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EVALUATION OF ANTIOXIDANT AND ANTICANCER ACTIVITIES OF FAMILIES LAMIACEAE AND CARYOPHYLLACEAE

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Article presents the results of experimental investigation of antioxidant and anticarcinogenic activity of plant extracts of families Lamiaceae and Caryophyllaceae. Selected species are: *Salvia officinalis*, *Origanum vulgare*, *Melissa officinalis*, *Stellaria media*. The determination of ascorbic acid content and total phenols content is carried out. Cytotoxic activity of plant extracts is evaluated using WISH cell lines. The following methodologies are concidered: spectrophotometry; photoelectric colorimetry; microscopic method based on inhibition and quantification of cancer cell lines.

Key words: extraction, bioactive compounds, antioxidants, anticancer activity, ascorbic acid, phenols, WISH cell line.

Introduction. Nowadays natural antioxidants have gained much attention from consumers, as they became considered safer than synthetic ones. Polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer actions, and they are widely distributed in food and medicinal plants [1].

In biological systems, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as hydroxyls, superoxides, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells. Normally, antioxidant system occurring in human body can scavenge these radicals, which

would keep the balance between oxidation and anti-oxidation. However, the exposure of cigarette smoking, radiation, alcohol, or environmental toxins increases the production of excessive ROS and RNS, which disrupt the balance between oxidation and anti-oxidation and result in some chronic and degenerative diseases [1]. The increment of intake of exogenous antioxidants, such as those that derive from fruits, vegetables, cereals, beverages, flowers, spices and traditional medicinal herb would decrease the damage caused by oxidative stress, acting as free radical scavengers, catchers of singlet oxygen and reducing agents.

To extract antioxidants from plant material, various techniques may be used. In laboratory studies hot water bath and maceration can be applied, but they are very time-consuming, so in industry ultrasound, microwave, pressurized liquid, enzyme hydrolysis, supercritical fluids, high hydrostatic pressure, pulsed electric field, and high voltage electrical discharges are chosen. The effective extraction is crucial to explore the potential of antioxidant sources and promote their application.

Plant families Lamiaceae and Caryophyllaceae were chosen for the studies, as they are known to be abundant on antioxidants and serve as medicinal substances and food supplements. Common sage (*Salvia officinalis*), oregano (*Origanum vulgare*), lemon balm (*Melissa officinalis*) and common chickweed (*Stellaria media*) were taken for the experiments as they are medicinal, food, herbaceous plants that are widespread in Ukraine. Health promoting bioactive compounds they contain are the following: vitamin E, vitamin C, rutin, niacin, riboflavin, thiaminascorbic acid, β-carotene, γ-linolenic, gallic, caffeic, coumaric, rosmarinic acid, naringenin, quercetin, kaempferol and chicoric acid, main essential oil components (sabinene, eucalyptol and caryophyllene oxide), and main fatty acids and hydrocarbons (cyclotetracosane, eicosanol etc.) [2–4].

Purpose of the work is to evaluate and compare antioxidant and anticancer activity of extracts of plants.

Material and methods. Plant material characteristics. For the experimental outlines there were took 5 samples: *Salvia officinalis* dry medicinal herb, *Origanum vulgare* dry wild herb and fresh cultured herb, *Melissa officinalis* fresh cultured herb

and *Stellaria media* fresh wild herb. Aboveground part of herbs were collected during the flowering, in May 2019. Before the experimental outline herbs were washed with distilled water and dried according to the procedure. Only fully evolved leaves were used for the experiments. Leaves were cut and weighted for the usage.

WISH cell line characteristics. WISH is a HeLa derivative cell line (WISH ATCC-№ CCL-25 was taken for the experiment) that is used for the evaluation of anticancer activity. It is originally derived from human amnion tissue and has been used in virus studies, as it is susceptible to VSV, adenovirus and poliovirus. It is also usefull in differentiating virulent and avirulent measles virus. This cell line was found to be indistinguishable from HeLa by STR PCR DNA profiling. Therefore, the cell line must be considered as derived from HeLa. WISH cell culture (Figure 1 and 2) is adherent monolayer epithelial culture that is incubated on RPMI-1640 medium [5].

