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Hedwigia ciliata (Hedw.) P. Beauv. moss extracts - Chemical characterization and *in vitro* testing of immunomodulatory potential

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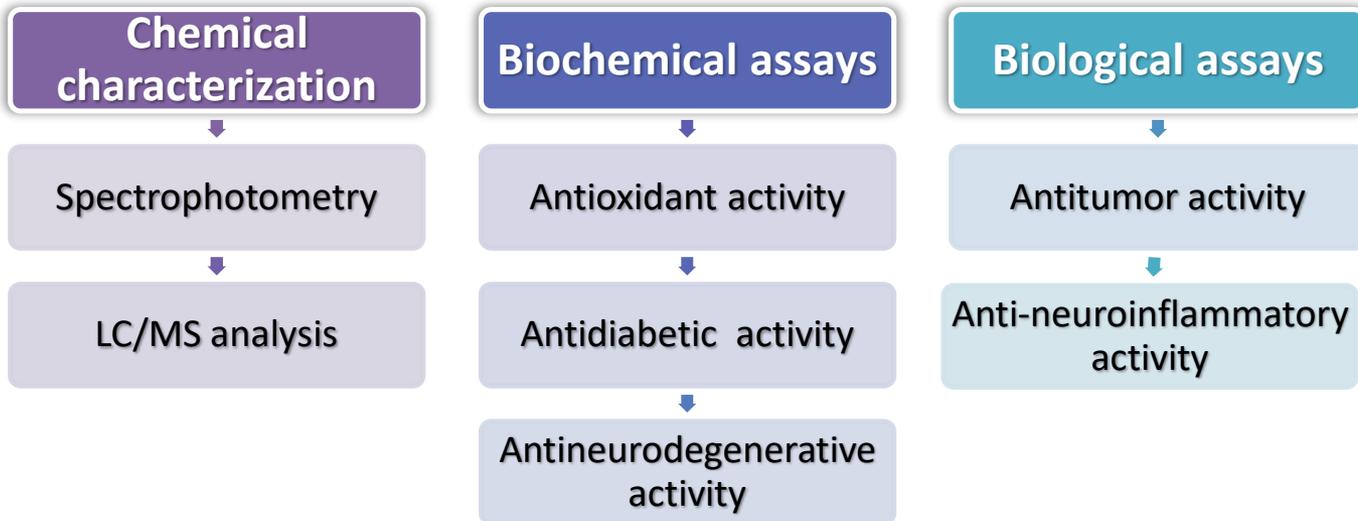
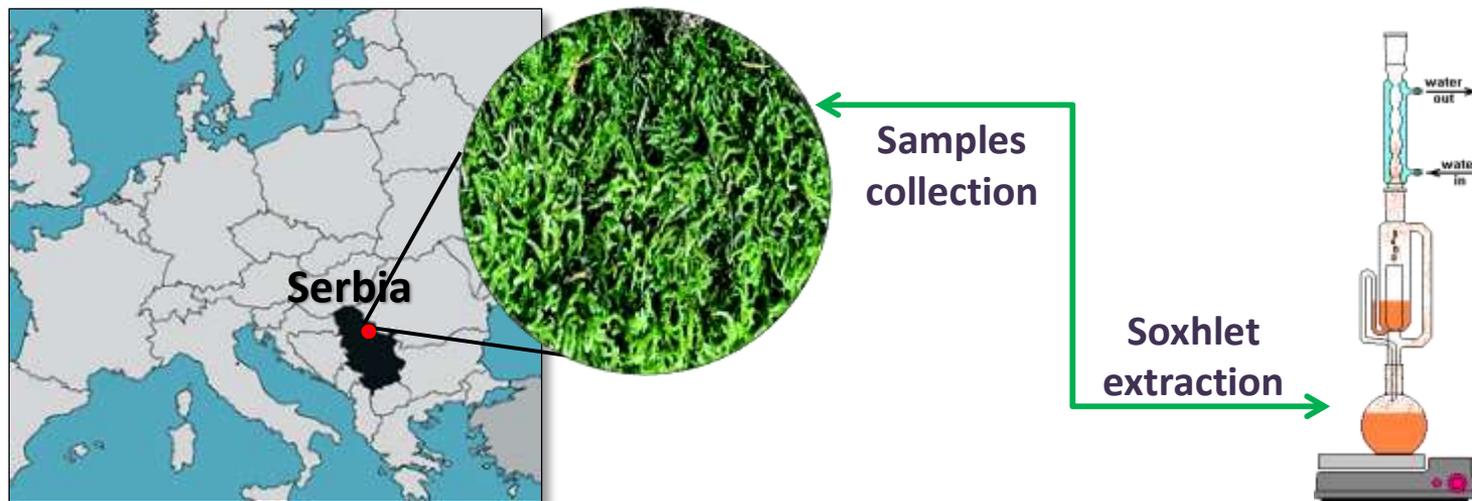
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Hedwigia ciliata (Hedw.) P. Beauv. moss extracts - Chemical characterization and *in vitro* testing of immunomodulatory potential



