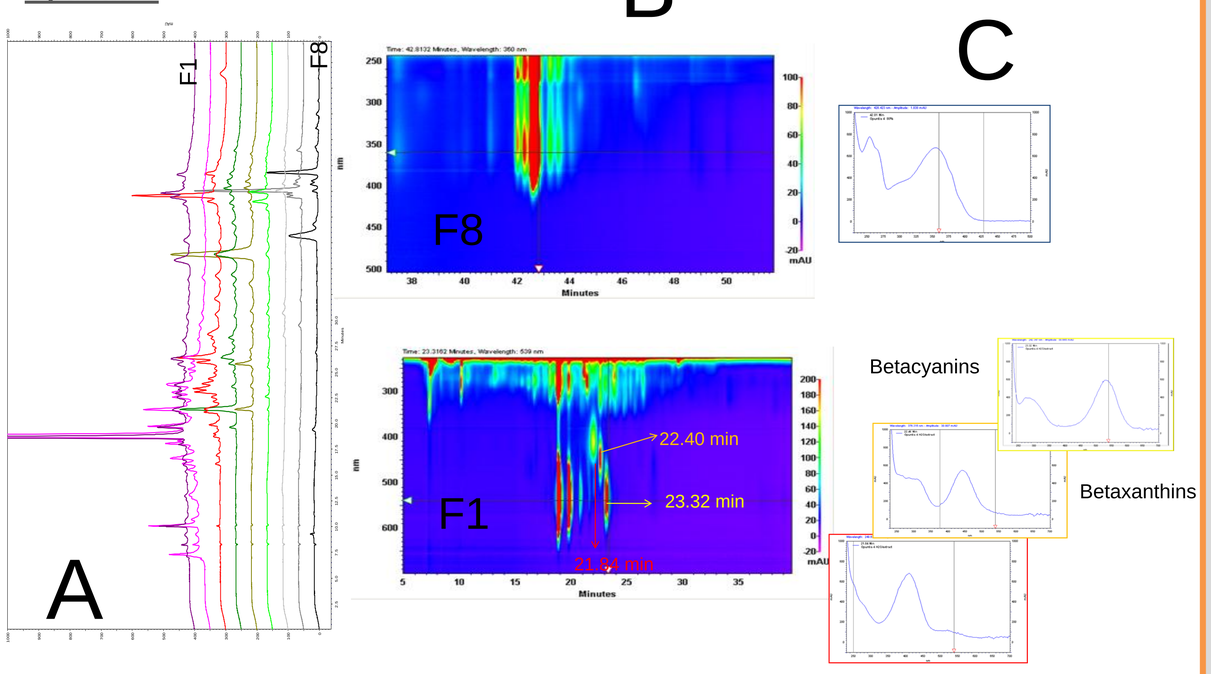
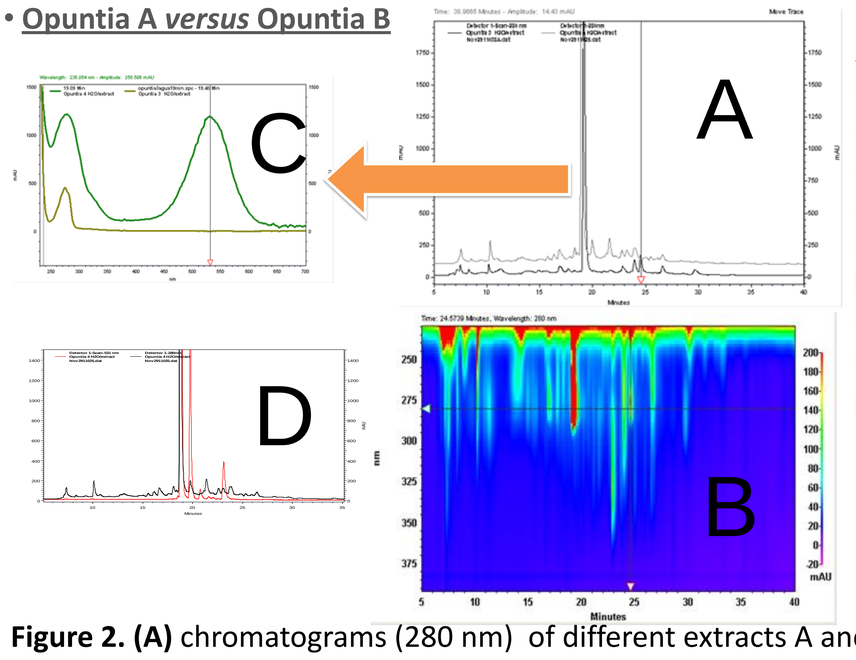


Figure 1. Extract B. **(A)** chromatograms (280nm) of different fractions (see table 2); **(B)** biplot of fractions F1 and F8; **(C)** spectra of different peaks in F1 and F8

• **Opuntia A *versus* Opuntia B**



At ±19.30 min in extract B are detected at least 2 compounds with different UV-vis spectra as shown in figure 2. One of them corresponds to a betacyanin and the other one was identified by LC-MS/MS (Figure 3) as piscidic acid.

