CANPE– Analysis of antiproliferative effect of four plant extracts on cancerous SH-SY5Y cell line using impedance-based real-time monitoring assay

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Introduction

Cancer is one of the top three causes of mortality in Europe and worldwide. Although in the last few decades there has been progress in early detection of cancer and improve standard therapy (surgery , radio- and chemotherapy), duration of survival after treatment is limited. Currently one of the directions of research to improve anti-tumor therapy is the use of natural products in combination with standard anti-tumor agents to overcome the resistance of cancer cells.

The aim of present work was to investigate the antiproliferative activity of four plant extracts on SH-SY5Y human neuroblastoma cell line using the method of real-time monitoring of cell growth by impedance measurements.

Methods

- SH-SY5Y cells were grown in direct contact with gold electrodes integrated to the bottom surface of the microtiter plate well.
- Low intensity AC signals (μA) were periodically applied to electrodes and the magnitude of the electric impedance was measured.

No.	Species name	Common name	Family	Plant extract composition
1.	*Geranium robertianum* (GE) - *herba*	**Herb-Robert, Red Robin, Death come quickly**	*Geraniaceae*	5 mg total phenols, expressed as gallic acid equivalents [GAE], per 1 mL sample; ethanol 40%
2.	*Epilobium hirsutum* (EP) - *herba*	**Great willowherb, Great hairy Willowherb**	*Onagraceae*	
3.	*Fagus sylvatica* (FG) - *folium*	**European beech, common beech**	*Fagaceae*	5 mg total phenols, expressed as gallic acid equivalents [GAE], per 1 mL sample; ethanol 20%
4.	*Juglans regia* (JG) - *folium*	**Persian walnut, English walnut, common walnut**	*Juglandaceae*	

Working protocol

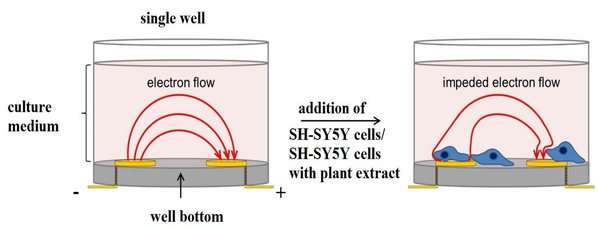
SH-SY5Y adherent monolayer culture

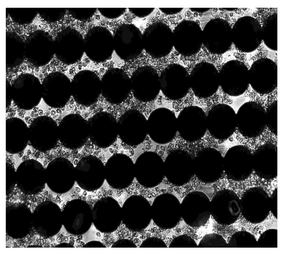
↓

24 h seeding

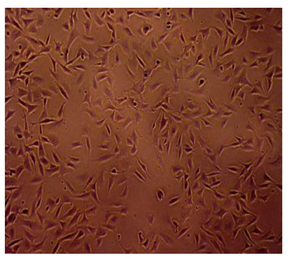
Cells were incubated with 6 concentrations of GE, EP, FG and JG, 72h

0.5 ug GAE/mL | **5 ug GAE/mL** | **10 ug GAE/mL** | **20 ug GAE/mL** | **30 ug GAE/mL** | **40 ug GAE/mL**