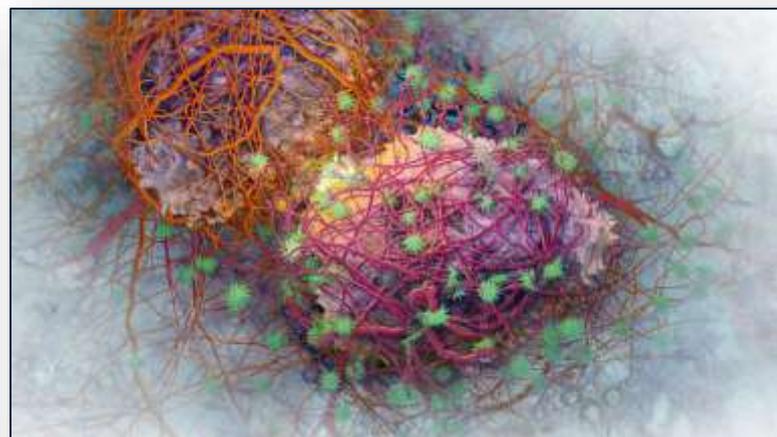
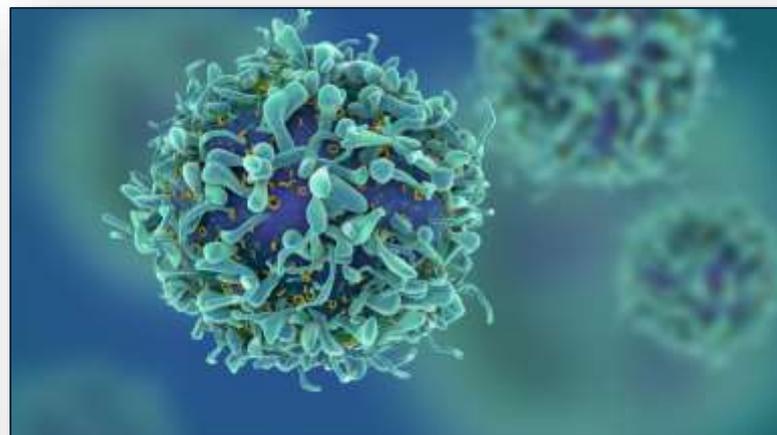
Abstract: Owing to structural diversity and potential pharmacological activity, bioactive compounds derived from natural sources are in the focus of intensive exploration. In this study, the chemical composition of moss *Hedwigia ciliata* P. Beauv. extracts was examined, and further tested for **antioxidant**, **antineurodegenerative**, **antidiabetic**, and **antiproliferative** activity. The extracts were prepared by Soxhlet extractor using solvents of different polarity. Chemical analysis revealed the presence of **phenolics**, **flavonoids**, and **triterpenoids**. Antioxidant activity of extracts was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl), total reducing power, and β -carotene/linoleic acid assay. To evaluate the enzyme-inhibitory activity, extracts were screened for α -amylase, α -glucosidase, acetylcholinesterase, and tyrosinase inhibitory effects. The *H. ciliata* extracts displayed significant antiproliferative activity (~ **50% inhibition**) against the MDA-MB-231 (**human breast adenocarcinoma**) cells. The potential anti-neuroinflammatory activity was observed due to significant reduction of the NO production by LPS-stimulated BV2 (normal murine microglia cell line), being in line with previously recognized antineurodegenerative potential, as measured by the inhibition of acetylcholinesterase and tyrosinase activity. Furthermore, the extracts displayed significant antidiabetic effect *via* α -glucosidase inhibition. In general, the obtained results suggest the **potential immunomodulatory activity** of the moss *H. ciliata* extracts, remaining to be characterized in more detail in search for mechanisms underlying the immunomodulation processes.

