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BIOACTIVE PROPERTIES OF ETHANOL EXTRACTS *TAGETES PATULA* L. FLOWERS

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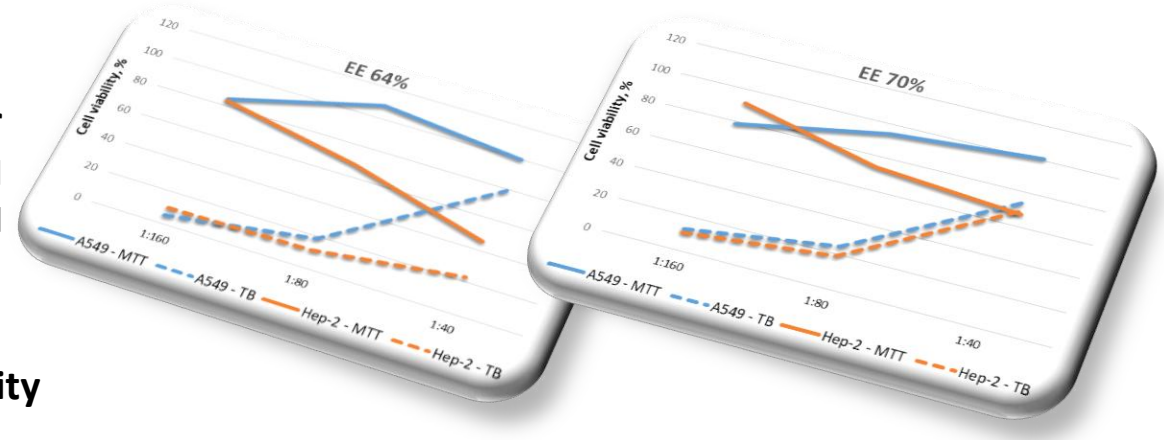
Graphical Abstract

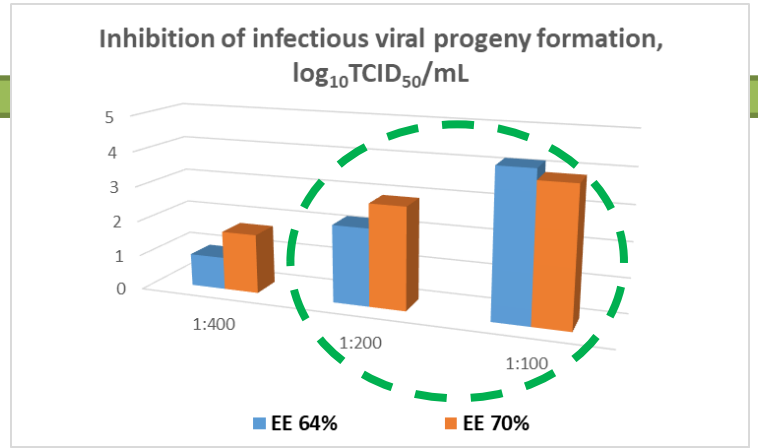
BIOACTIVE PROPERTIES OF ETHANOL EXTRACTS *TAGETES PATULA* L. FLOWERS

64 and 70 %
EtOH extracts of marigold

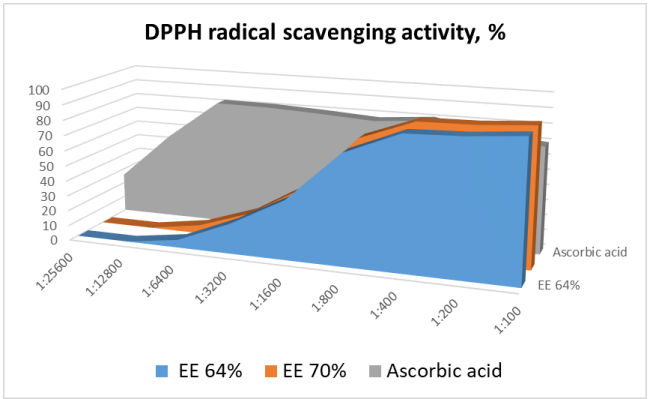
Significant **inhibition of cancer cells viability**, their mitochondrial activity and increasing of cell membrane permeability.