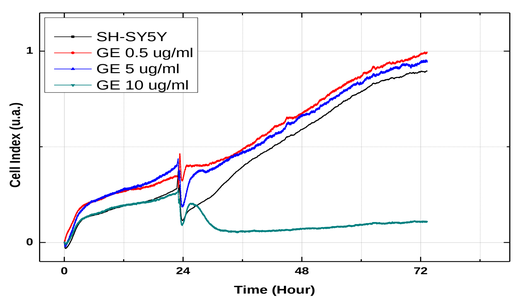
A

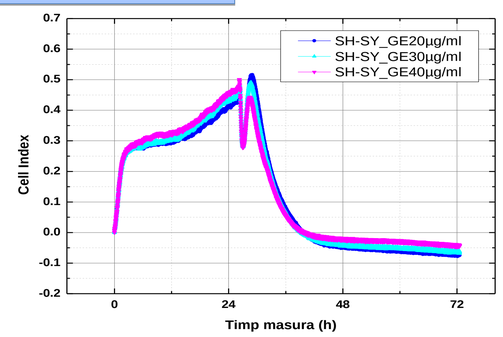


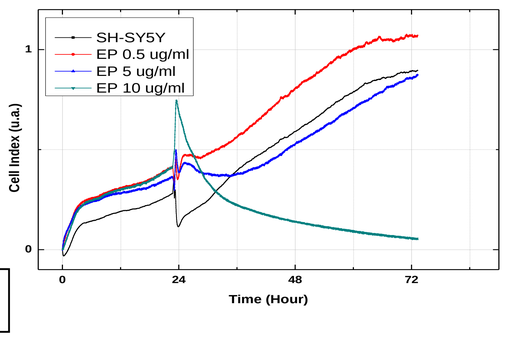
B

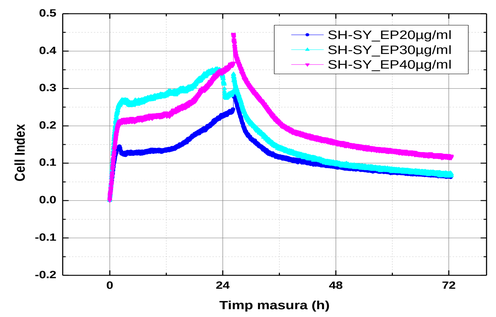
Microscopical images: A - of a standard E-Plate with cells and vegetal extract **(FG 10 μg/mL, 72h) (10X);** B – of a Petri dish with cells and no plant extract **(25X)**.

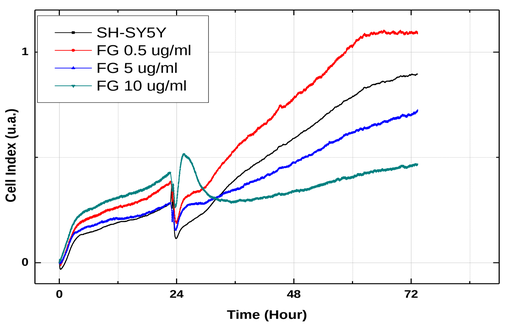
Results

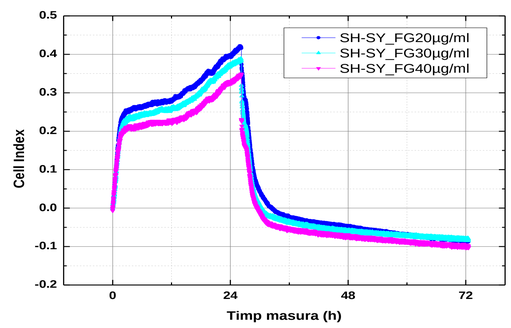


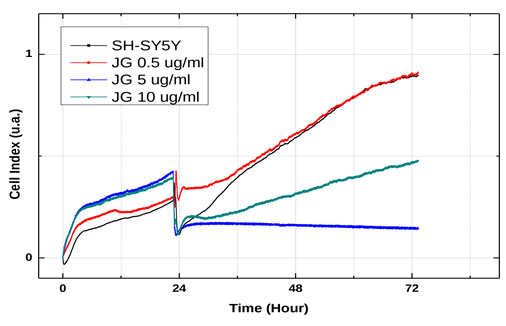


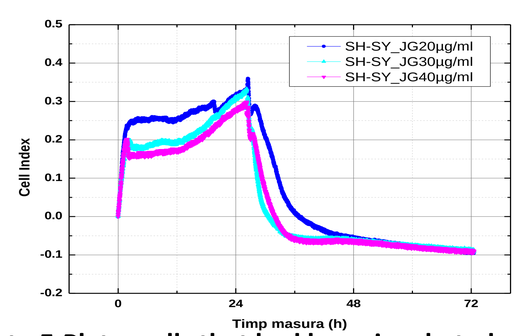












Real-time monitoring of SH-SY5Y cells adhesion to E-Plate wells that had been incubated with of 0.5, 5, 10, 20, 30, 40 μg GAE/mL of plant extract (GE, EP, FG, JG) for 72h

Conclusion

- **The extracts of 0.5, 5, 10 μg GAE/mL showed no antiproliferative effect, while at higher concentrations (20, 30, 40 μg GAE/mL) the cellular growth decreased rapidly.**
- **10 μg GAE/mL was the concentration for which the antiproliferative effect of the extracts on SH-SY5Y become significant.**
- **These experiments will be continued with the evaluation by the same technique of antiproliferative-antitumor effect of combinations of these plant extracts with conventional cytostatic agents for identification of new combinations with potentiated activity.**
- **Our results represent the first evidence of antiproliferative-anticancer activity of the 4 extracts using a method of real-time monitoring of cellular growth.**

Acknowledgement - This work was supported by the ANCSI, program NUCLEU, project no. 1627 03 01