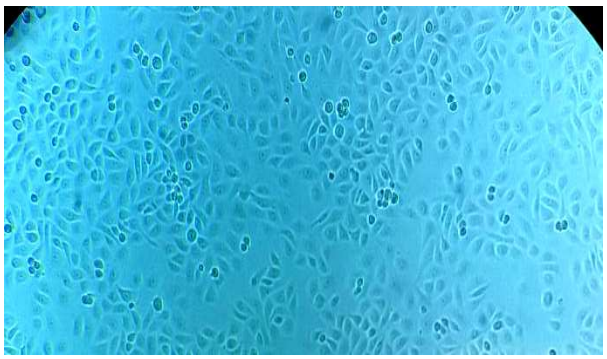

Fig. 1. Living cells of WISH (K+) (x100)

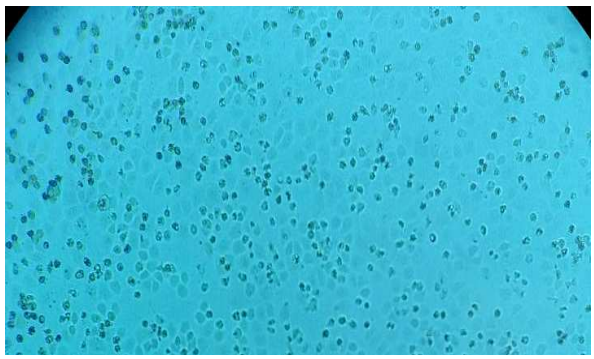

Fig. 2. Dead cells of WISH cell line under the action of plant extracts (x100)

Aqueous extracts preparation. To the incubation for WISH cell culture (human amnion cells) for the evaluation of anticancer activity, water extracts must be prepared. Plant material was chopped with scissors, weighted (5 g each) and grinded. 1 g of each sample was taken and transferred to glass bottles of 50 ml and added with 20 ml of water (plant material: water = 1 : 20). The materials were left for 24 hours incubation. The 1st (*Salvia officinalis*) and 2nd (*Origanum vulgare* wild) were heated according to the procedure on water bath: 30 min, 400 rpm, 45°C. Before the experiment, extracts were filtered through cloth, filter paper and glass filters succesively.

Ethanol extracts preparation. To the incubation for the evaluation of antioxidant activity, ethanol extracts must be prepared. Plant material was chopped with scissors, weighted (5 g each) and grinded. 1 g of each sample was taken and transferred to glass beakers of 50 ml and added with 10 ml of ethanol 96 % (plant material: ethanol = 1 : 10). The materials were left for 24 hours incubation.

Determination of vitamin C content in plant extracts. To evaluate the concentration of ascorbic acid in plant extracts, spectrophotometry with 2,4-dinitrophenylhydrazine was used [6].

Reagents: metaphosphoric acid – acetic acid; 2,4-dinitrophenylhydrazine solution and thiourea solution; standard vitamin C (ascorbic acid, AA) solution, 500 ppm; 85 % sulfuric acid solution. Fifteen grams of solid metaphosphoric acid are dissolved in mixture of 40 mL glacial acetic acid and 450 mL of distilled water, in a 500 mL volumetric flask. The solution is filtred and collected. 2 g of 2,4-dinitrophenylhydrazine and 4 g thiourea are dissolved in 100 mL 4,5M H_2SO_4.

After the reagent solutions preparation, five grams of samples are weighted using the electronic weights, transmitted to the plastic tubes and are homegenized with 25 mL of metaphosphoric acid – acetic acid solution. Then filtred and centrifuged at 4000 rpm for 15 minutes (Figure 3), after what the supernatant solution is used for spectrophotometric determination of vitamin C content.

