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Pharmacological activities of Lichens from Russia

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Lichen ingredients inhibit proliferation of human glioblastoma cells by MAP-Kinase modulation and they synergize with Temozolomide

A.Shcherbakova[1,2], A.Koptina[1,3], G.UlrichMerzenich[2]

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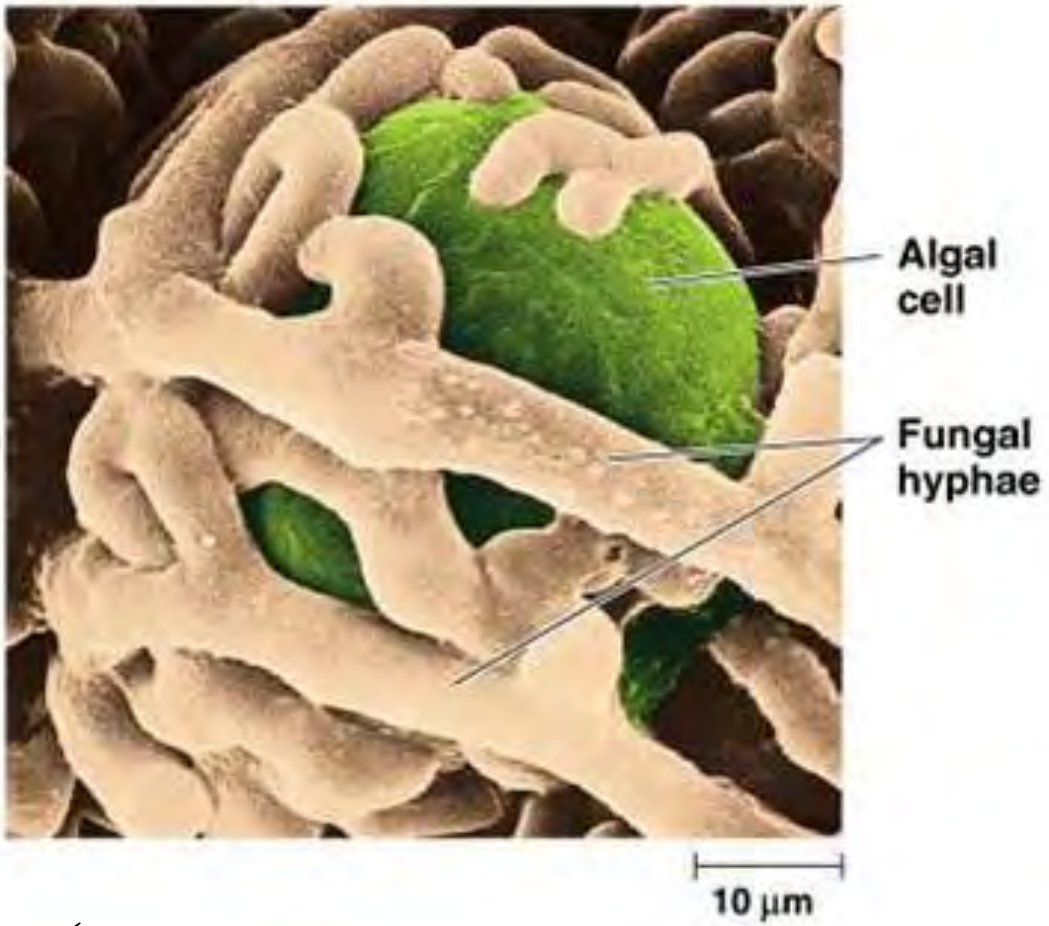
What are lichens?



- ✓Widespread in the world
- ✓Capability to grow in extreme conditions
- ✓Unique biologically active substances

Biological activities of lichens substances

Secondary metabolites

(depsides, depsidones, dibenzofurans and the pulvinic acid derivative family)

- Antibacterial
- Antifungal
- Antiviral
- Antitumor
- Antipyretic [1,2,]

Primary metabolites

(amino acids, vitamins, polysaccharides)

- Antitumor[3]
- Immunomodulation [4,5]

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2. Mitrovic, T., S. Stamenkovic, and V. Cvetkovic, *Lichens as source of versatile bioactive compounds* Biologica Nyssana, 2011. **1**: p. 6.
3. Omarsdottir S, Freysdottir J, Olafsdottir ES. Immunomodulating polysaccharides from the lichen Thamnolia vermicularis var. subuliformis. Phytomedicine. 2007 Feb;14(2-3):179-84.
4. Freysdottir J, Omarsdottir S, Ingolfsdottir K, Vikingsson A, Olafsdottir ES. In vitro and in vivo immunomodulating effects of traditionally prepared extract and purified compounds from Cetraria islandica. Int Immunopharmacol. 2008 Mar;8(3):423-30.
5. Kim HS, Kim JY, Lee HK, Kim MS, Lee SR, Kang JS, et al. Dendritic cell activation by glucan isolated from umbilicaria esculenta. Immune Netw. 2010 Dec;10(6):188-97.

Cancer and malignant glioma

- Cancer is the most morbid and mortal disease worldwide (4 million new cases and 8.2 million cancer related deaths in 2012 (WHO))
- The number of cancer cases increasing
- Glioblastoma belongs to the most mortal ones with a poor prognosis: glioblastoma patients survive 7-15 months after diagnosis

