

# Proline-based DESs - Greener Alternative for Obtaining Polyphenol-Rich Extracts

from *Satureja kitaibelii* Wierzb. ex Heuff. (Lamiaceae)

J. Arsenijević<sup>1</sup>, N. Kovačević<sup>1</sup>, M. Drobac<sup>1</sup>, S. Ražić<sup>2</sup>, F. Emhemmed<sup>3</sup>, C. Muller<sup>3</sup>, C. Marcic<sup>3</sup>, E. Marchioni<sup>3</sup>

<sup>1</sup> Department of Pharmacognosy, University of Belgrade - Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia; <sup>2</sup> Department of Analytical Chemistry, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia; <sup>3</sup> Institut Pluridisciplinaire Hubert Curien, UMR 7178 CNRS, Université de Strasbourg, Illkirch, France

Using natural deep eutectic solvents (DESs) for preparation of extracts is in line with the principles of green chemistry. In that regard, their applicability needs to be confirmed concerning the extraction efficiency and toxicity.

DESs tested in this work were good extracting agents for phenolic compounds from aerial parts of *Satureja kitaibelii*, especially for higher caffeic acid oligomers such as clinopodic acid O. Additionally, their low cytotoxicity is a good starting predictor of their safety.

## Natural Deep Eutectic Solvents (DESs)

Environmentally sustainable solvents, composed of two or more compounds that are primary metabolites, i.e. organic acids, sugars, alcohols, amines and amino acids.

## *Satureja kitaibelii* - Rtanj's tea



Aerial parts traditionally used to treat various respiratory, urinary and other disorders [1].

Extracts exhibit a significant bioactivity [2].