Figure 2. (A) chromatograms (280 nm) of different extracts A and B; **(B)** biplot of extract A; **(C)** UV-vis spectra of peak at ±19.30 min in extract A and B ; **(D)** Chromatograms of extract B at 280 nm and 531 nm

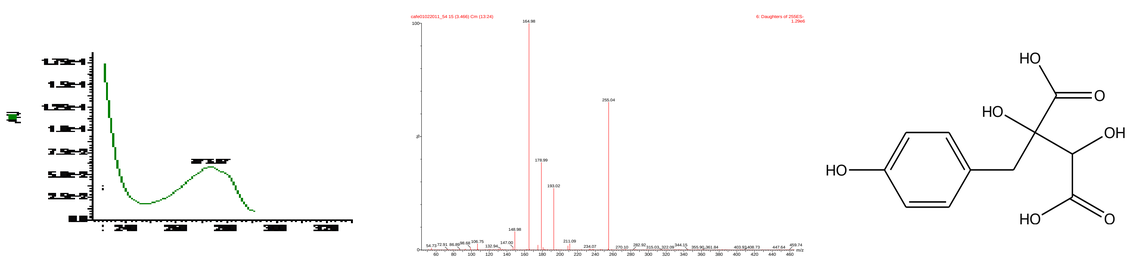


Figure 3. (A) UV spectra; **(B)** MS/MS spectra; **(C)** structure of piscidic acid

TPC AND ANTIOXIDANT ACTIVITY

Table 1. Total Phenolic content, ORAC and HORAC of extracts

Original Extracts	TPC	ORAC (µmol TEAC/L)	HORAC (µmol CAE/L)
A	9701 ± 163	315173 ± 2512	217723 ± 5021
B	2135 ± 101	23005 ± 1111	38220 ± 665

Table 2. Total Phenolic content of extracts and fractions

Extract A	TPC (mg GAE/L)	Extract B	TPC (mg GAE/L)
Original	2135 ± 101	Original	9701 ± 163
F1 (0% EtOH)	451.9±7	F1 (0% EtOH)	196.3±3.0
F2 (2.5% EtOH)	204.3±4.1	-	
F3 (5.0% EtOH)	50.0±0.1	-	
F4 (7.5% EtOH)	27.4±0.7	-	
F5 (10% EtOH)	14.5±0.1	F2 (10% EtOH)	40.2±0.8
F6 (20% EtOH)	12.3±0.3	F3 (20% EtOH)	38.4±0.8
F7 (30% EtOH)	15.0±0.1	F4 (30% EtOH)	18.2±0.5
F8 (40% EtOH)	15.6±0.3	F5 (40% EtOH)	14.3±0.3
F9 (50% EtOH)	17.4±0.1	F6 (50% EtOH)	9.4±0.5
F10 (80% EtOH)	13.1±0.1	F7 (80% EtOH)	24.9±0.7
F11 (100% EtOH)	15.8±0.5	F8 (100% EtOH)	25.3±0.4

ANTIPROLIFERATIVE ACTIVITY

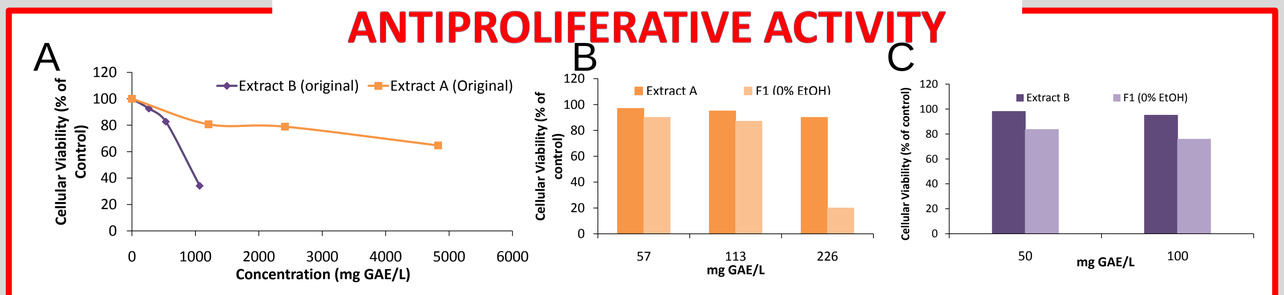


Figure 4. Antiproliferative effect of extracts against (A)- Extract A versus Extract B; (B)- Extract A and fraction F1; (C)- Extract B and franction F1

Table 3. Effective dose values of extracts and fractions

Extracts		ED50 (mg GAE/L)
Extract A	Original	nd (>4800)
	F1 (0% EtOH)	171,8
Extract B	Original	870,9
	F1 (0% EtOH)	nd (> 100)

Nd- not detectable for the concentrations tested

Extract B presents higher antiproliferative effect than extract A (Figure 4A)

For both extracts, Fraction 1 seems to present compounds responsible for the antiproliferative effect (Figures 4 B-C)

CONCLUSIONS

- Separation with preparative column using Sephadex LH-20 enabled to obtain fractions separating different families of compounds.(flavonols and betalains);
- The compounds identified in fraction 1 (mainly betalains) present antiproliferative effect on HT29 cells;
- Piscidic acid was identified by LC-MS/MS in the extract from residue A.

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[1] Ahmed, E. M. *et al*, *J. Agric. Food Chem.*, 26, 187-191, (1978); **[2]** Canbas, A., *et al*, *Journal of Food Composition and Analysis*, 17, 789-796, (2004); **[3]** Feliciano et al. *Food Anal Methods* 2: 149-161(2009) ; **[4]** Serra et al. *J Funct Foods* 2: 46-53 (2010) ; **[5]** Serra *et al.* (2010) *J Supercr Fluids*, 55, 1007-1013 (2011)