Pronounced anti-herpetic activity



Antioxidant potential



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Abstract:

Introduction. Marigolds are characterized by a rich composition of biologically active substances, a complex effect on the body and low side effects. They are widely cultivated in Ukraine. However, activity of plants extracts depending on many factors, including the part of the plant, geographical source, extraction method, etc. So, we prepared extracts from marigold *Tagetes patula* L. flowers in ethanol. The effects of the extracts on the morphological and functional properties of cells, antioxidant potential and antiviral activity were studied.

Methods. The influence of the extracts on viability of tumor cells were studied using fluorescence microscopy, MTT and trypan blue assays. The *in vitro* antioxidant activity of the extracts was measured by the free radical scavenging capacity according to the DPPH test. Antiviral activity was determined using yield reduction assay.

Results. Morphological changes of human tumor cell were detected, namely their rounding, granularity, increase in size, and swelling. The mitochondrial activity of the cells at the used concentrations of extracts was within 45 - 100%. However, a study of cell membrane permeability revealed inhibition of A549 and Hep-2 cells viability under the influence of extracts by 18-79% and 19-69%, respectively. In addition, analysis of the mitotic activity of tumor cells under the action of the extracts showed a significant mitotic index changes. High antiradical activity and the ability of extracts to absorb DPPH radical were shown. Also, a similar pronounced antiherpetic efficiency of both extracts was established, the decreasing of herpes simplex virus type 1 infectious titer was in the range of 1.7 - 4.7 $\log_{10}$ $TCID_{50}$/ml.

Conclusions. These findings suggest, that extracts obtained from marigold *Tagetes patula* L. flowers may hold promise as a potential therapeutic agents.

Keywords: antiherpetic efficacy; ethanol marigold extracts; free radical scavenging activity; tumor cell morphologal changes

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Introduction

A fundamental direction in modern medical biology, pharmacology, and biotechnology is the search for new sources of biologically active substances (BAS).

Marigolds (Latin name *Tagetes*) belong to the *Asteraceae* family and are promising medicinal and ornamental plants with a wide range of pharmacological activity.

Marigold extracts are characterized by the presence of various compounds (carotenoids, flavonoids, phenylpropanoids, thiophenes, essential oils, and others), which contributes to their broad biological activity.

The properties and benefits of marigold extracts

Marigolds are quite common plants in the world, but there has been insufficient research into the phytochemical composition and biological characteristics of their extracts, specifically from plants grown in Ukraine.

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https://plants.ces.ncsu.edu/plants/tagetes-patula/

Objects

Extracts: Extracts were prepared from marigold *Tagetes* patula L. flowers using 64% and 70% ethanol (EE 64% and EE 70%).

Cell cultures:

- Vero cells (african green monkey kidneys)
- A549 (lung cancer)
- Hep-2 (laryngo carcinoma)

Viruses:

- Herpes simplex virus type 1 (HSV-1)

Methods

- The influence of the extracts on viability of cells were studied using fluorescence microscopy, MTT and trypan blue assays.
- The *in vitro* antioxidant activity of the extracts was measured by the free radical scavenging capacity according to the DPPH test.
- Antiviral activity against HSV-1 was determined using yield reduction assay.