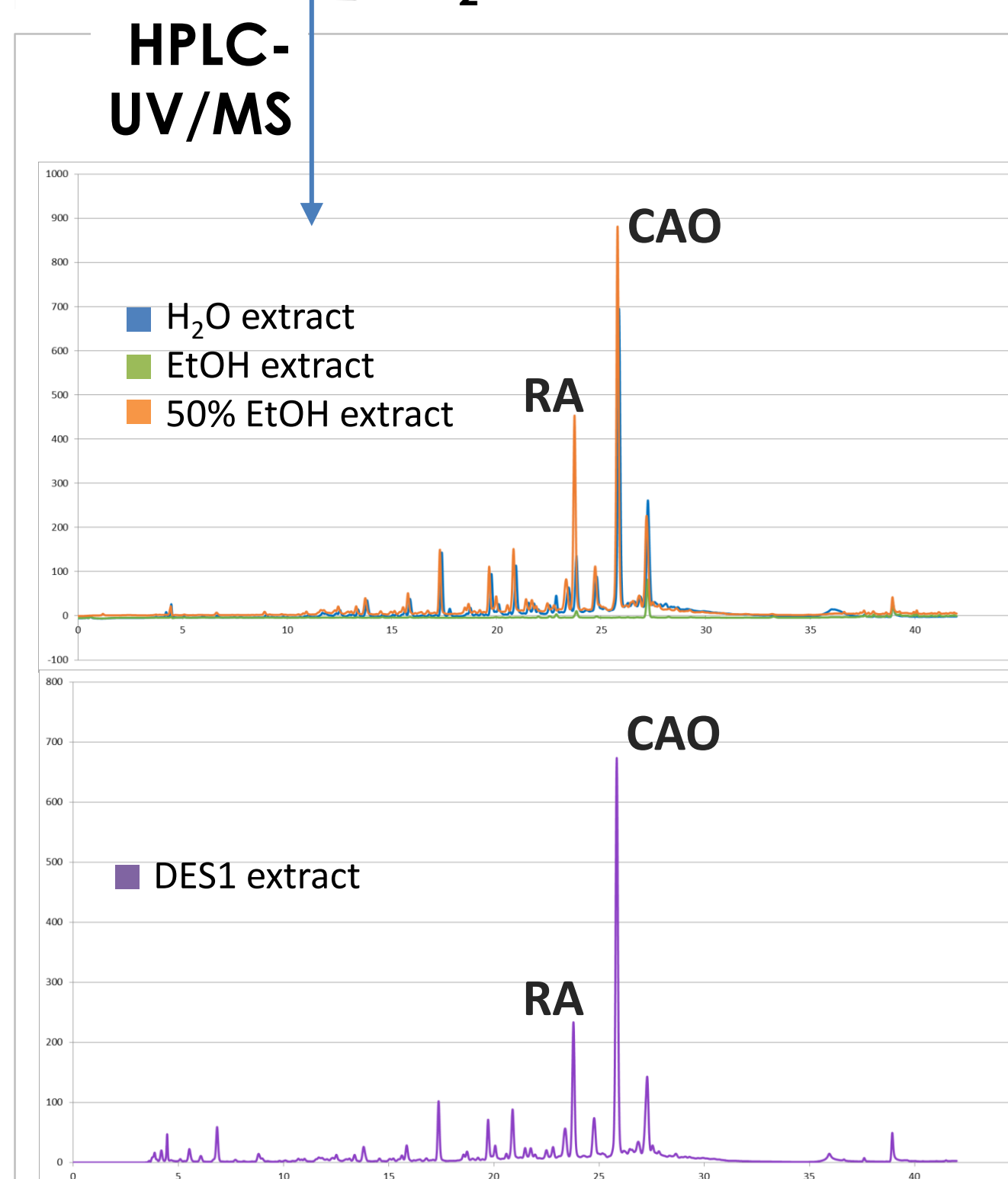
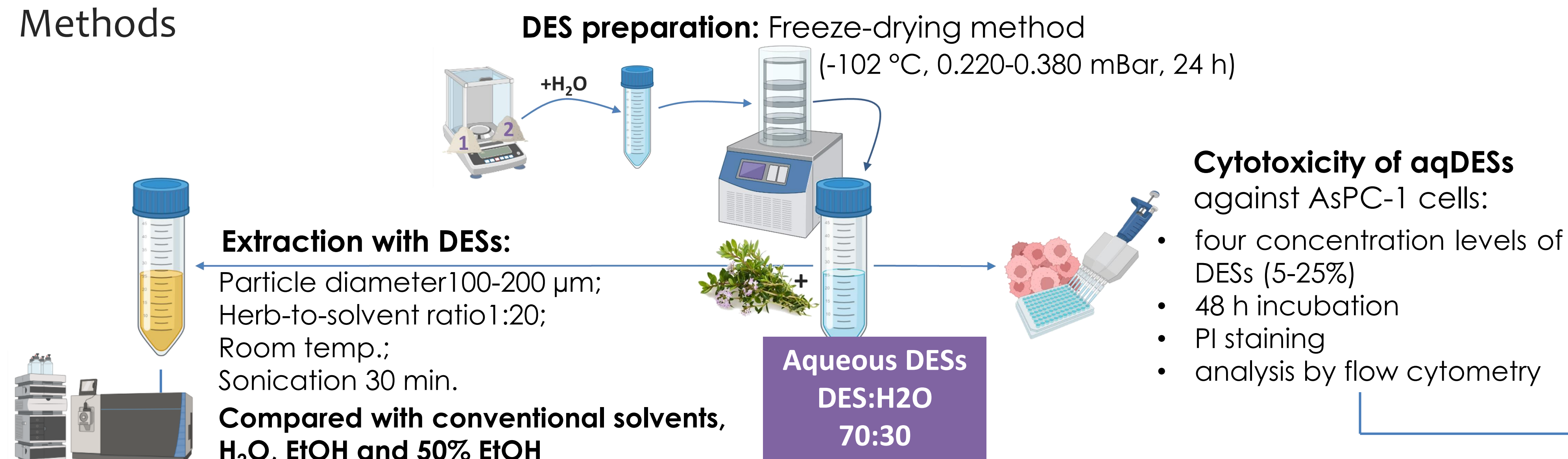
Composition: phenolic acids, flavonoids, essential oil [2].

## Objectives

To assess the polyphenol-extracting ability of proline (Pro) and sugar/sugar alcohol based natural DESs from commercially available Rtanj's tea.

To evaluate cytotoxicity of these solvents against AsPC-1 cells.