Fig. 3. Homogenized plant material with metaphosphoric – acetic acid sol. 1 – *Salvia officinalis* dry medicinal herb, 2 – *Origanum vulgare* dry wild herb, 3 – *Melissa officinalis* fresh cultured herb, 4 – *Origanum vulgare* fresh cultured herb, 5 – *Stellaria media* fresh wild herb

After that 0,23 mL of 3 % bromine water are added into 4 mL of centrifuged sample solution to oxidize the ascorbic acid to dehydroascorbic acid and after that 0,13 mL of 10 % thiourea to remove the excess of bromine. Then 1 ml of 2,4-DNPH solution is added to form osazone. All standards, samples and blank solution are kept at 37 °C temperature for 3 hours in a thermostatic bath. After it all are cooled in ice bath for 30 minutes and treated with 5 mL chilled 85 % H_2SO_4, with constant stirring. As a result, a colored solution's absorbance is taken at 521 nm (Figure 4).

Fig. 4. Samples after incubation (S – ascorbic acid sample solution)

Determination of total phenolic content. The total phenolic content (TPC) can be measured using specrophotometric procedure. For this, 0,2 mL of ethanol extract solution and 1 mL of 10 % Folin–Ciocalteu reagent are mixed and after six minutes is added 0,8 mL of 7,5 % Na_2CO_3. A standard curve was prepared using 0–100 $\mu g/mL^{-1}$solutions of gallic acid in ethanol [7]. The total phenolic content of each extract was determined from the standard curve and expressed as mg gallic acid equivalent per dried plant material. Absorbance was recorded at 740 nm after two hours incubation (Figure 5). The same procedure is repeated for standard gallic acid in order to construct calibration curve. Phenolic content of samples is calculated from standard curve equation and expressed as gallic acid equivalents (mg GAE/g dry extract).

Fig. 5. Samples (1–5) and gallic acid (serial dilution) after incubation

Anticancer activity evaluation. For the anticancer activity of plant extracts evaluation and comparison, WISH cell lines were used and analyzed using BioTek Multifunctional Fluorometer Cytation3 with automated digital microscopy. WISH cells were examined using dying with toluidine blue stain and microscopy (invert microscope Optika XDS-3) and incubated in 75 cm^2 bed with 15 ml of RPMI-1640 nutrition medium. After the growing of monolayer, the culture was examined again. The cells were evenly attached to the surface of bed, homogeneous on their appearance, close to spherical form with chiasms. After reseeding of cell cultures, previously prepared plant extracts (plant material : water = 1 : 20) were introduced in the quantity of 100 μL to the cells in 96-well plates and incubated for 3 days. On the 3rd day the dead cells (cytotoxic activity of plant extracts) are washed out, living cells are dyed with crystal violet and examined by absorption using multifunctional fluorometer.

Results and discussion. Determination of vitamin C content in plant extracts. The obtained solutions were spectrophotometrically evaluated using Spectrophotometer UV-1800 comparing to the blank solution (4 ml of distilled water + 0,23 ml of bromine water + 0,13 ml of thioureate + 1 ml of 2,4-DNPH incubated for 3 hours and chilled with 5 ml of 85 % H_2SO_4). The measured optical density of samples was used for the formula:

$$x = \frac{A_1 \times m_o \times P}{A_0 \times 100 \times 100} \times \frac{V}{m_1,}$$

where:

A_0 – optical density of standard ascorbic acid solution;

A_1 – optical density of examined solution;

m_0 – weight of ascorbic acid, mg;

m_1 – weight of sample, g;

P = 99,8%;

V – volume of solution dilution factor.

Calculated ascorbic acid content is presented in figure 6.