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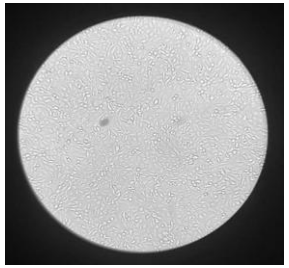
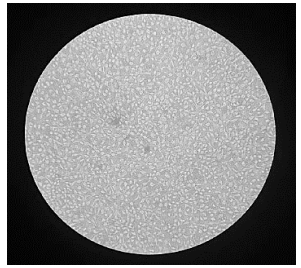
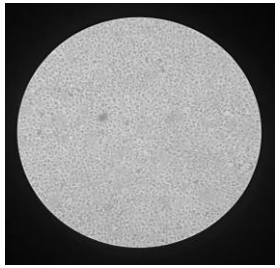
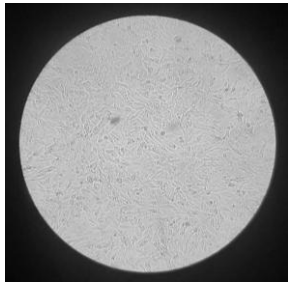
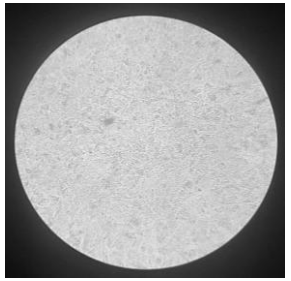
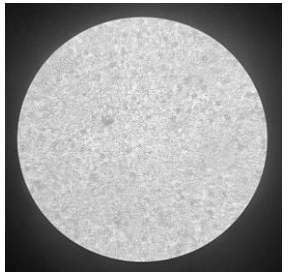
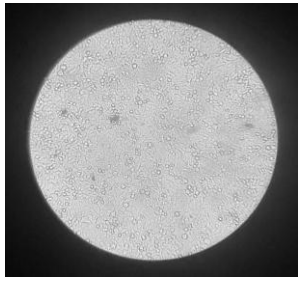
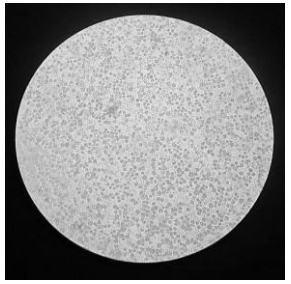
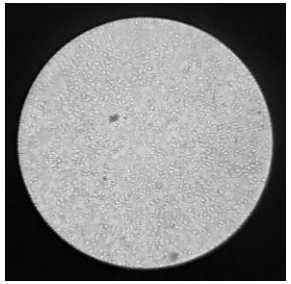
Results and discussion

In the absence of extracts, at 24 h the cells displayed typical shape and intact morphology. Whereas in the presence of extracts in lowest dilution changes in the cell morphology (especially A549 and Hep-2) were observed, namely their rounding, granularity, increase in size, and swelling. It should be noted that at high dilutions extracts did not affect on cell morphology.

