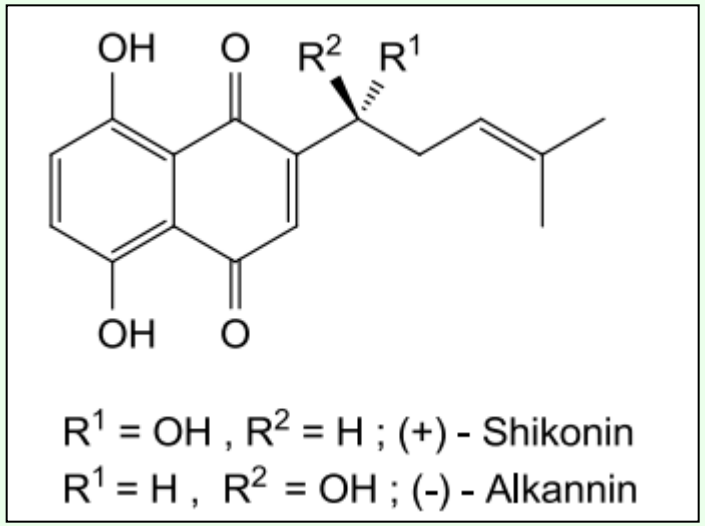
FEATURES OF BIOMASS GROWTH AND NAPHTHOQUINONES ACCUMULATION IN THE CELL LINE 3Ep OF *ECHIUM PLANTAGINEUM* TISSUE CULTURE

Iryna Konvalyuk[1], Oleksandr Bieda[1,2], Oksana Poronnik[1], Sergiy Yarmoluk[1], Viktor Kunakh[1]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
[2] Scientific Service Company OTAVA, LLC, Kyiv, Ukraine

Echium plantagineum, a medicinal plant belonging to the Boraginaceae family, contains phenolic compounds, terpenoids, and naphthoquinones, including shikonin and its derivatives. The presence of these biologically active compounds (BACs) gives the plant wound-healing, antibacterial, fungicidal, anti-inflammatory activities. Given the lack of industrial sources of shikonin in Ukraine, *E. plantagineum* tissue culture could serve as an innovative source of BACs with wound-healing properties.

