Subcritical Water Extraction of *Actinidia arguta* leaves: radical scavenging capacity and cell effects

Ana Sofia Luís[1,2], Ana Margarida Silva[2], Cristina Delerue-Matos[2], Ricardo Ferraz[1,3], Jaroslava Švarc-Gajić[4], Francisca Rodrigues[2*]

[1]Polytechnic of Porto –School of Health, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal; [2]REQUIMTE/LAQV, Polytechnic of Porto –School of Engineering, Rua Dr. António Bernardino de Almeida, 4249-015 Porto, Portugal; [3]Health and Environment Research Center, School of Health of the Polytechnic Institute of Porto, Porto, Portugal; [4]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia

*francisca.rodrigues@graq.isep.ipp.pt

INTRODUCTION

- Kiwiberry is known to be a small fruit produced by *Actinidia arguta* vine, native from Asian region [1];
- Kiwiberry has aroused commercial interest due to its richness in bioactive compounds, that are frequently associated to beneficial effects in human's health [2];
- During its production and harvesting processes are generated different by-products, such as leaves, enriched in bioactive compounds;
- Antioxidant, antimicrobial, anti-inflammatory and radical scavenging activities are examples of properties linked to *A. arguta* by-products [3, 4, 5, 6];

Actinidia arguta plant [3]

- The biocompounds present can be recovered by green extraction techniques, such as Subcritical Water Extraction (SWE) [3];
- SWE is classified as a green and sustainable extraction technique that employs water as solvent [7];
- Water is a clean, cheap and widely available solvent, that under subcritical conditions (100°C - 374°C) maintains its liquid state above the boiling point [7,8];
- Extracts without associated toxicity by the solvent, less extraction time and good extraction efficiencies are some of SWE advantages [7].

OBJECTIVES

- Evaluate the bioactivity, antioxidant and radical scavenging activity of *A. arguta* leaves extracts obtained at different temperatures by SWE and effects on HT29-MTX and Caco-2 cell viability.

MATERIAL AND METHODS

***Actinidia arguta* leaves**

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Subcritical Water Extraction

Temperature: 110°C - 160°C
Pressure: 20 bars
Time: 30 minutes

- Condition 1: 110°C
- Condition 2: 123°C
- Condition 3: 135°C
- Condition 4: 148°C
- Condition 5: 160°C

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Lyophilization

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Total Phenolic Content (TPC) assay

Total Flavonoids Content (TFC) assay

Hypochlorous acid (HOCl) radical scavenging assay

DPPH$^{\cdot}$ radical scavenging activity (DPPH) assay

Superoxide ($O_2^{\cdot-}$) radical scavenging assay

Oxygen Radical Absorbance Capacity (ORAC) assay

2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

RESULTS AND DISCUSSION

Table 1: TPC, TFC, DPPH free radical scavenging, $O_2^{\cdot-}$, HOCl and peroxyl radical (ROO$^{\cdot}$) scavenging capacity results of *A. arguta* leaves extracts by SWE. Values are expressed as mean ± standard deviation (n=3). Different letters in the same column mean significant differences (p<0.05) between samples.