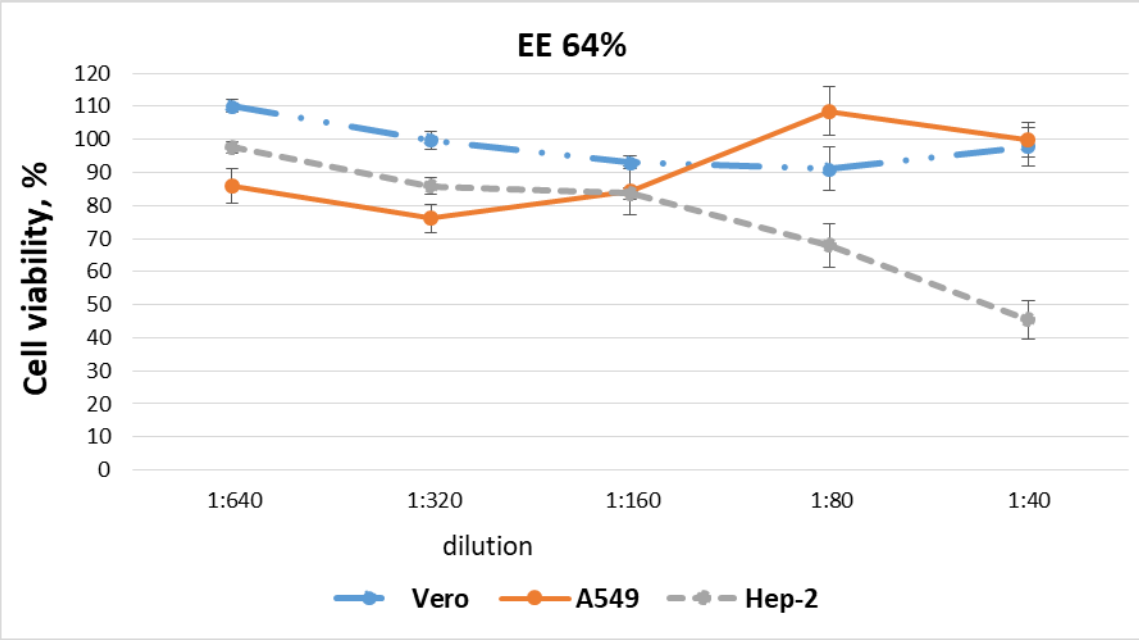
Cell Morphology Characterization

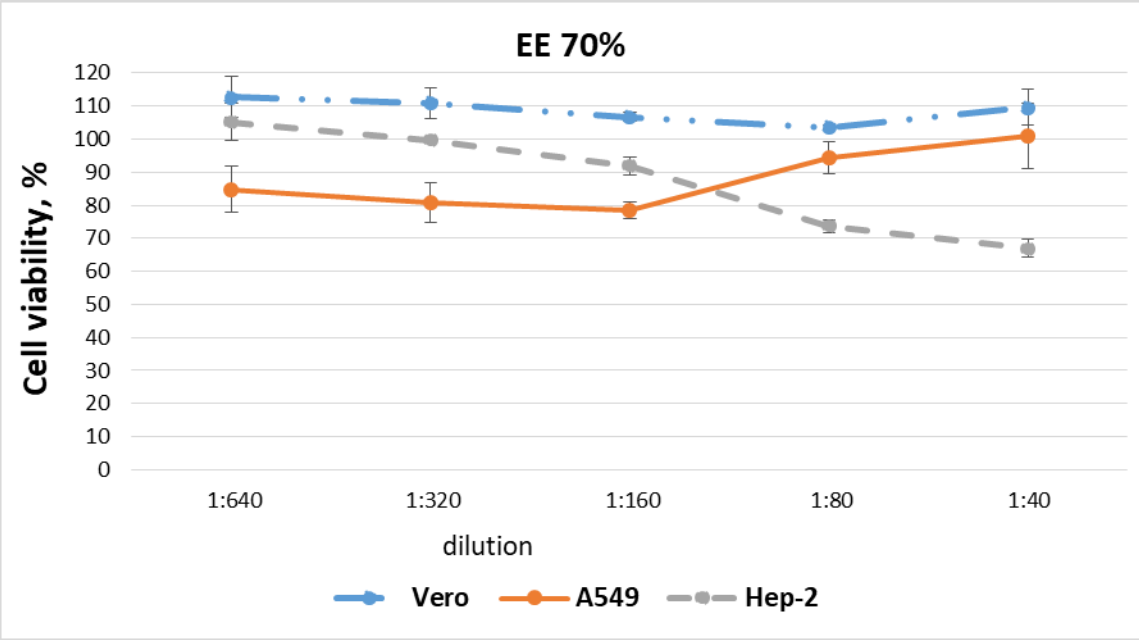
Cells type	Cells control	EE 70% (1:20)	EE 64% (1:20)
Vero			
A549			
Hep-2			

The morphological changes were observed using a light inverted microscope at 70 × magnification.

Results and discussion

Effect of the extracts on mitochondrial activity of the cells (MTT-test)