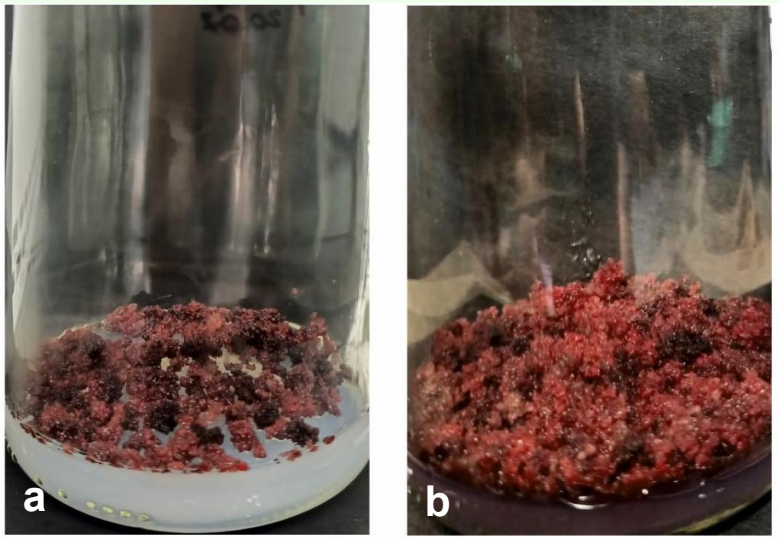


***Echium plantagineum* in nature and its biologically active compounds**

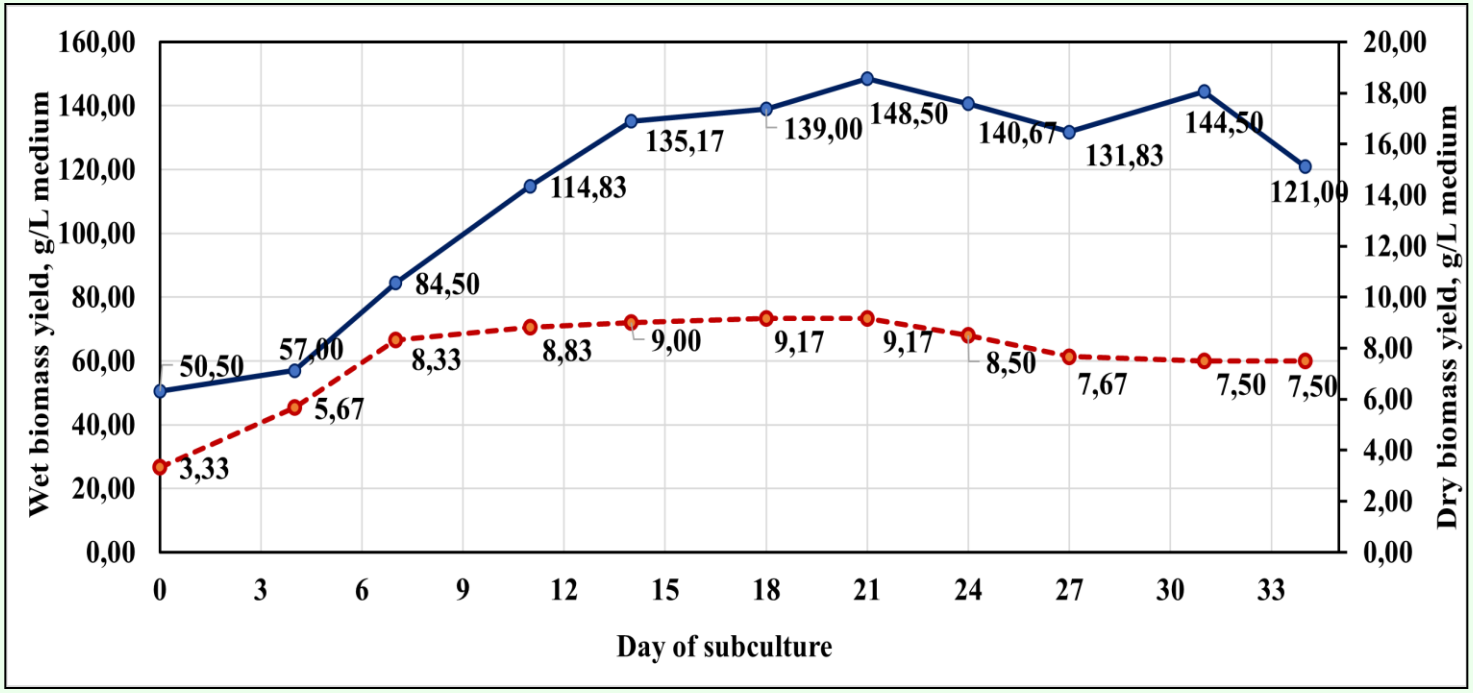
The **aim** of the study was to investigate features of biomass growth in the cell line 3Ep of *E. plantagineum* tissue culture and the content of naphthoquinone compounds in the cell biomass.

Methods. The starting material was the cell line 3Ep of root origin, which had been cultivated for 5 years on the Linsmaier and Skoog medium in glass jars [*Poronnik, Shablij, Kunakh, 2008*].
The subculture duration was 21 days. To determine the wet and dry biomass yield, the callus was weighed during subculturing and every three days thereafter for a period of 34 days. The content of naphthoquinone compounds was studied in ethyl acetate extracts from dry biomass using an HPLC.

Results. The tissue culture of the cell line 3Ep consists of dedifferentiated cells that exhibit no signs of morphogenesis, has a brownish-red color and fine-grained conglomerates. The growth index was 3–4. The growth curve of the culture was S-shaped, reaching the stationary phase on the 14th day of subculture.