Lichens

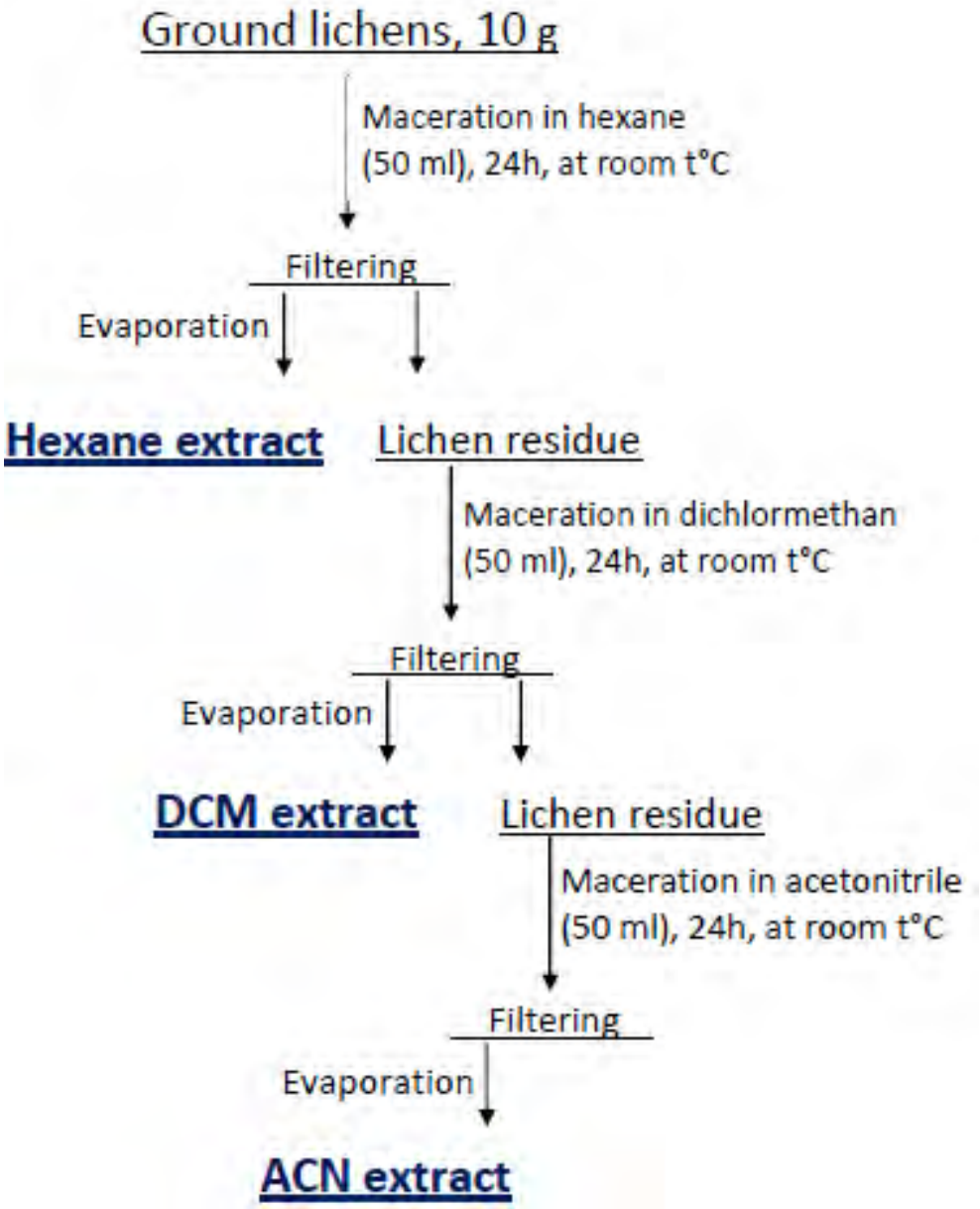
Evernia prunastri (L.)

usnic acid, evernic acid, atranorin, salazinic acid

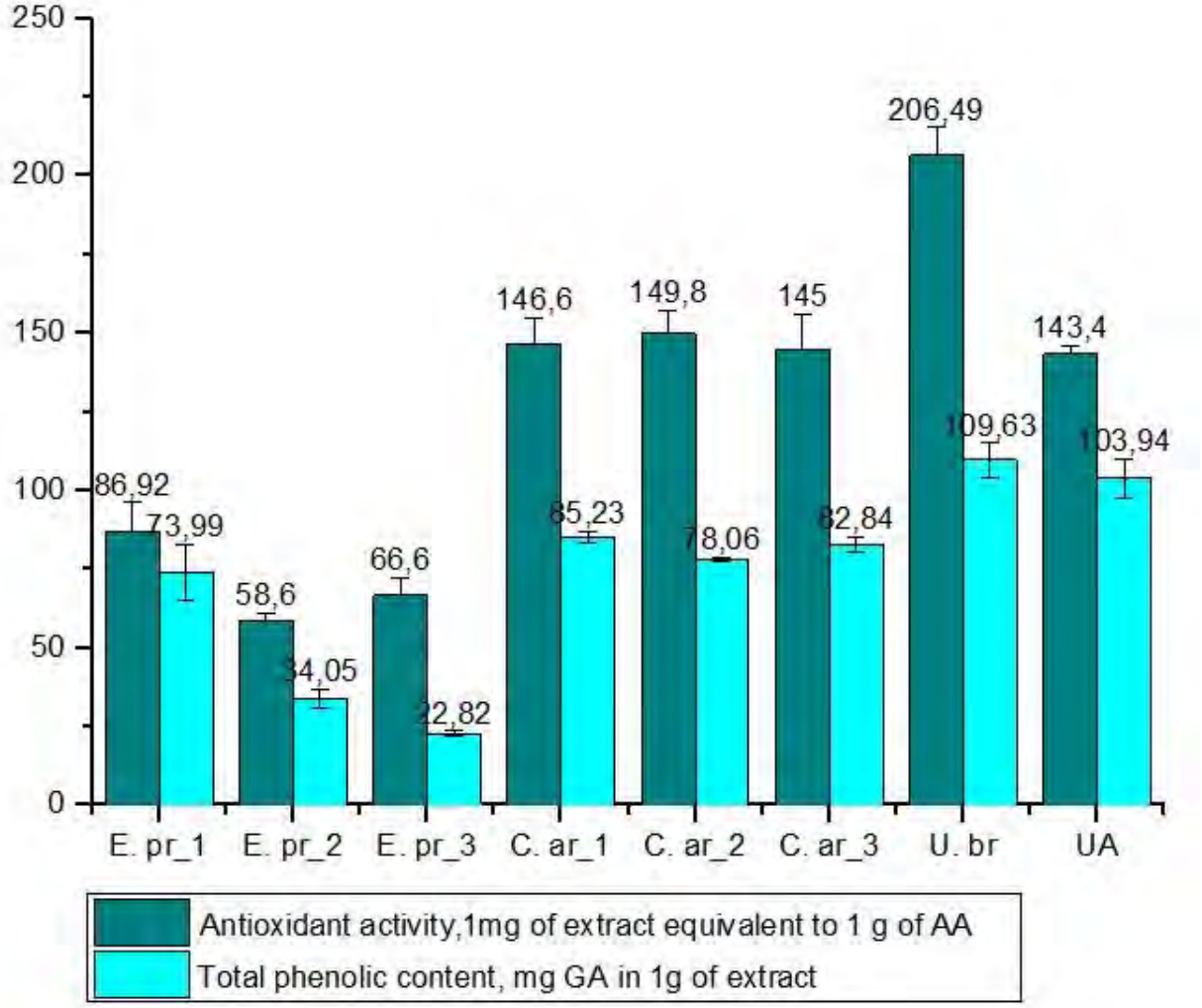
Cladonia arbuscula (Wallr.)