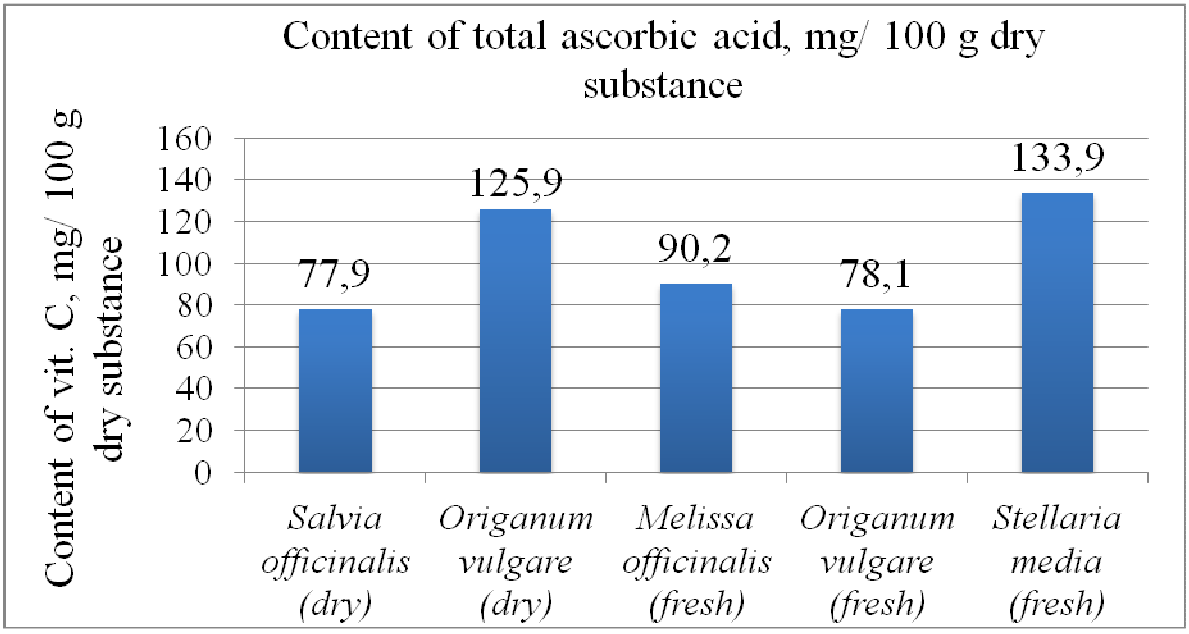

Fig. 6. Content of total ascorbic acid (AA) in the investigated samples

Determination of total phenolic content. The total phenolic content (TPC) was measured using photoelectric colorimetry procedure with the help of КФК-3. As listed in procedure, callibration graph of standard gallic acid was made. The callibration curve is presented on figure 7. The resulting equation obtained is: y = 325,38x - 39,245; R^2 = 0,9583.

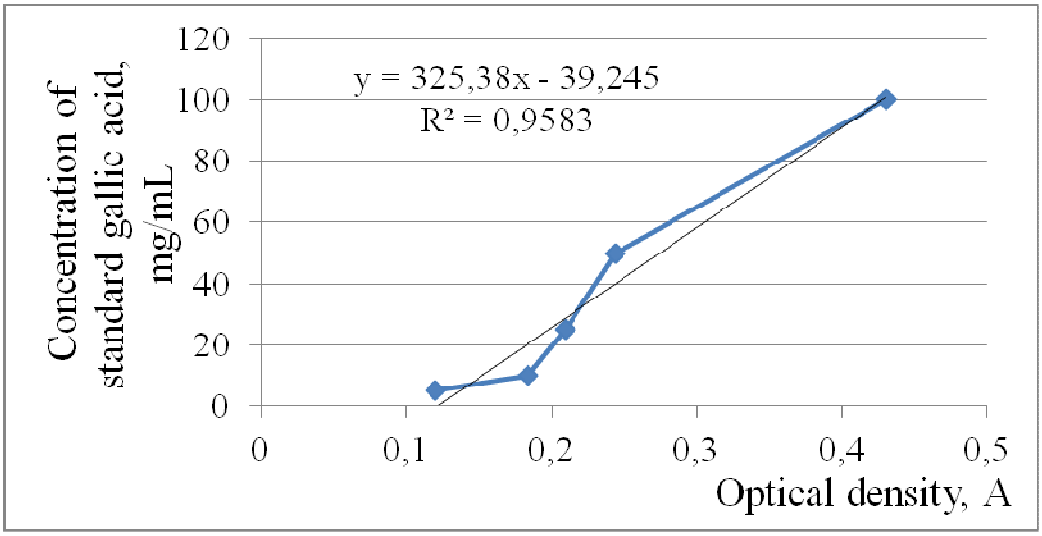

Fig. 7. Calibration graph of gallic acid, 0-100 mg/mL

With the help of obtained equation and having measured the optical density of extracts, phenols content in the form of gallic acid equivalents (mg GAE/g) can be calculated (Figure 8).