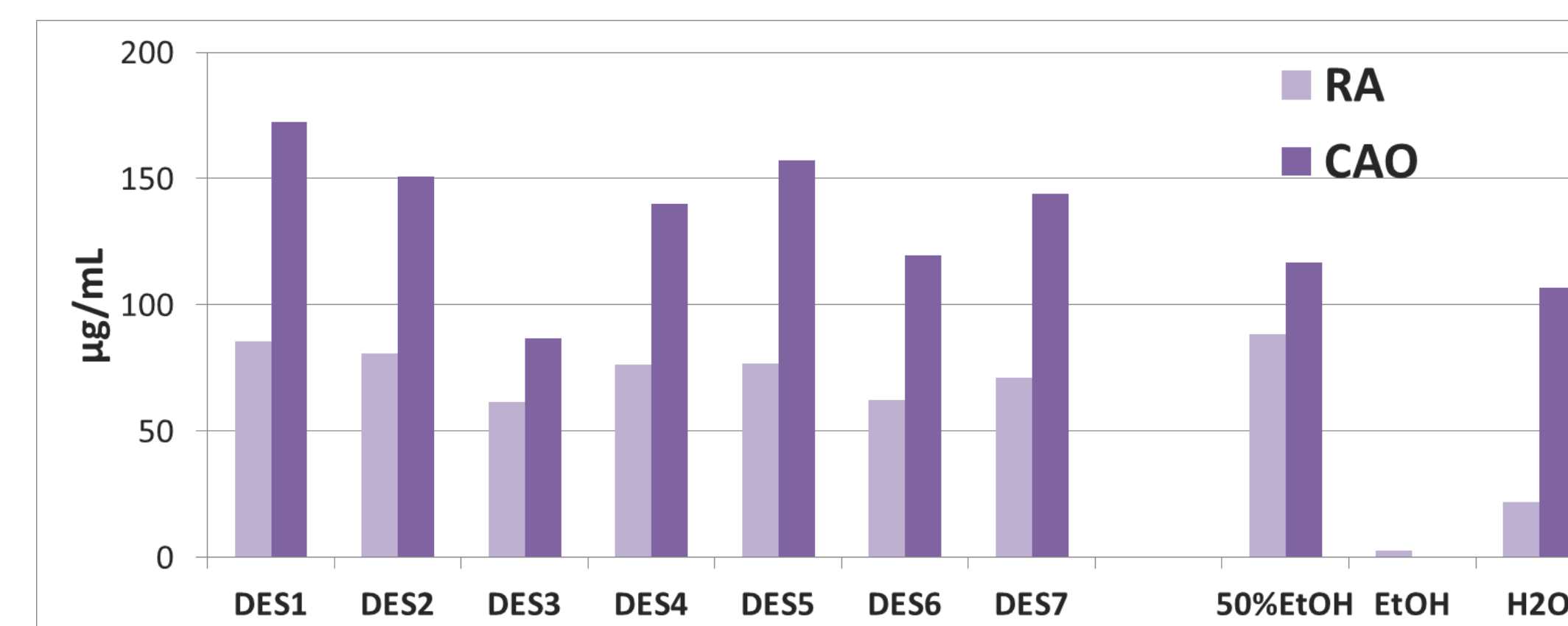
## Methods



**Fig. 1.** Chromatographic profiles (280 nm) of extracts obtained with H<sub>2</sub>O, EtOH and 50%EtOH, and DES1

Peak	UV $\lambda_{max}$ (nm)	ESI-MS data (m/z)	Assignment
RA	328; 294	359 [M-H] <sup>-</sup> ; 197; 179; 161	Rosmarinic acid
CAO	286; 330	1075 [M-H] <sup>-</sup> ; 877; 519; 339	Clinopodic acid O

## Results



**Fig. 2.** Concentration of rosmarinic acid (RA) and clinopodic acid O (CAO) in the obtained extracts

The content of RA and CAO was determined by external calibration using RA as the standard compound.