A. arguta extracts	TPC (mg GAE/g dw)	TFC (mg CE/g dw)	IC_{50} (µg/mL)			ROO$^{\cdot}$ (S_{sample}/S_{Trolox})
			DPPH$^{\cdot}$	$O_2^{\cdot-}$	HOCl	
110°C	106.48 ± 4.71^{a}	46.07 ± 4.11^{b}	583.43 ± 29.48^{a,b}	344.53 ± 23.09^{c}	18.61 ± 0.72^{b}	0.13 ± 0.00^{c}
123°C	109.72 ± 4.98^{a}	53.11 ± 4.52^{a}	497.13 ± 39.46^{b}	335.23 ± 11.71^{c}	17.06 ± 0.92^{b}	0.15 ± 0.02^{c}
135°C	68.78 ± 2.72^{c}	33.68 ± 3.38^{c}	539.63 ± 40.13^{a,b}	440.67 ± 2.51^{b}	20.56 ± 0.11^{b}	0.10 ± 0.00^{c}
148°C	72.92 ±1.18^{b,c}	32.69 ± 1.60^{c}	625.60 ± 49,73^{a}	473.07 ± 6.57^{b}	20.28 ± 1.41^{b}	0.10 ± 0.00^{c}
160°C	77.37 ± 3.01^{b}	32.72 ± 1.27^{c}	574.73 ± 19.54^{a,b}	563.73 ± 24.13^{a}	26.93 ± 1.34^{a}	0.11 ± 0.00^{c}
Positive controls						
Trolox	-	-	30.57 ± 2.08^{c}	-	-	-
Catechin	-	-	-	137.67 ± 7.19^{d}	0.95 ± 0.03^{d}	6.25 ± 0.28^{a}
Gallic acid	-	-	-	99.46 ± 2.12^{d}	11.06 ± 0.40^{c}	1.32 ± 0.08^{b}

dw: dry weight; GAE: gallic acid equivalents; CE: catechin equivalents; IC_{50} = in vitro concentration required to decrease in 50% the reactivity of the studied reactive species in the tested media

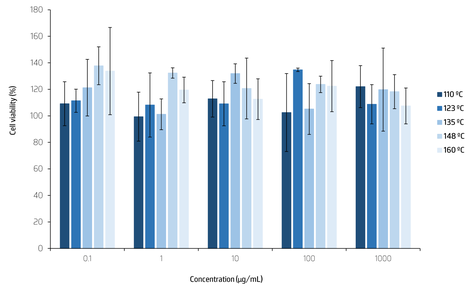

Figure 2 - Effect of *A. arguta* leaves extracts on the viability of HT29-MTX measured by an MTT assay, at different concentrations

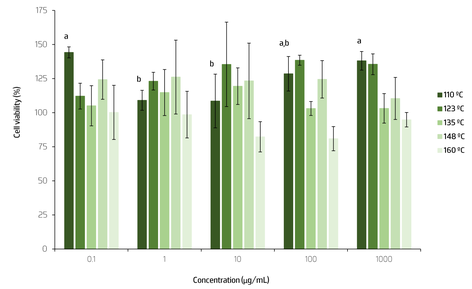

Figure 3 - Effect of *A. arguta* leaves extracts on the viability of Caco-2 cells measured by an MTT assay, at different concentrations

- In the TPC assay, the best results were achieved with condition 1 (106.48 mg GAE/g dw) and 2 (109.72 mg GAE/g dw) and in the TFC assay, the highest result was observed with condition 2 (53.11 mg CE/g dw);
- The results ranged between 497.13 µg/mL and 625.60 µg/mL for DPPH assay. Almeida *et al.* and Marangi *et al.* obtained lower results [5, 9];
- For $O_2^{\cdot-}$ assay, the best results were found in condition 1 (344.53 µg/mL) and 2 (335.23 µg/mL) and no significant differences were observed between them (p>0.05);
- Regarding HOCl assay, no significant differences were observed between conditions 1, 2, 3 and 4 (p>0.05)
- In the ORAC assay, all conditions presented lower results than catechin and gallic acid (positive controls);
- No inhibition effects were detected on the viability of HT29-MTX cells, at the highest tested concentration (1000 µg/mL). Relatively to Caco-2 cells, condition 5 displayed viabilities of 80.93% and 82.29% at concentrations of 10 µg/mL and 100 µg/mL, respectively.

CONCLUSION

- *A. arguta* leaves are rich in bioactive compounds with radical scavenging activity;
- SWE proved to be an efficient extraction technique to recover high-value compounds from *A. arguta* leaves;
- The best condition to extract bioactive compounds is at 123°C (condition 2), according to the results. The degradation of polyphenols can occur with increasing temperature [10];
- To identify and quantify the bioactive compounds and ensure the extracts' safety, further analysis, such as liquid chromatography, should be performed.

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