usnic acid, fumarprotocetraric acid
Antimycobacterial activity

Extract preparation



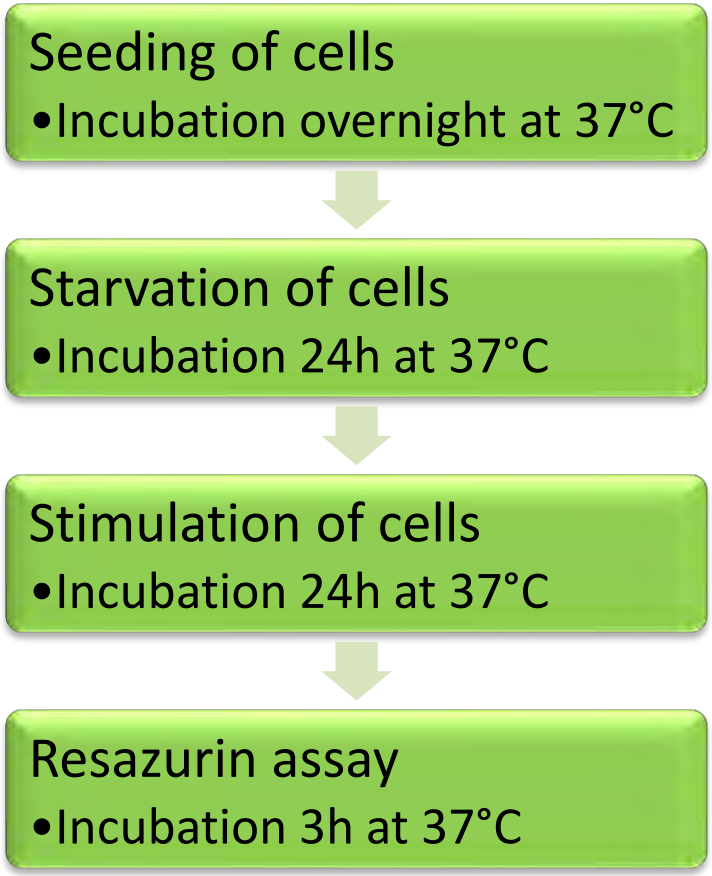
Antioxidant capacity and content of total phenolics



E. pr_1 – hexane extract from *E. prunastri*
E. pr_2 – dichlormethan extract from *E. prunastri*
E. pr_3 – acetonitrile extract from *E. prunastri*
C. ar_1 – hexane extract from *C. arbuscula*
C.ar_2 – dichlormethan extract from *C. arbuscula*
C.ar_3 – acetonitrile extract from *C. arbuscula*
U.br – chloroform extract from *U. barbata*
UA – usnic acid
AA – ascorbic acid
GA - gallic acid

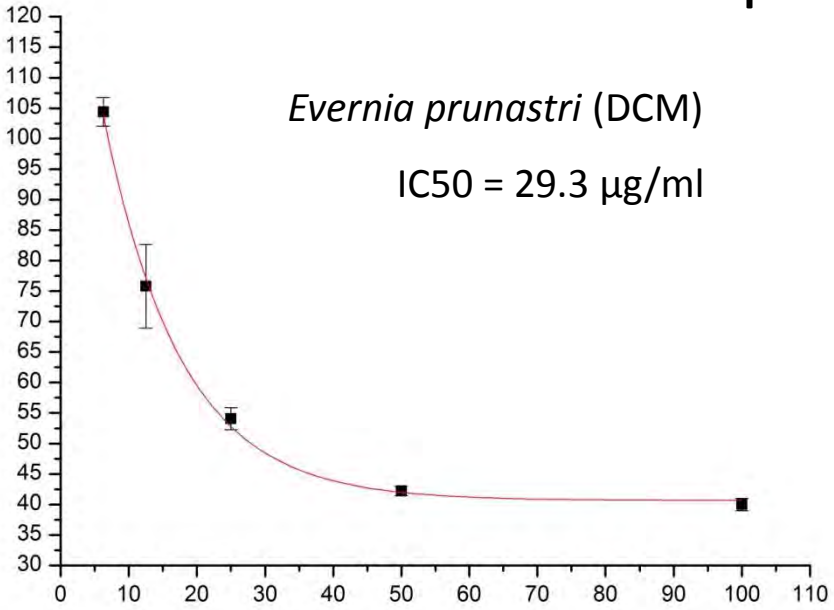
Cytotoxic assay

(method)



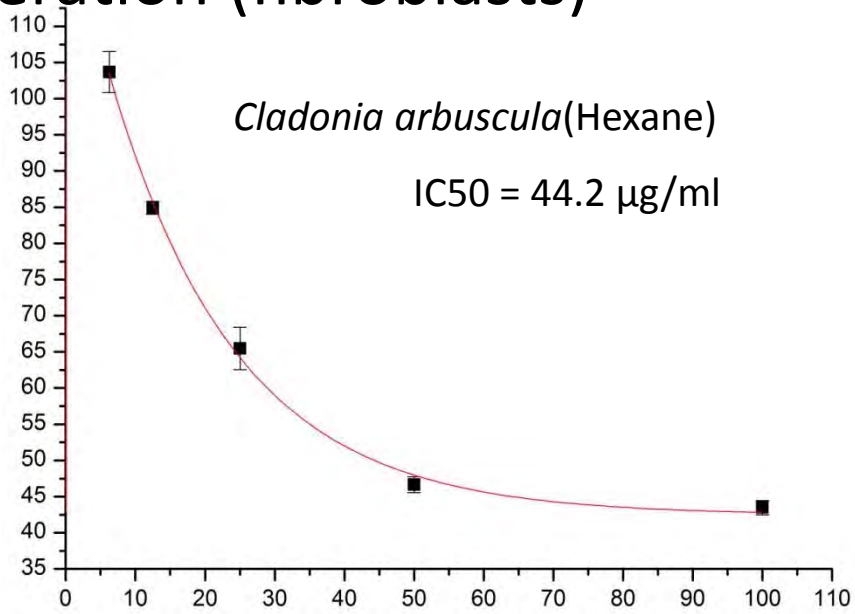
- Cells: Human skin fibroblasts
  Human glioblastoma U87
- Concentration of cells:
  $5x10^3$ cells/well
- Concentration of extracts:
  6.25 – 100 µg/ml

Inhibition of cell proliferation (fibroblasts)



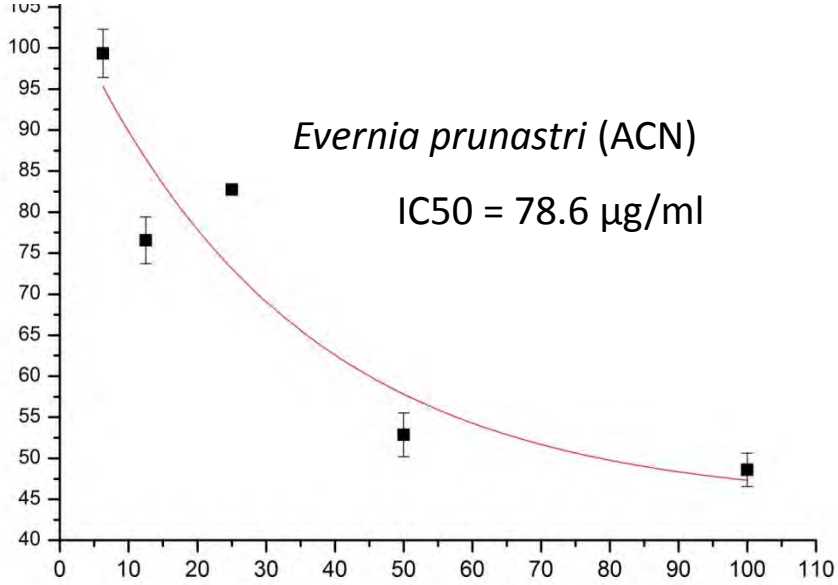
Evernia prunastri (DCM)