### Concentration of RA:

50%EtOH > DES extracts (62-86 µg/mL) > H<sub>2</sub>O >> EtOH

### Concentration of CAO:

DES1,2,4-7 (120-172 µg/mL) > 50%EtOH > H<sub>2</sub>O > DES3 >> EtOH

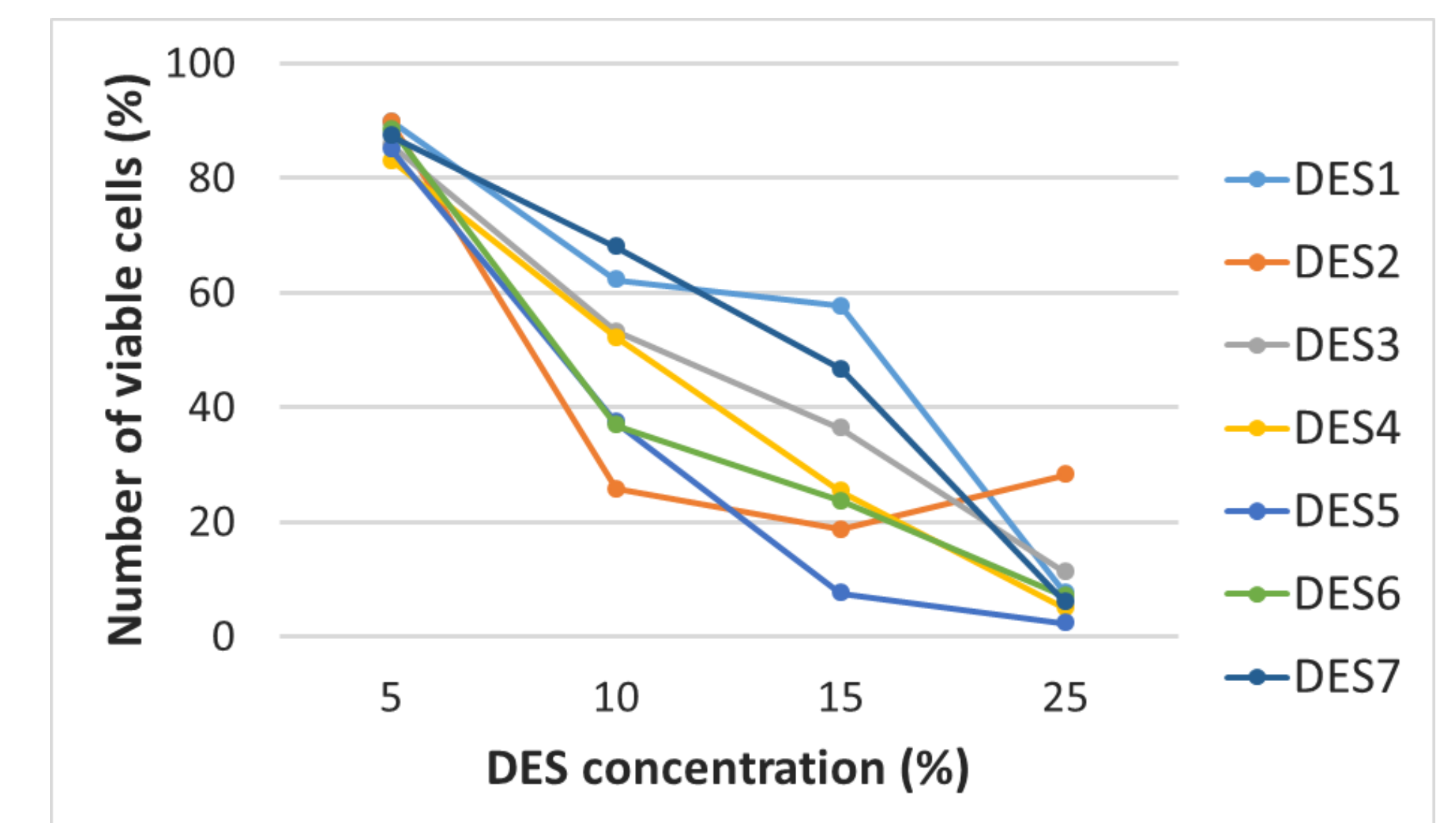
**Table 1.** Composition of prepared DESs

	Component 1	Component 2	Mol ratio
DES1	Proline	D-Glucose	1:1
DES2		D-Glucose	5:3
DES3		D-Fructose	1:1
DES4		D-Fructose	5:3
DES5		D-Sorbitol	1:2
DES6		Sucrose	2:1
DES7		Sucrose	3:2

## Viability of AsPC-1 cells

At the lowest tested concentration (5%), DESs did not significantly affect survival of AsPC-1 cells in comparison to the untreated cells

DESs 83.1-90.0%  $\approx$  untreated cells 86,6%



**Fig. 2.** Viability of AsPC-1 cells after 48 h incubation with DESs