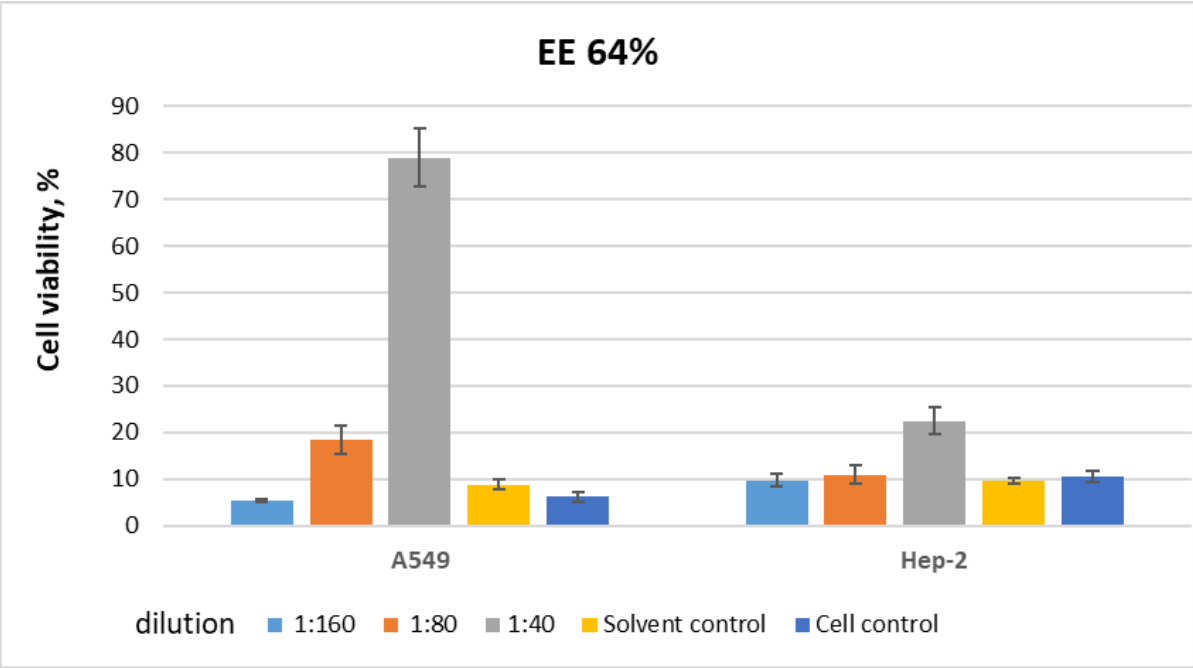


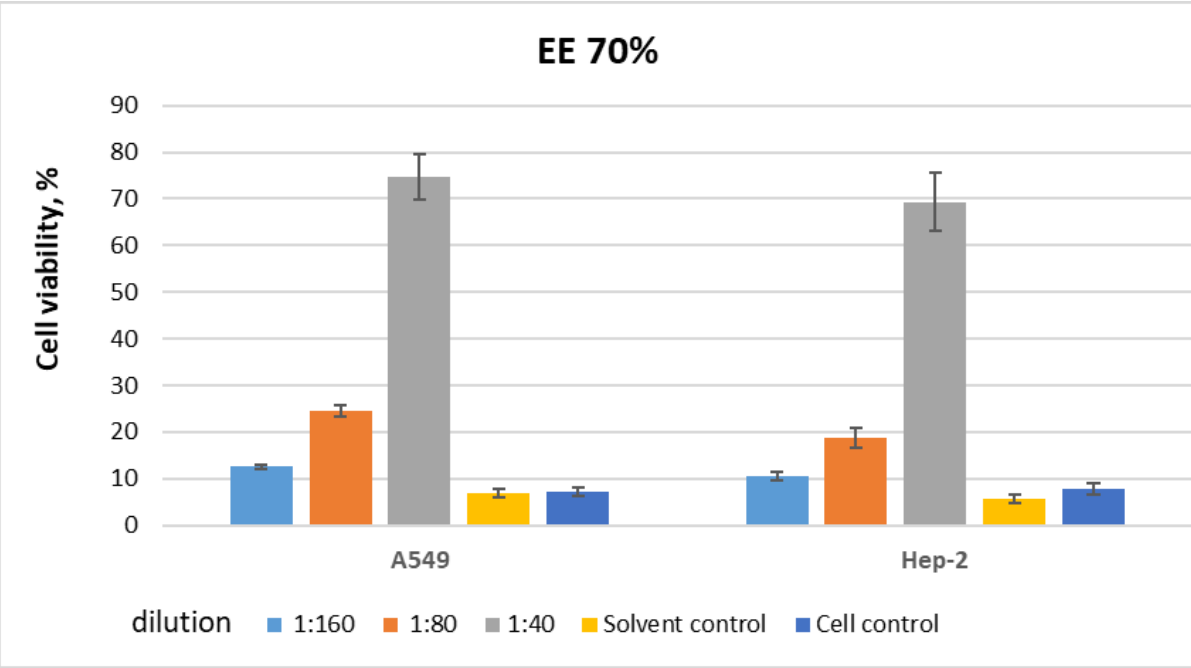


The mitochondrial activity of A549 and Hep-2 cells after extracts treatment was within 45 - 100%. While for Vero cells regardless of the dilution of the extracts it remained similar to the control cells.

Results and discussion

The influence of the extracts on cell membrane permeability (trypan blue assay)

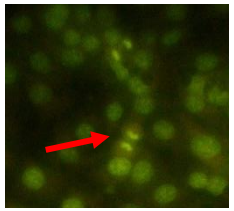




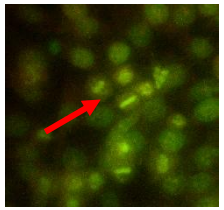
Revealed significant inhibition of A549 and Hep-2 cells viability under the influence of extracts, since cell membrane permeability increased by 18-79% and 19-69%, respectively. The 70% extract showed a greater toxic effect for both used tumor cell lines.

Results and discussion

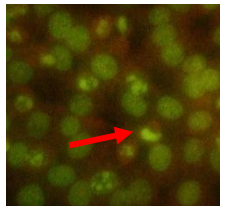
Detection of cells mitotic activity (fluorescent microscopy and acridine orange staining)