IC50 = 29.3 µg/ml



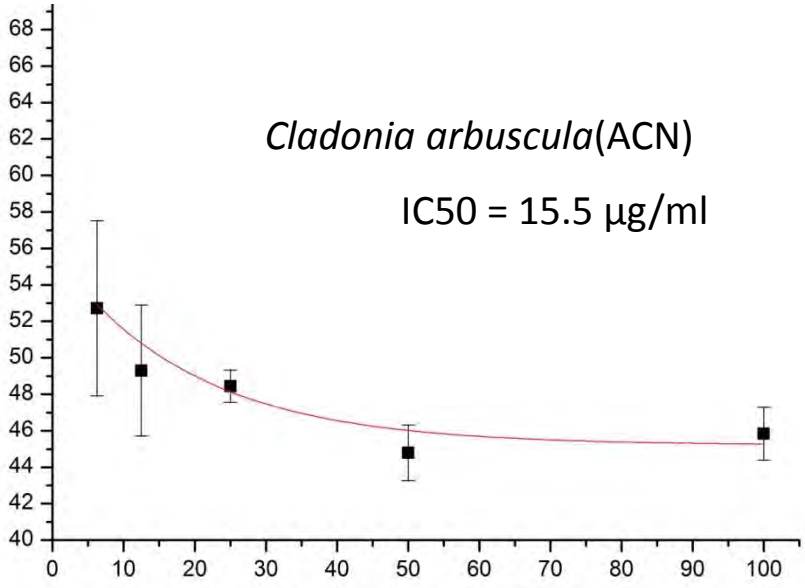
Cladonia arbuscula(Hexane)

IC50 = 44.2 µg/ml



Evernia prunastri (ACN)

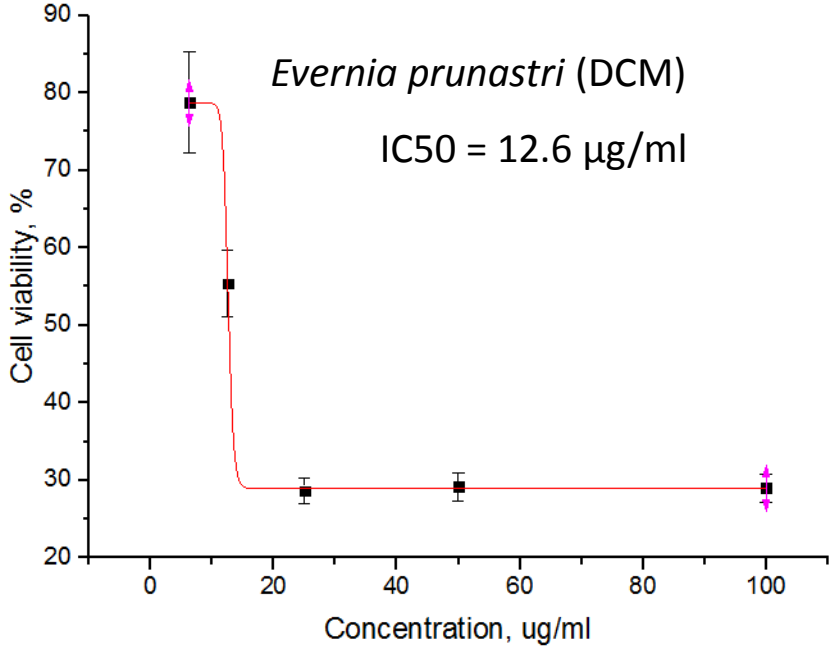
IC50 = 78.6 µg/ml

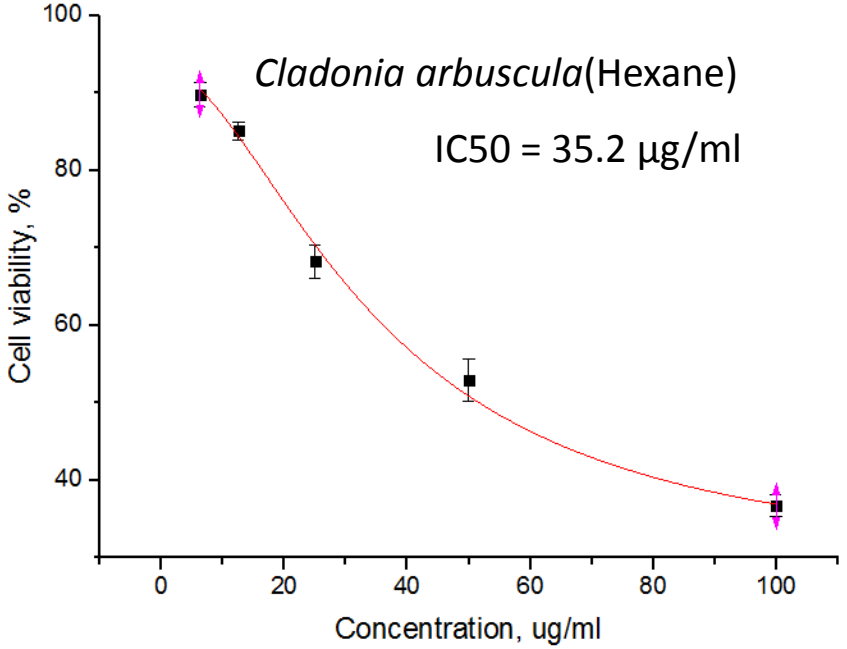


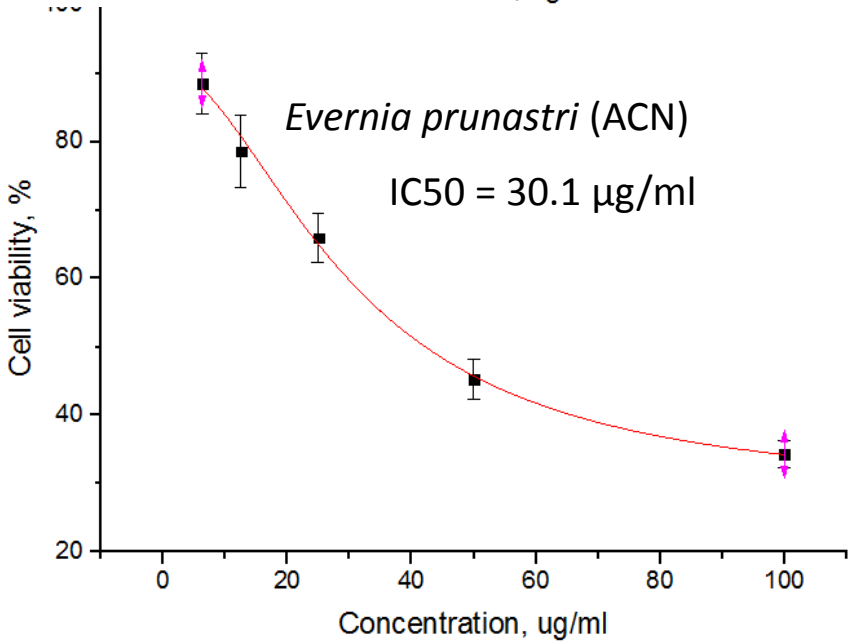
Cladonia arbuscula(ACN)