Keywords: antidiabetic, antineurodegenerative, antiproliferative, moss extracts

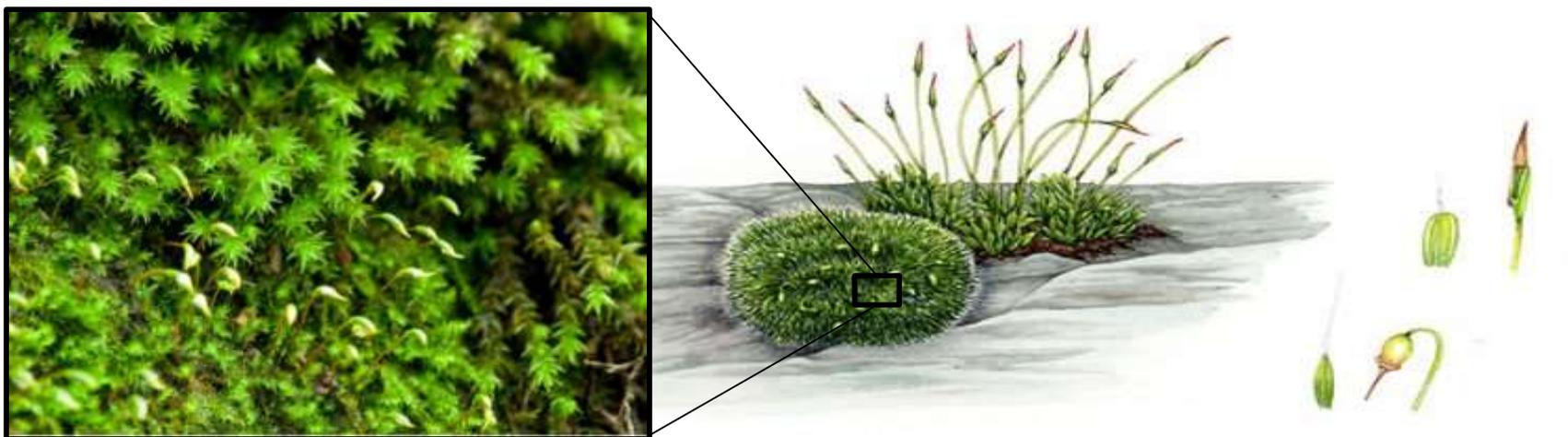


Introduction

- As a result of an excessive inflammatory response, various acute and chronic diseases can occur, as well as tumors
- The increased rate of tumor diseases has led to an expanding number of studies aimed at prevention, diagnosis, prognosis and therapy
- Bioactive compounds act as **immunomodulators**



- **Immunomodulators** are present in various plant extracts
- Bioactive compounds from natural sources are of great importance because of their potential pharmacological activities and tremendous structural diversity
- Some of the currently identified moss secondary metabolites belong to phenolics, flavonoids and terpenoids which are considered to have antioxidative, antiproliferative and anti-inflammatory properties



- **Mosses** represent a large group of plants that are widespread in almost every part of the world
- one of the oldest plants on the planet
- there are about 20,000 species which include:
 1. ***Anthocerotophyta*** (hornworts, ~ 300)
 2. ***Marchantiophyta*** (liverworts , ~ 6,000)
 3. ***Briophyta*** (mosses ~ 14,000)
- mosses have been used in traditional medicine since ancient times for the treatment of various pathological conditions





Results and discussion

- Chemical characterization of selected secondary metabolite content in extracts of *Hedwigia ciliata*

Extract	Solvent	Phenols (mg GAE/g extract)	Phenolic acids (mg CAE/g extract)	Flavonols (mg QE/g extract)	Flavonoids (mg QE/g extract)	Triterpenoids (mg UAE/g extract)
E1	ethanol	11.46	56.33	<5	28.75	134.80
E2	water:ethanol	16.04	57.56	<5	20.60	94.38
E3	ethyl acetate	14.04	151.14	15.50	49.54	79.37