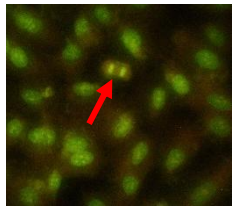
Hep-2



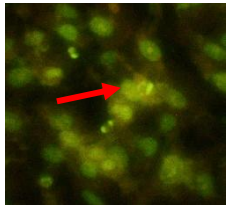
Hep-2+ EE 64% 1:160



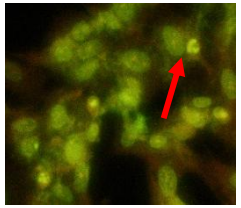
Hep-2+ EE 70% 1:160



A549

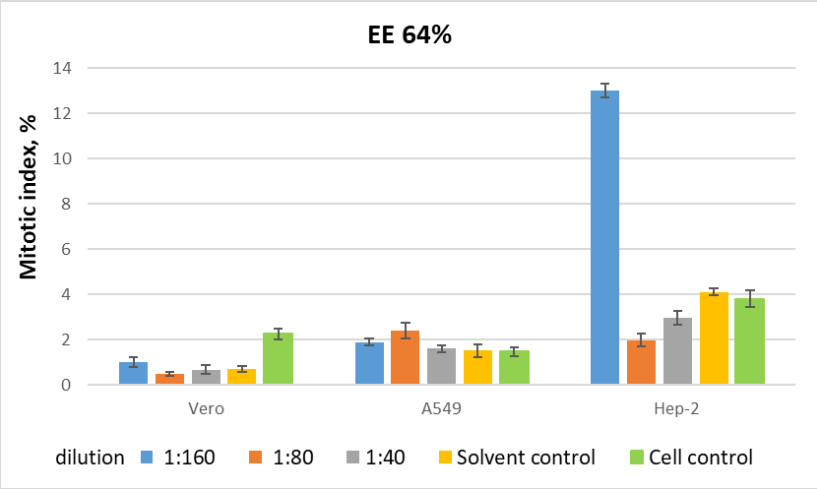


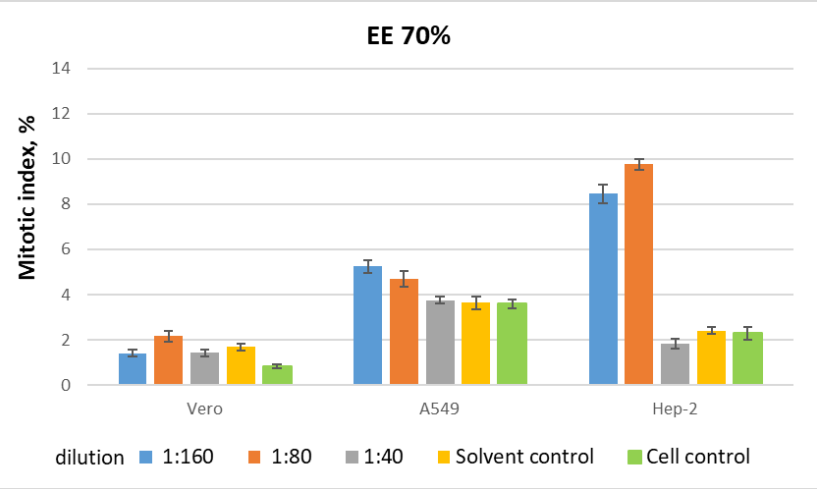
A549+ EE 64% 1:160



A549+ EE 64% 1:160

Red arrows show mitotic cells (280× magnification)

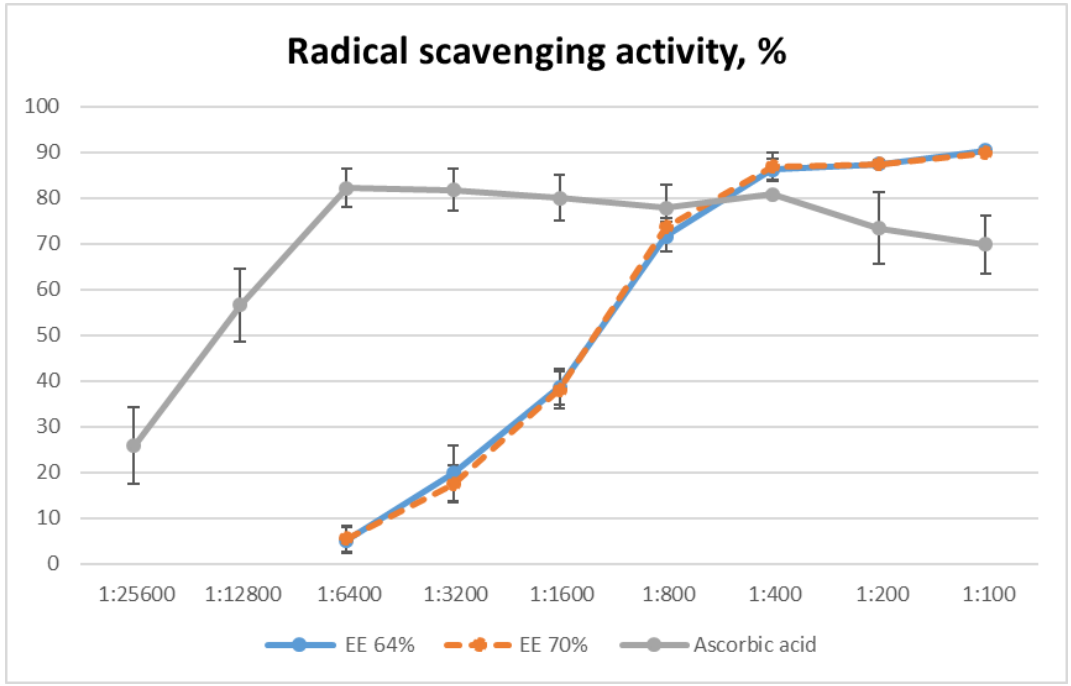




Extracts used at higher dilutions significantly increased the mitotic index of tumor cells. However, a high number of abnormal mitoses was detected.