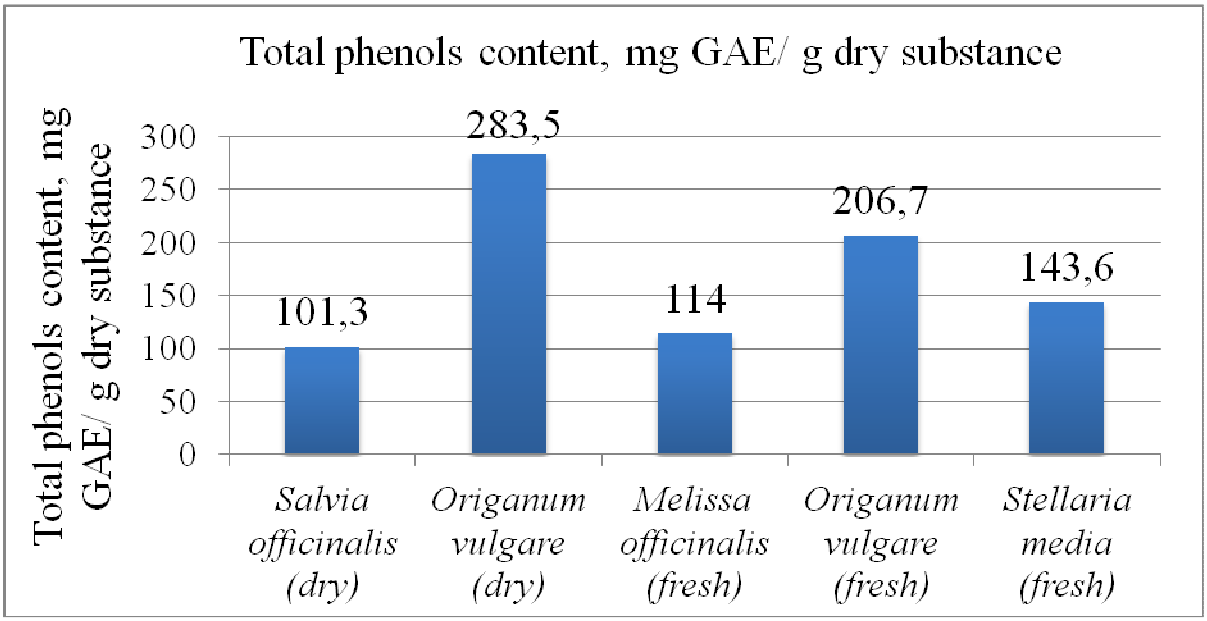

Fig. 8. Content of total phenols in the investigated samples

Anticancer activity evaluation. The 96-well plates (Figure 9 and 10) were examined using 2 methods:

1) microscopy with the help of invert microscope Optika XDS-3;

2) absorption using BioTek Multifunctional Fluorometer Cytation3 with automated digital microscopy.

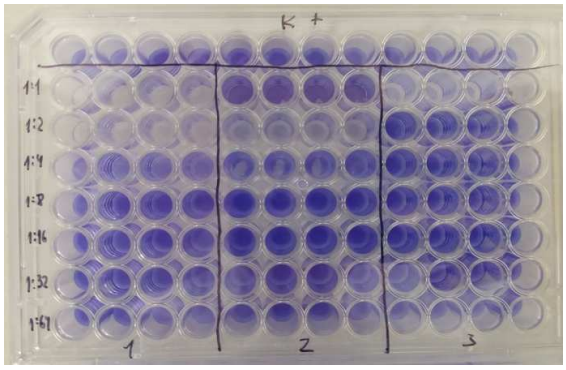

Fig. 9. WISH culture after dying with toluidine 1 – *S. officinalis*; 2 – *O. vulgare* (dry); 3 – *M. officinalis*