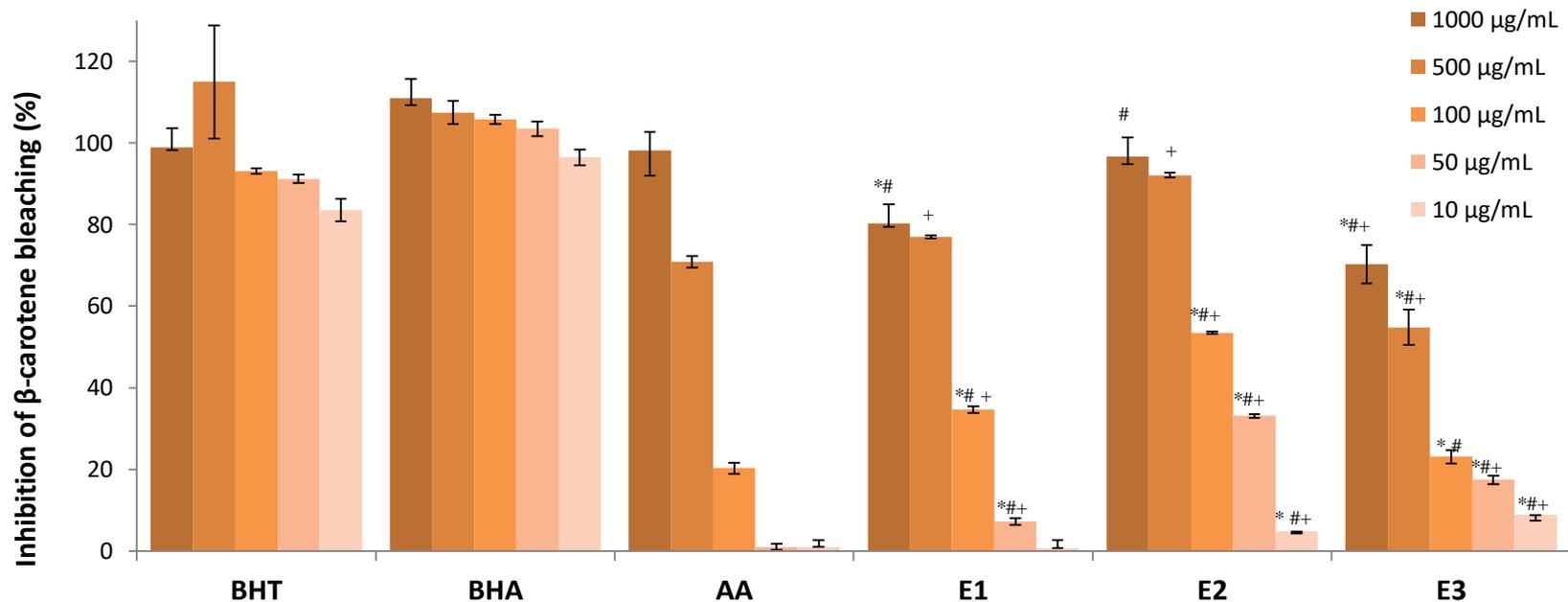


- Liquid Chromatography – Mass Spectrometry analysis of moss extracts

	Ethanol [mg per 100 g weight]	water:ethanol [mg per 100 g weight]	ethyl acetate [mg per 100 g weight]
Gallic acid	1.48	1.16	0.49
Protocatechuic acid	8.11	4.01	0.83
5-O-Caffeoylquinic acid	0.12	0.08	0.02
p-Hydroxybenzoic acid	6.97	9.21	0.60
Caffeic acid	1.04	0.51	0.08
Quercetin 3-O-rutinoside	0.87	1.29	0.03
p-Coumaric acid	0.99	0.42	0.26
Quercetin 3-O-glucoside	0.69	0.23	0.14
Isorhamnetin 3-O-glucoside	0.37	0.16	0.23
Eriodictyol	0.46	0.38	0.07
Apigenin	1.83	0.86	0.15
Naringenin	2.42	1.09	0.07
Kaempferol	2.45	1.00	0.10
Acacetin	0.23	0.08	0.04



- **Antioxidant activity** - the capacity of the extracts to inhibit lipid peroxidation by measuring the inhibition rate of **β-carotene** bleaching (%).