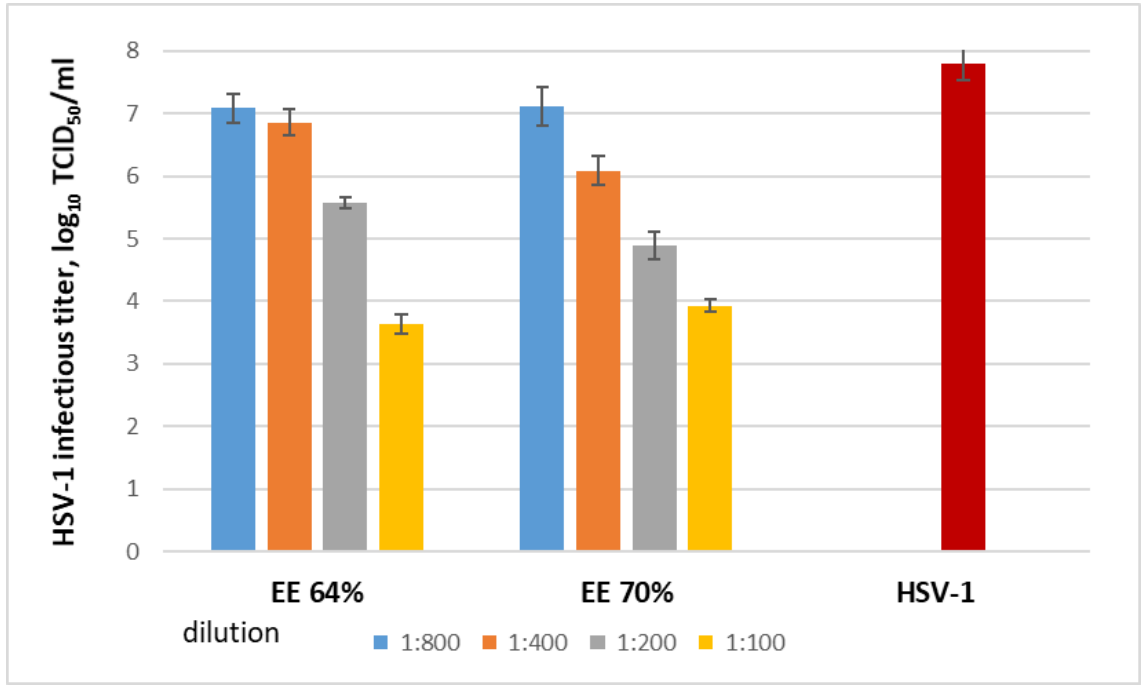
Results and discussion

DPPH radical scavenging activity



Extracts shown high antioxidant effect, since at dilutions 1:1600-1:100 its radical scavenging activity was 38-90%. IC_{50} indexes in dilution for EE 64% and EE 70% were ≈1:1320, for reference compound it was 1:16593.

Antiviral activity (yield reduction assay)



Pronounced antiherpetic activity of both extracts was observed, since depending on the dilution the decreasing of HSV-1 infectious titer was in the range of 1.7 - 4.7 $\log_{10}$ $TCID_{50}$/ml.

Conclusions

This study confirms that ethanol marigold extracts induces changes in cancerous Hep-2 and A549 cells. The influence on mitochondrial activity of such cells types and their membrane permeability was dose dependent and more pronounced with a decrease in extracts dilution. At the same time, the result of the fluorescent microscopy and acridine orange staining of the cells showed significant increasing of the mitotic index of tumor cells and number of abnormal mitoses with increasing dilution of the extracts. Screening of the antioxidant activity of extracts showed high antiradical activity and the ability of both extracts to absorb DPPH

The effect of obtained ethanol extracts of marigold on the reproduction of DNA-containing herpes simplex virus type 1 *in vitro* was studied. Both extracts were found to affect on the formation of infectious full-fledged viral progeny. Infectious titer of HSV-1 was decreased by 1.7 - 4.7 $\log_{10}$ $TCID_{50}$/ml.

These findings suggest, that extracts obtained from marigold *Tagetes patula* L. flowers may hold promise as a potential therapeutic agent.