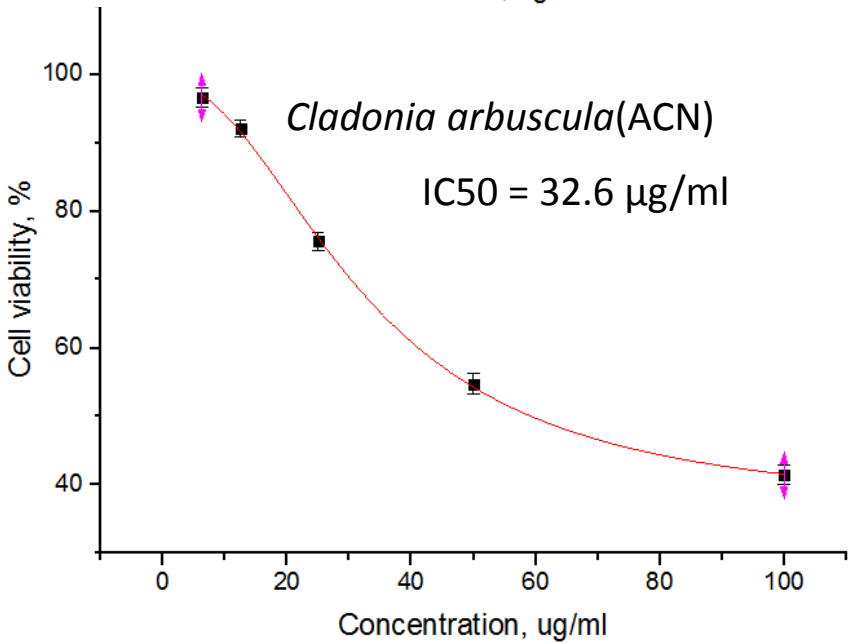
IC50 = 15.5 µg/ml

Inhibition of cell proliferation (U87 glioblastoma)