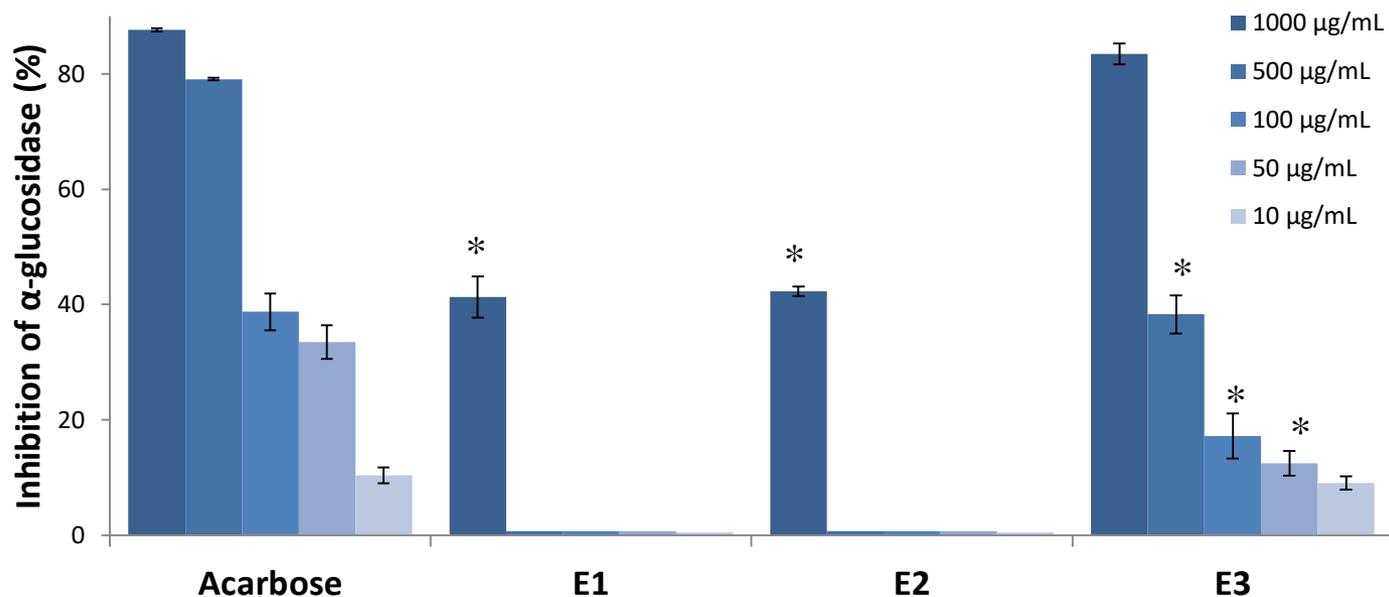


➤ Significant activity was measured for **water:ethanol (E2)** extracts

p<0.05; different concentrations of extracts vs. * BHT (3,5-di-tert-butyl-4-hydroxytoluene);
 # BHA (2-tert-butyl-4-hydroxyanisole);
 + AA (ascorbic acid)



- Antidiabetic activity - the effects of extracts against α -glucosidase

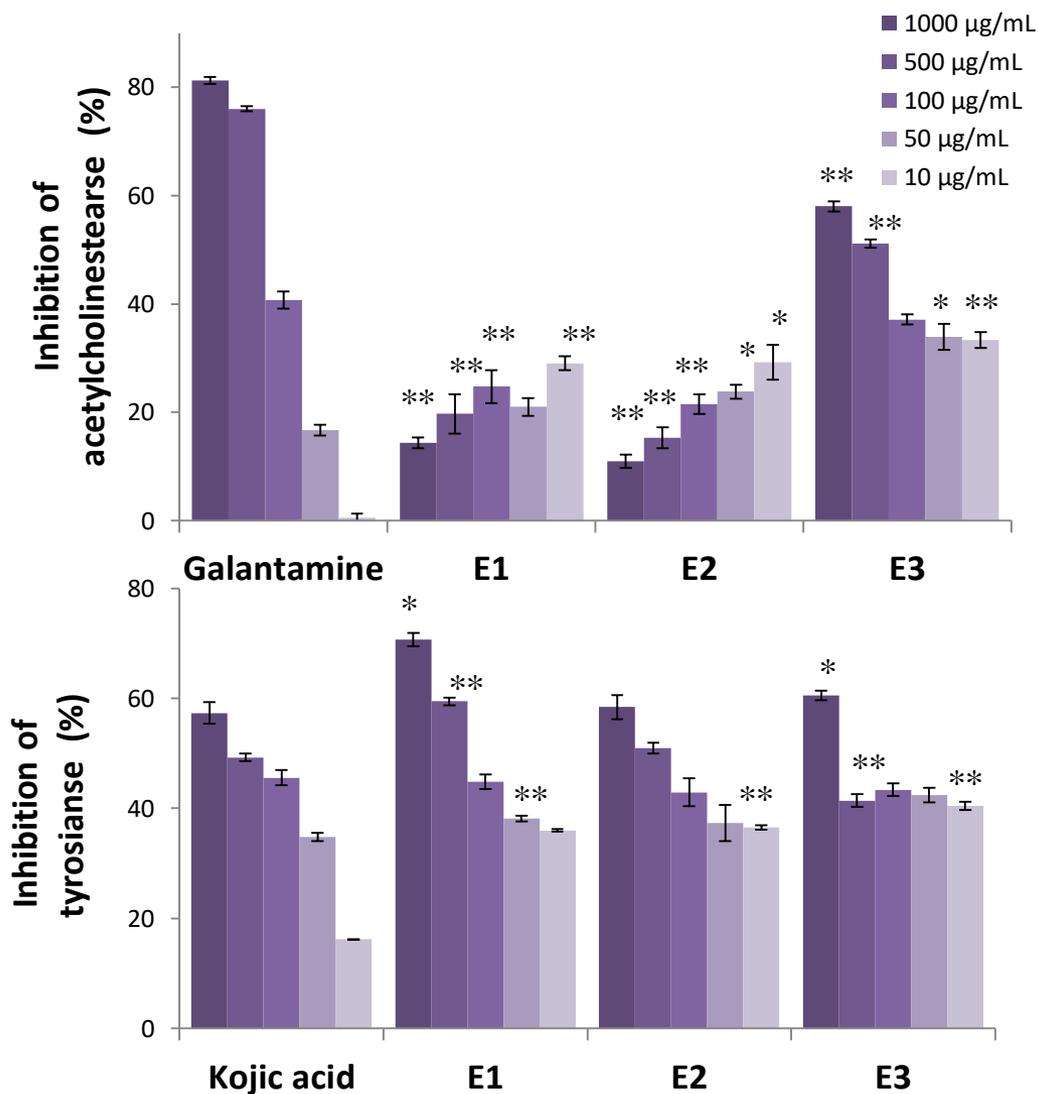


➤ All of the tested extracts exhibited **inhibitory effect** against α -glucosidase with the highest inhibition detected in **ethyl acetate** extract