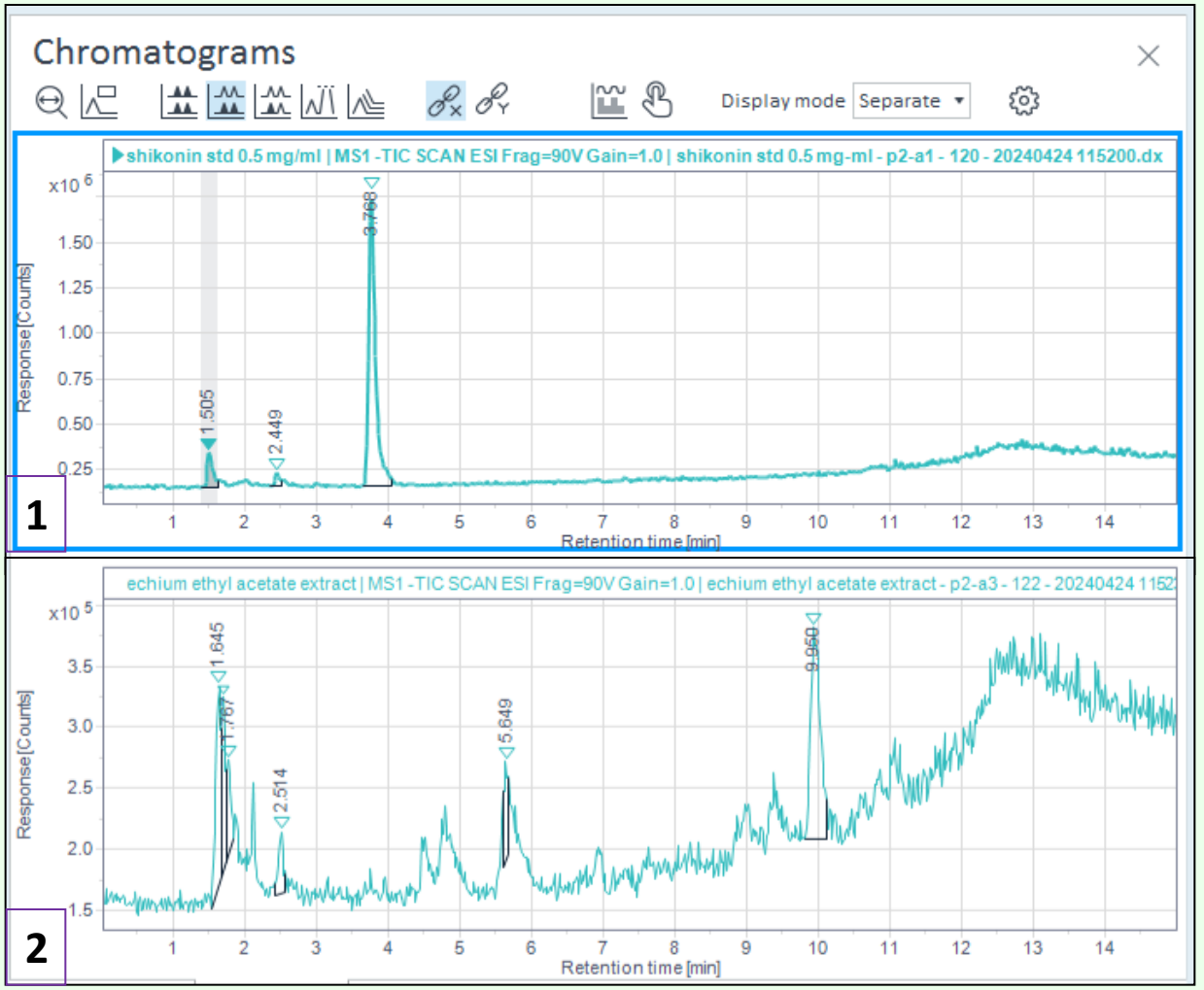
Cell line 3Ep of *E. plantagineum* tissue culture: a – on the 1st day, b – on the 21st day of subculture.



The maximum wet biomass yield was observed on the 21st day of subculture, reaching 148.5 g/L of medium, while the dry biomass yield was 9.2 g/L. The dry matter content in the wet biomass during the stationary phase ranged from 6.2 to 6.7 % dry biomass.

The content of dry biomass in the wet in the stationary phase of growth was 5.4–7.8 %.

Shikonin and its derivatives: acetylshikonin, isovalerylshikonin, and hydroxyvalerylshikonin were identified in the *E. plantagineum* tissue culture extracts.
The total content of naphthoquinone compounds was estimated at 2.11–2.43 % of the dry weight, which is several times higher than in the roots of natural plants, which accumulate up to 0.45 % of naphthoquinone compounds according to literature [*Papageorgiou et al., 1999*].



Chromatogram of the shikonin standard (1) and chromatogram of the ethyl acetate extract from dry biomass *E. plantagineum*

Conclusions. It was found that during subculture, the line 3Ep of *E. plantagineum* tissue culture accumulates 148.5 g/L of wet biomass and 9.2 g/L of dry biomass, which contains 2-3 % shikonin and its derivatives, which significantly exceeds the indicators of wild-grown plants and confirms the potential of its use in the pharmaceutical and biotechnological industries.