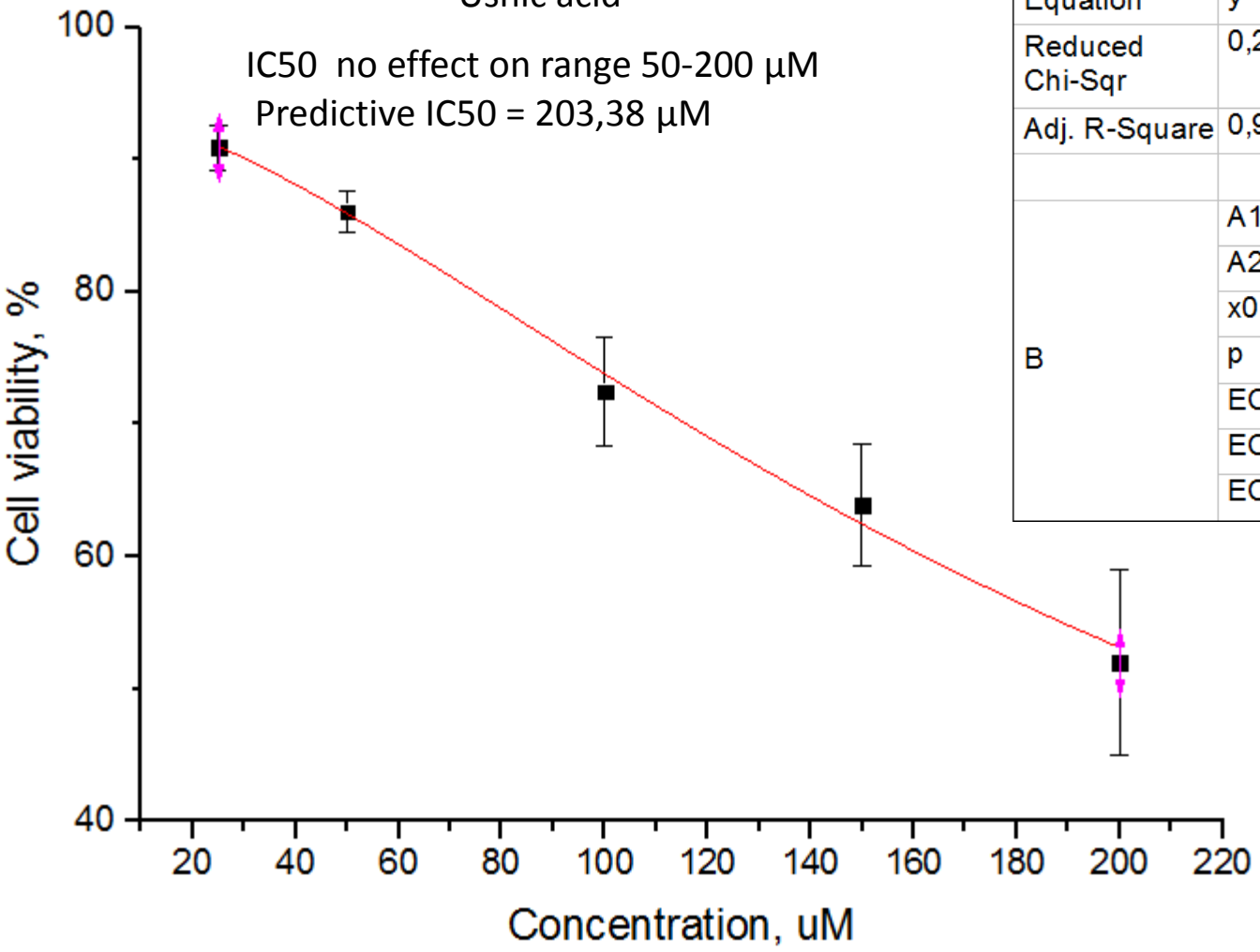






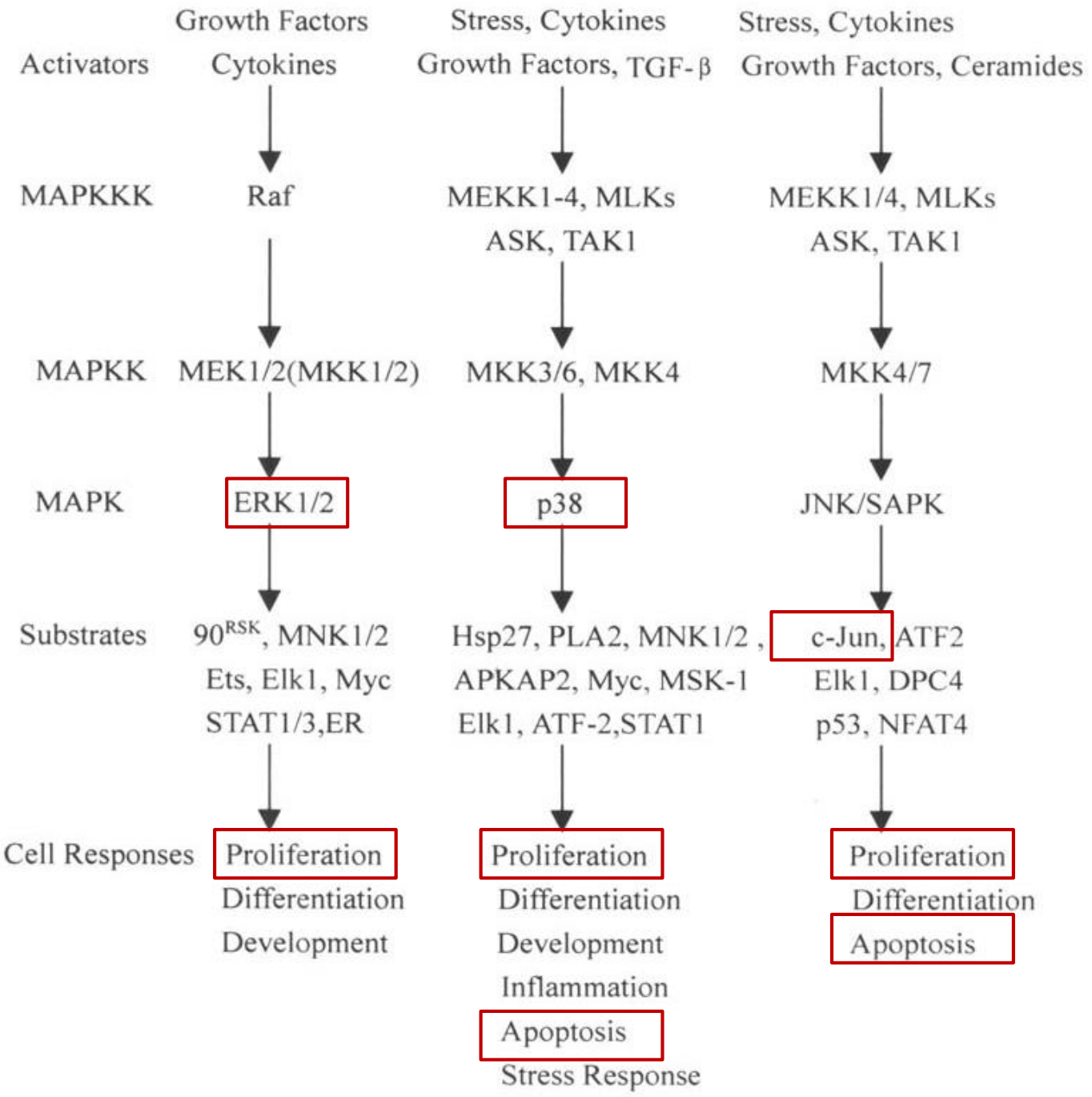


Inhibition of cell proliferation (U87 glioblastoma)



Model	Logistic		
Equation	y = A2 + (A1-A2)/(1 + (x/x0)^p)		
Reduced Chi-Sqr	0,24842		
Adj. R-Square	0,98482		
		Value	Standard Erro
B	A1	93,72648	2,97055
	A2	11,23688	76,78268
	x0	203,9245	213,86629
	p	1,60628	0,73922
	EC20	86,03072	58,3479
	EC50	203,9245	213,86629
	EC80	483,3762	692,97962

Cell signaling (phosphorylation of MAPK)

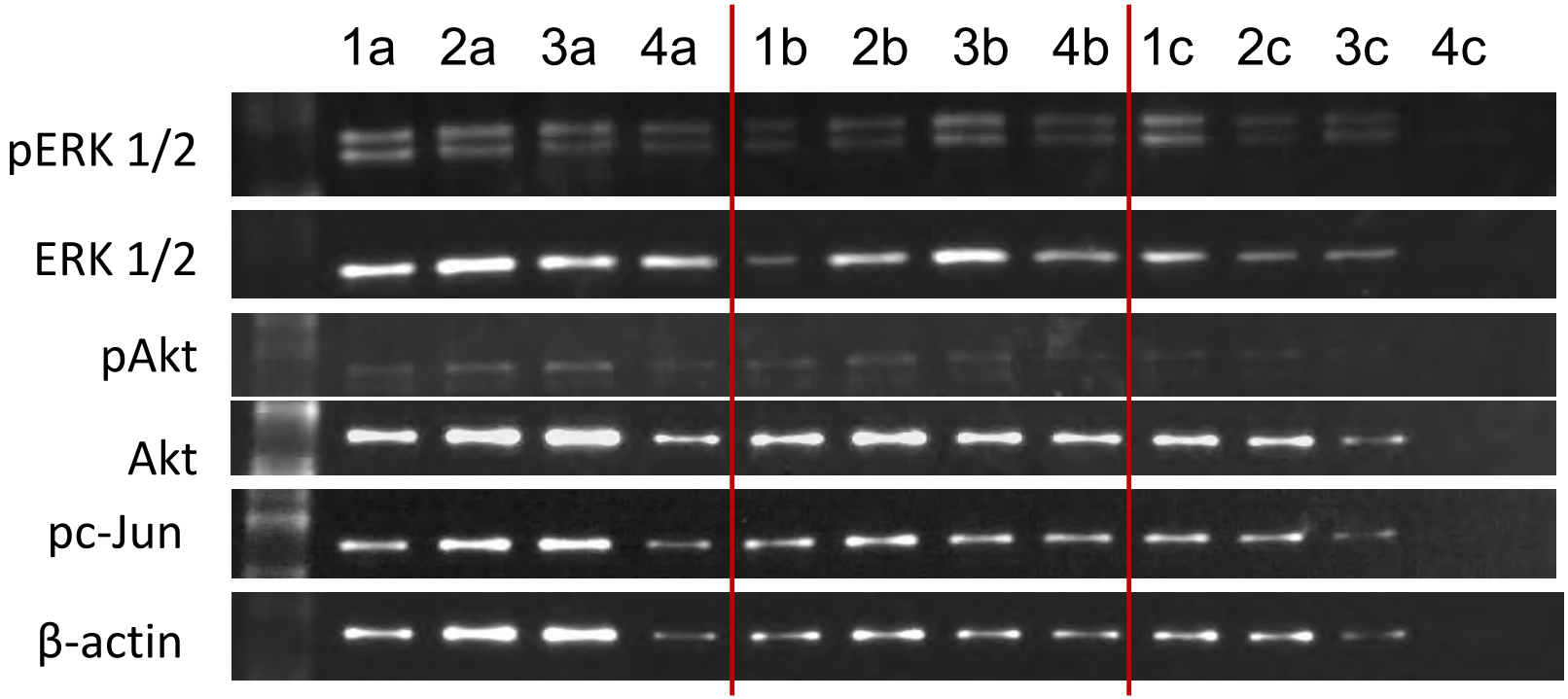


[1]Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res. 2002 Mar;12(1):9-18.