* $p < 0.05$; different concentrations of extracts vs. acarbose



- Antineurodegenerative activity - the effects of extracts towards **acetylcholinesterase** and **tyrosinase**



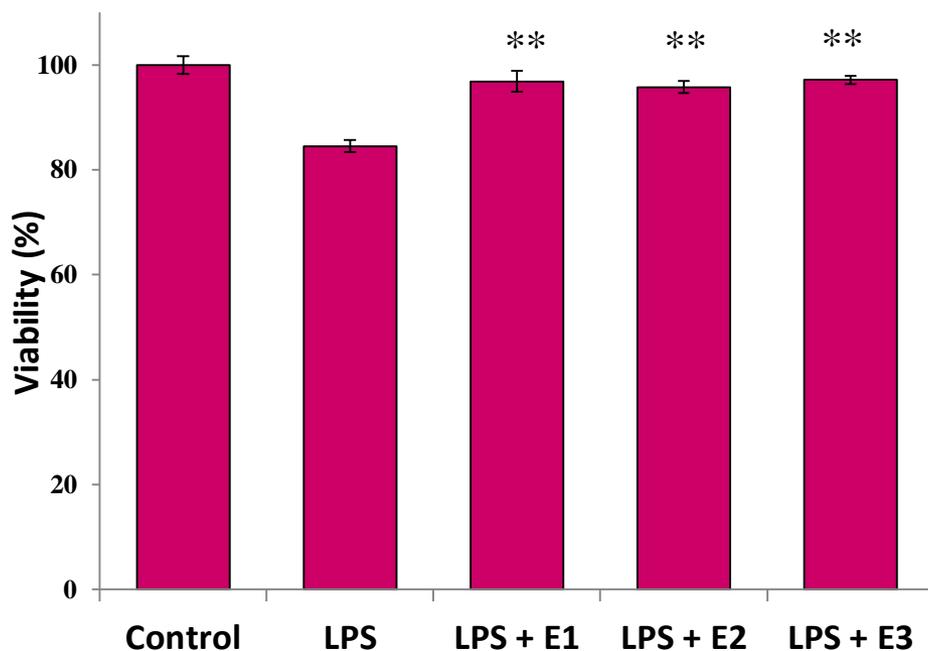
➤ The extracts exhibited **inhibitory** effects against **acetylcholinesterase** and **tyrosinase**, enzymes associated with the development of **Alzheimer's** and **Parkinson's** disease

* p < 0.05; ** p < 0.01 different concentrations of extracts vs. standard galantamine / kojic acid



- **Anti-neuroinflammatory** activity towards **LPS-stimulated BV2 (microglia)** cell line – the effect of extracts on the cell viability *in vitro* after 24 h of exposure

Cell viability - **MTT** assay



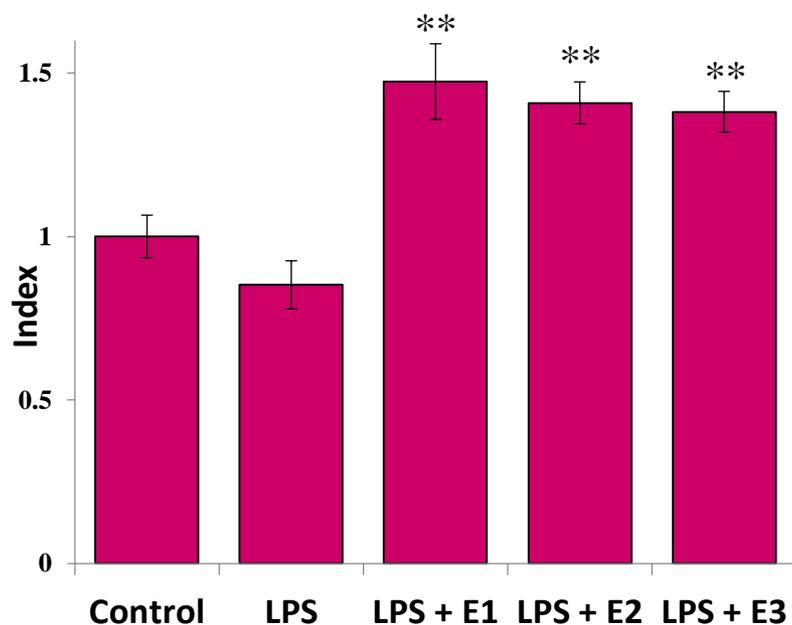
➤ Extracts increased the viability of LPS-stimulated BV2 cells