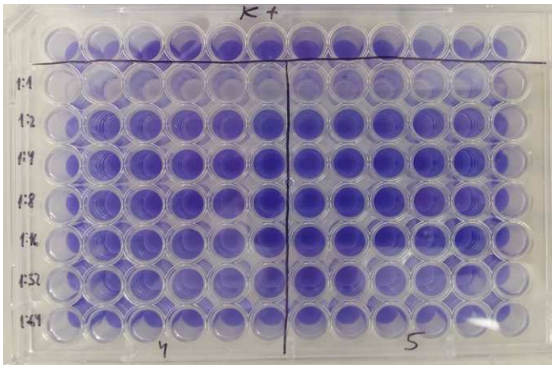

Fig. 10. WISH culture after dying with toluidine 4 – *Origanum vulgare* (fresh); 5 – *Stellaria media*

Microscopy results. The best cytotoxic result was shown by *Salvia officinalis* (dry medicinal herb) 1:1, 1:2, 1:4, 1:8 dilutions; medium result by *Origanum vulgare* (both dry wild herb and fresh cultured herb) 1:1, 1:2, 1:4 dilutions; the least promising results were seen by *Stellaria media* (fresh wild herb) and *Melissa officinalis* (fresh cultured herb) 1:1, 1:2 dilutions.

Absorption measurement. The dead cells (cytotoxic activity of plant extracts) were washed out with the phospate buffer and put into thermoshaker for 3 min at 900 rpm. The remaining living cells were dyed with 300 μL of crystal violet for 5 min. After that the excessive dye was washed out with 100 μL of of ethnol and put into thermoshaker for 4 min at 600 rpm. Next, after pouring out the dye with ethanol, plates were thoroughly washed with distilled water and put for drying for 30 min. The results were examined by absorption and are present on figure 11.

The same as by microscopy, the best cytotoxic result is shown by *Salvia officinalis* and *Origanum vulgare*; the least cytotoxic results were seen by *Stellaria media* (and *Melissa officinalis*.