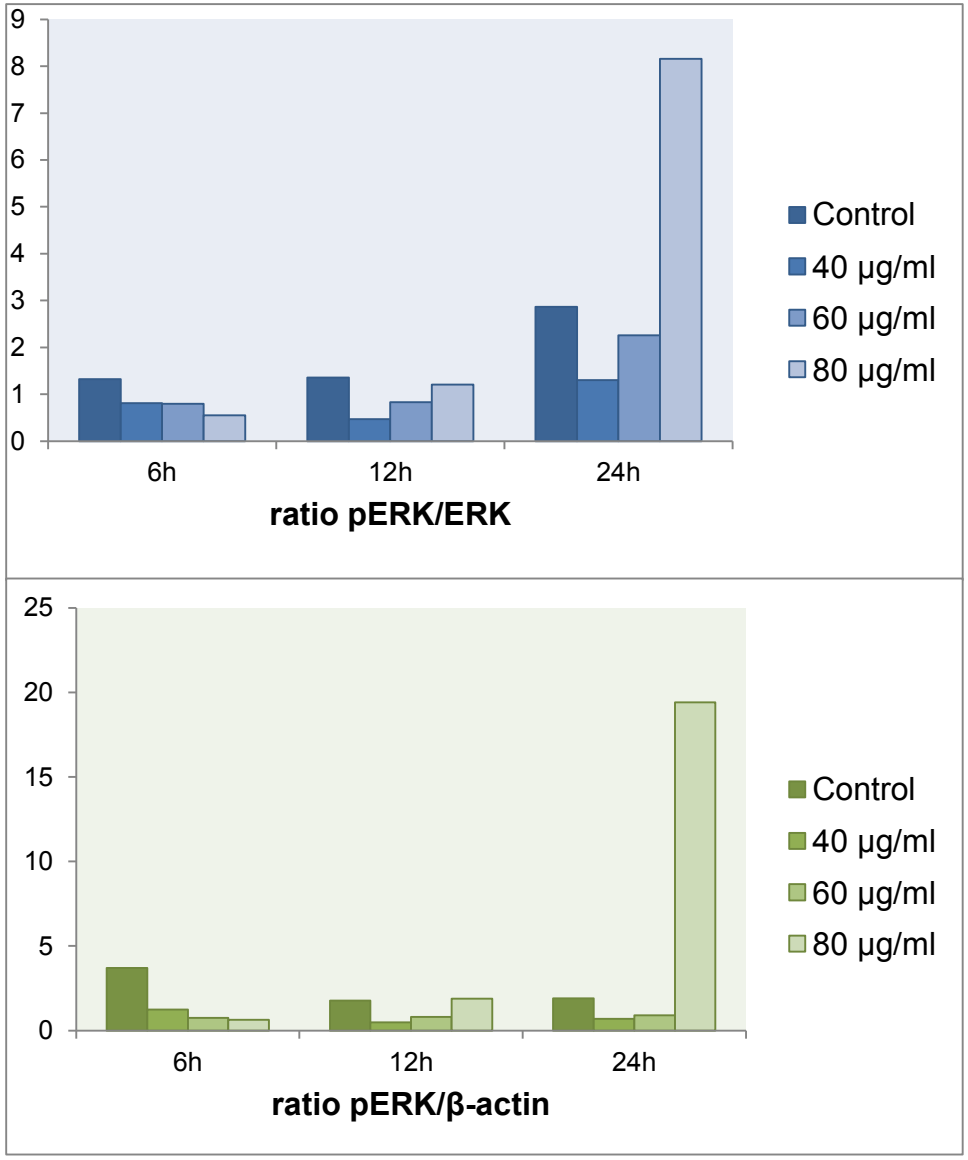
Experimental design for the evaluation of cell signaling activities

	Control	40 µg/ml	60 µg/ml	80 µg/ml
6 hours	1a	2a	3a	4a
12 hours	1b	2b	3b	4b
24 hours	1c	2c	3c	4c

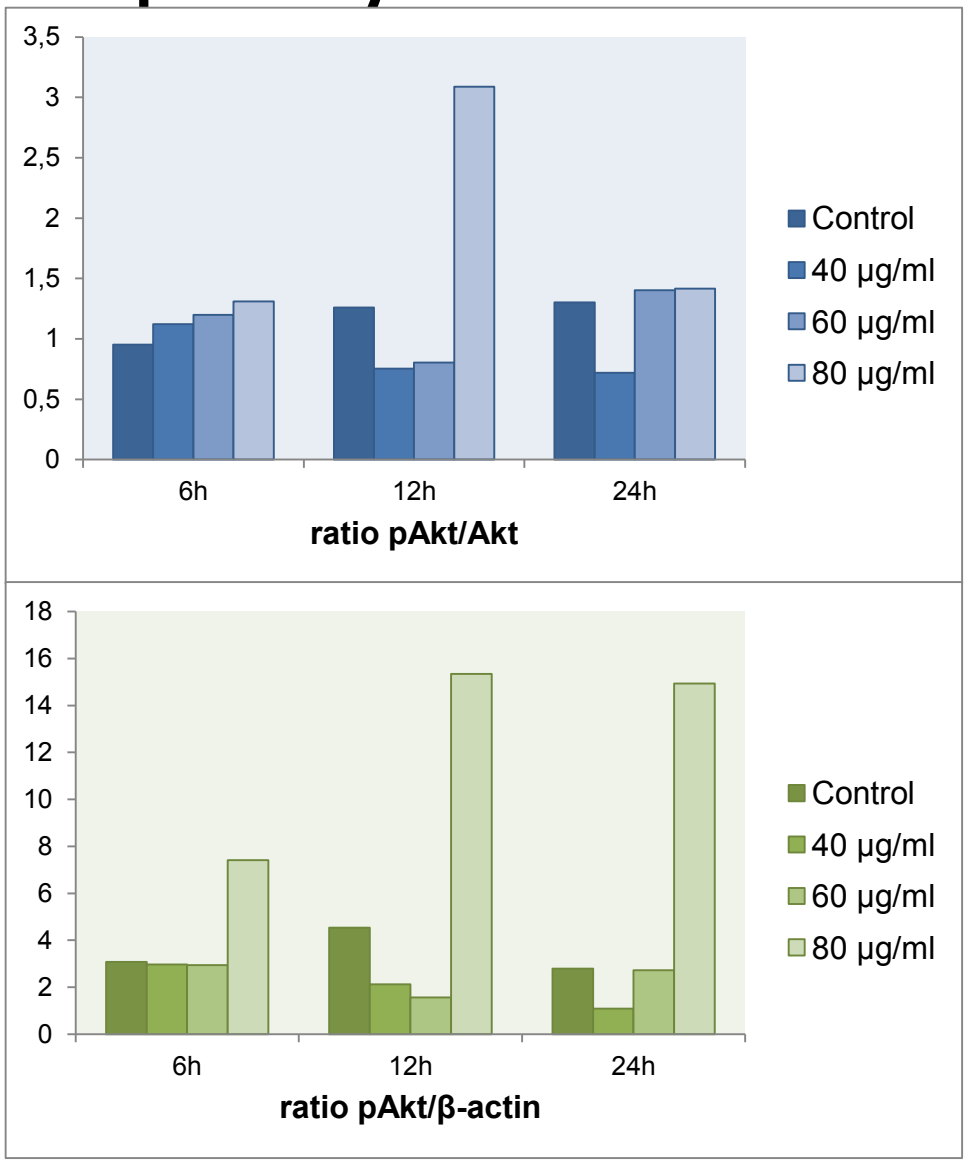
Western Blot analysis of MAPK-activities