** $p < 0.01$ extract vs. LPS-treated cells



- **Anti-neuroinflammatory** activity – the effect of extracts on the reactive oxygen species (**ROS**) production *in vitro* after 24 h of exposure

ROS production - NBT assay



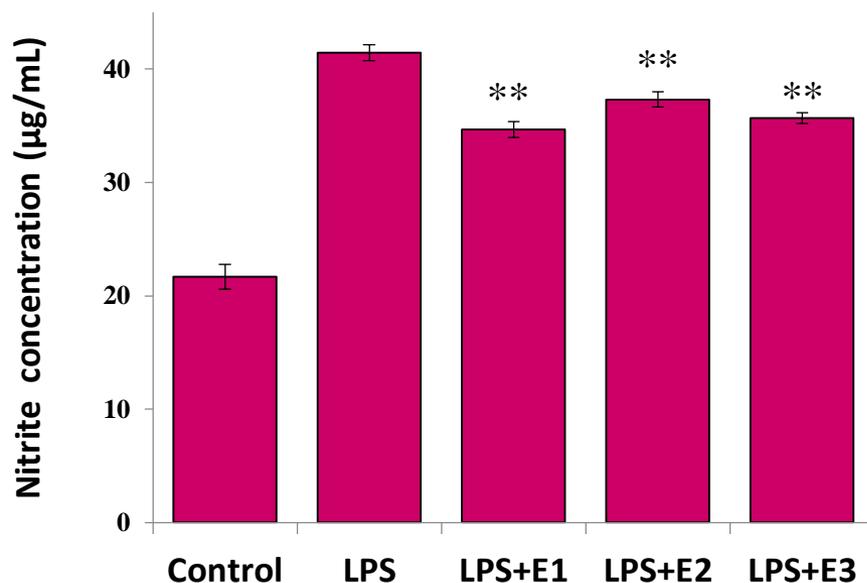
** p<0.01 extract vs. LPS-treated cells

➤ The extracts exhibited low antioxidant potential according to their inability to **decrease** the concentration of **ROS**



- **Anti-neuroinflammatory** activity – the effect of extracts on the **NO** production (measured by nitrite level) *in vitro* after 24 h of exposure

NO production - Griess assay



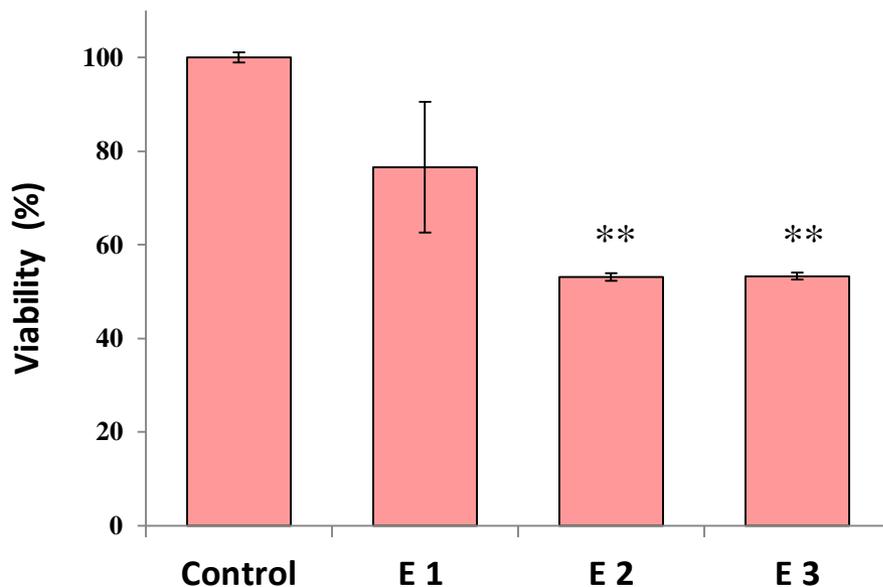
➤ The extracts **reduced** the production of **NO**

** $p < 0.01$ extract vs. LPS-treated cells



- **Antitumor activity towards MDA-MB-231 (breast cancer) cell line *in vitro* after 24 h of exposure**