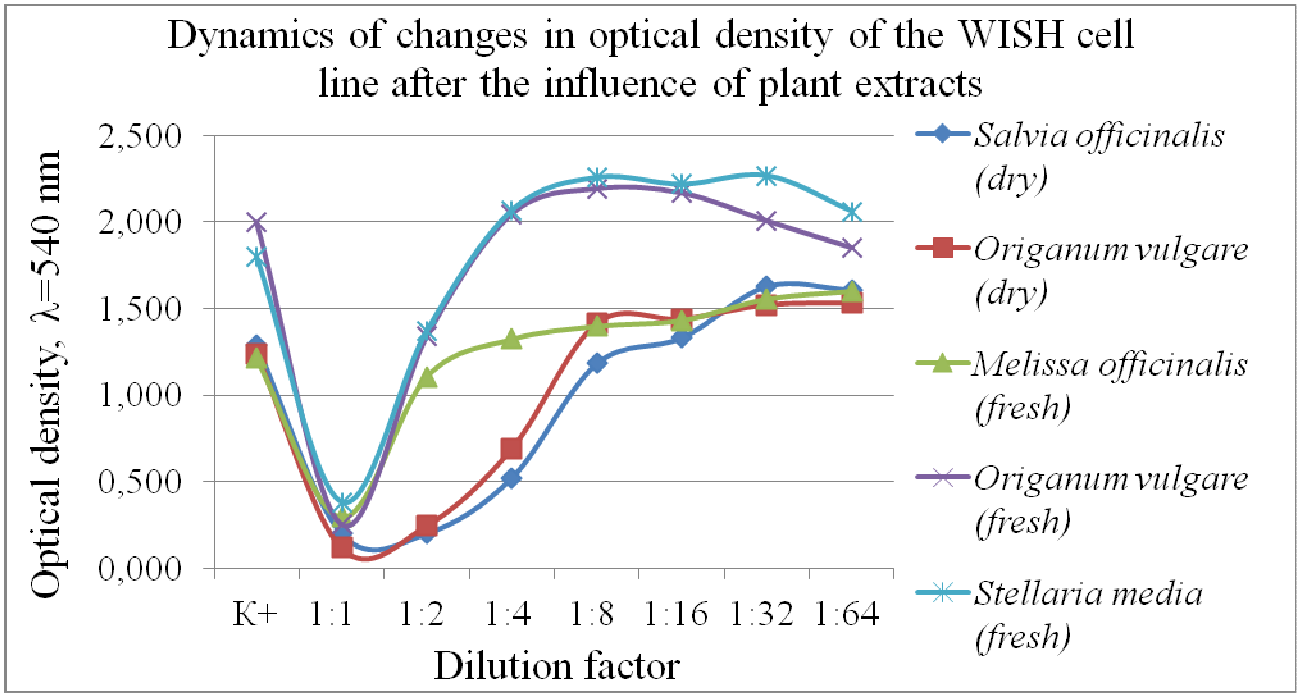

Fig. 11. Dependence of optical density of WISH from plant extracts dilution

CONCLUSION

Capacity of Lamiaceae and Caryophyllaceae extracts usage as antioxidants and anticarcinogenic medicinal substances is proved. As for antioxidant activity evaluation, the biggest amount of vitamin C was in the samples of *Stellaria media* and *Origanum vulgare*; the biggest content of phenols was in the sample of *Origanum vulgare* as well. As for anticancer activity evaluation, the best cytotoxic result was shawn by *Salvia officinalis* and *Origanum vulgare*.

Having analyzed all procedures of evaluation of antioxidant and anticarcinogenic activity, the most promising species for usage is *Origanum vulgare*.

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ОЦІНКА АНТИОКСИДАНТНОЇ ТА АНТИКАНЦЕРОГЕННОЇ АКТИВНОСТІ РОДИН ГЛУХОКРОПИВОВІ ТА ГВОЗДИЧНІ

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У статті наведено результати експериментального дослідження антиоксидантної та антиканцерогенної активності екстрактів рослин з родин Глухокропивові (Lamiaceae) та Гвоздичні (Caryophyllaceae). Обрані для дослідження види: *Salvia officinalis*, *Origanum vulgare*, *Melissa officinalis*, *Stellaria media*. Проведено визначення вмісту аскорбінової кислоти та загального вмісту фенолів. Цитотоксична активність рослинних екстрактів оцінена з використанням клітинної лінії WISH. Були використані наступні методи досліджень: спектрофотометрія, фотоелектроколориметрія, мікроскопічний метод, що ґрунтується на інгібуванні та кількісному визначенні ракових клітинних ліній.

Ключові слова: екстракція, біологічно активні речовини, антиоксиданти, антиканцерогенна активність, аскорбінова кислота, феноли, клітинна лінія WISH.

ОЦЕНКА АНТИОКСИДАНТНОЙ И АНТИКАНЦЕРОГЕННОЙ АКТИВНОСТИ СЕМЕЙСТВ ЯСНОТКОВЫЕ И ГВОЗДИЧНЫЕ

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В статье представлены результаты экспериментального исследования антиоксидантной та антиканцерогенной активности экстрактов растений из семейств Яснотковые (Lamiaceae) и Гвоздичные (Caryophyllaceae). Выбранные

для исследования виды: *Salvia officinalis*, *Origanum vulgare*, *Melissa officinalis*, *Stellaria media*. Проведено определение количества аскорбиновой кислоты и общей суммы фенолов. Цитотоксическая активность растительных экстрактов оценена с использованием клеточной линии WISH. Были использованы следующие методы исследований: спектрофотометрія, фотоелектро-колориметрія, микроскопический метод, который основывается на ингибировании и количественном определении раковых клеточных линий.

Ключевые слова: экстракция, биологически активные вещества, антиоксиданты, антиканцерогенная активность, аскорбиновая кислота, фенолы, клеточная линия WISH.