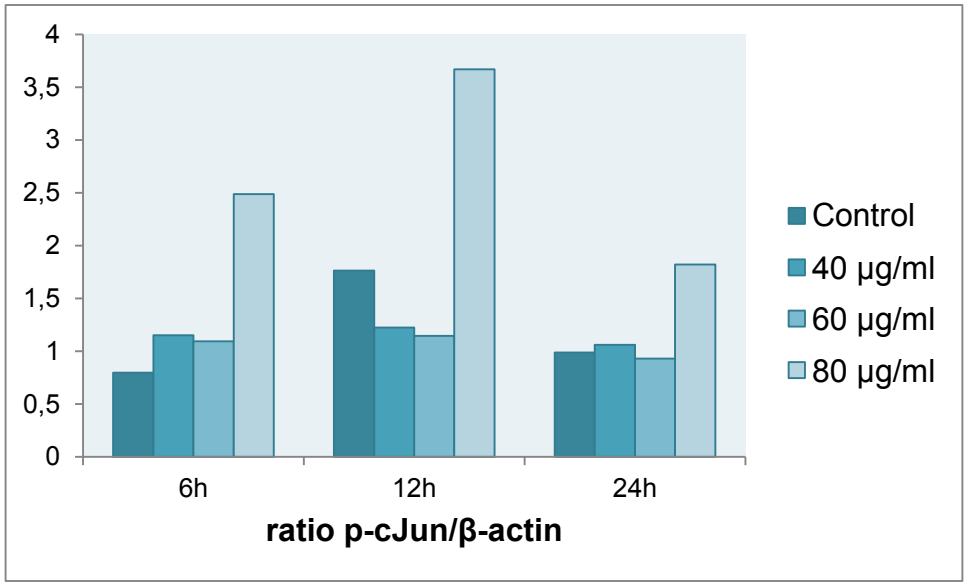
Phosphorylation of ERK1 and ERK2



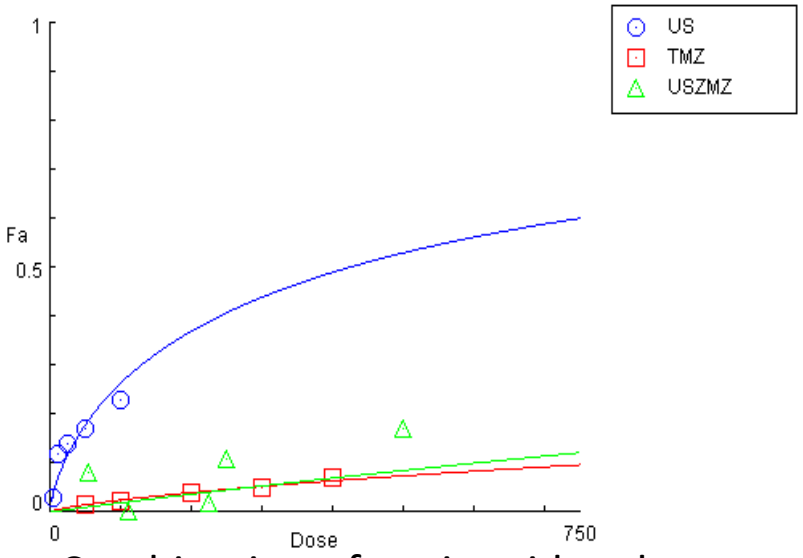
Phosphorylation of Akt



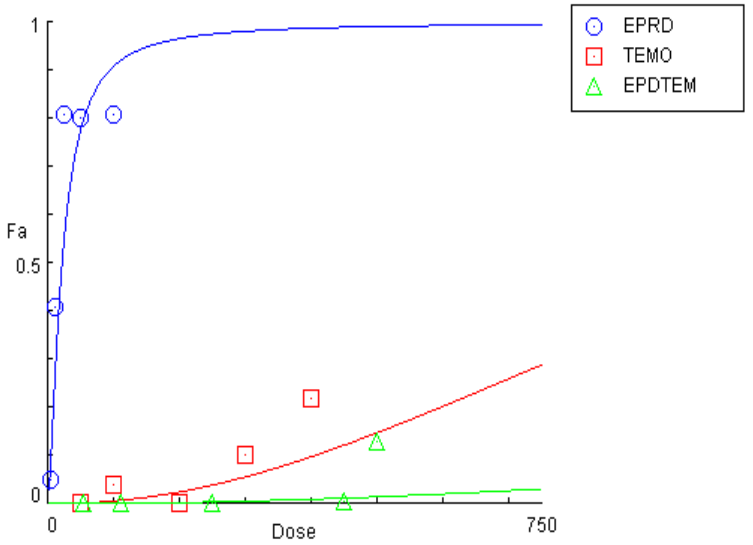
Phosphorylation of c-Jun



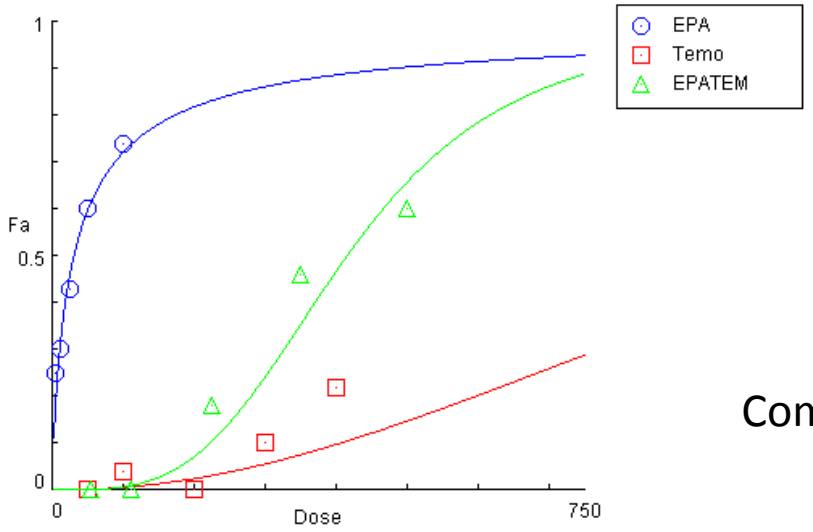
Dose-Effect Curves



Combination of usnic acid and temozolamide



Combination of E.pr DCM extract and temozolamide



Combination of E.pr ACN extract and temozolamide

Summary

- Examined Lichen extracts posses antioxidant activities which relate to their amount of phenols
- The *E. prunastri* ACN extract is the least toxic extract for human skin fibroblasts
- The highest toxicity was found for the *C. arbuscula* ACN extract against HSKS
- Lichen extracts inhibit the proliferation of glioblastoma U87 cells in a concentration range of 12.6 – 35.2 µg/ml
- The *E. prunastri* DCM extract showed in vitro the highest activity against U87 glioblastoma cells
- Usnic acid is less active against U87 cells
- The initiation of cell death occurs through a differential activation of MAP-Kinases
- The Lichen *E. prunastri* appears to be the most promising candidate for natural drug development against brain tumors

Acknowledgments

- German Academic Exchange service (DAAD) for the long-term scholarship at Bonn University (Medical Clinic III, University Clinic Centre Bonn, Germany), 2014/15
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Thank you for your attention!