Cell viability - MTT assay



** $p < 0.01$ extract vs. non-treated control cells

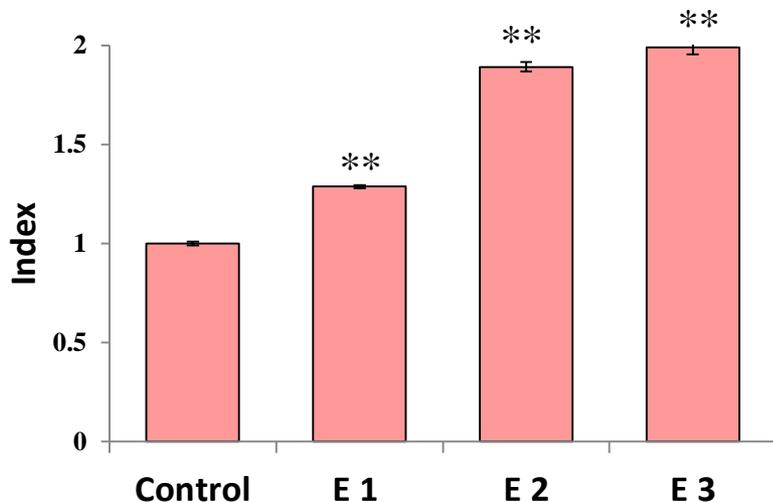
➤ This study showed for the first time that extracts of moss *H. ciliata* exhibited **antitumor activity** against the MDA-MB-231 cells

➤ All three extracts showed antiproliferative activity, among which **water:ethanol** and **ethyl acetate** extracts exhibited the highest activity, inhibiting up to **50%** of tumor cells

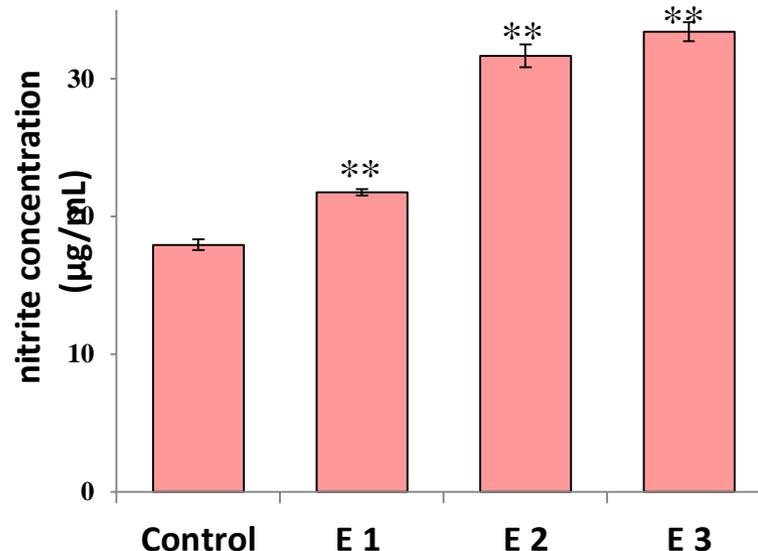


- **Antitumor** activity towards **MDA-MB-231** - the effect of investigated extracts on **ROS** and **NO** production

ROS production - **NBT** assay



NO production - **Griess** assay



- All extracts increased **ROS** and **NO** production ↑
- Potential **antitumor** agents in the prevention/treatment of **breast cancer**

** p<0.01 extract vs. non-treated control cells



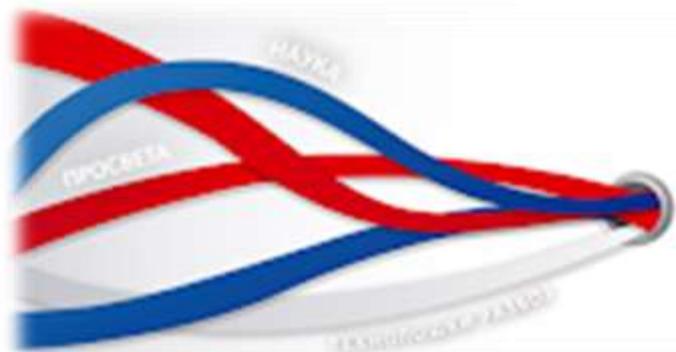
Conclusions

- The chemical characterization of the extracts revealed a wide range of **phenolic, flavonoid** and **triterpenoid** compounds as secondary metabolites of high biological activity
- The extracts showed **biocompatibility** towards the normal MRC -5 cell line
- The extracts demonstrated the **antioxidant activity** via β -carotene/linoleic acid assay, as well as an **inhibitory effect** against **α -glucosidase, acetylcholinesterase, and tyrosinase**
- In this paper is reported for the first time that *Hedwigia ciliata* moss extracts shows significant **antiproliferative activity** against **MDA-MB-231** cells (~ 50% inhibition)
- **Anti-inflammatory potential** - extracts reduced the production of NO by LPS-stimulated BV2 cells
- Different moss extracts could provide entirely new avenues for developing more efficient natural supplements for preventing and treating different inflammatory/degenerative and malignant processes



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