



DOI <https://doi.org/10.11603/2312-0967.2024.1.14441>

УДК 615.322:543.51:615.076.7

BIOLOGICALLY ACTIVE COMPOUNDS IN THE EXTRACTS FROM THE *SPERANSKIA TUBERCULATA* (BUNGE) BAILL HERB AND THEIR EFFECT ON THE VIABILITY OF CANCER CELLS OF FIVE DIFFERENT LINES

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ІНФОРМАЦІЯ

Надійшла до редакції / Received:
10.01.2024

Після доопрацювання / Revised:
20.02.2024

Прийнято до друку / Accepted:
26.02.2024

Ключові слова:

Speranskia tuberculata (Bunge)

Baill;

ethyl acetate extract;

petroleum ether extract;

LC/MS;

cancer cell lines;

MTT cytotoxicity test.

АНОТАЦІЯ

The aim of the work. To identify the most considerable bioactive compounds in the *Speranskia tuberculata* (Bunge) Baill extracts and study their effect on the viability of cancer cells of various origins.

Materials and Methods. The extracts from the *Speranskia tuberculata* herb were prepared using ethyl acetate and petroleum ether in the 12.5–75 µg/mL concentration range. The presence of compounds in the extracts was investigated by the tandem high-performance liquid chromatography-mass spectroscopy (LC/MS) method. The MTT cytotoxicity test was used to evaluate the effectiveness of extracts on five commercially available cell lines: A549 (human lung adenocarcinoma), HEPG2 (human hepatocellular carcinoma of the liver), A375 (human malignant melanoma), HELA (human pancreatic carcinoma), and RAW264.7 (macrophage-like cell line from mice transformed with Abelson's leukemia virus).

Results and Discussion. The peaks of about 300 compounds were identified in the mass spectra. Fifty-six compounds with the largest peaks were investigated from the point of view of manifestations of anticancer, antitumor, antioxidant and anti-inflammatory activity known from the scientific literature. Only 35 of the 56 compounds have a documented effect on the listed diseases; another 15 compounds are active on other diseases, and 6 showed no pharmacological activity. Of the 35 compounds belonging to 10 chemical classes, alkaloids, lipids, and polyphenols dominate. Chemical reactions for phytochemical screening of chemical classes found in extracts are presented. In MTT tests, the viability during treatment of 4 out of 5 cell lines significantly decreases with increasing concentration of extracts. Only RAW264.7 cells were stable – their viability did not fall below 75–85 %. Ethyl acetate extracts were the most effective. They maximally reduce viability to 18 %, and the IC₅₀ of this extract for all cell lines varied between 49–53 µg/mL. For petroleum ether, the IC₅₀ of three lines was 65–74 µg/mL (for A375, the approximate value reached 114 µg/mL).

Conclusions. Several hundred compounds were identified in the extracts from the *Speranskia tuberculata* herb using LC/MS. It was found in the scientific literature that certain compounds possess anticancer, antitumour, antioxidant, and anti-inflammatory effects. The MTT tests showed that the studied extracts significantly reduced the viability of cancer cells in four lines.

Introduction. Herbal remedies derived from plants are a crucial source for developing new drugs, such as active pharmaceutical ingredients (APIs). There are several reasons why herbal remedies are important. Firstly, there are vast areas around the world with a wide variety of plants, some of which have yet to be explored. This is particularly true in Asia, where there is a rich tradition of developing traditional medicines, such as Traditional Chinese Medicine (TCM) and Indian medical practices [1]. Consequently, traditional medicine often evolves faster than pharmaceutical science. Many pharmacopoeias don't include plants that have been used in traditional medicine for centuries.

The reason of such a situation lies in the poor research of the abovementioned plants. Chinese plant *Speranskia tuberculata* (Bunge) Baill (*Euphorbiaceae* Juss.) is a typical example. It is a perennial herbaceous plant that is endemic to Northeast China and grows on grassy slopes, meadows, or thickets, usually in dry places, 300-1900 m in height [2, 3]. *Speranskia tuberculata* is a drought-resistant plant. Hairy spherical buds along the stem produce tiny white flowers. On the one hand, it is a well-known plant for use in TCM. On the other hand, it is still not listed in the Pharmacopoeia of the People's Republic of China, let alone in the Pharmacopoeia of Ukraine.

Although *Speranskia tuberculata* is not included in the International Union for Conservation of Nature's (IUCN) Red List of Endangered Plants, its range is decreasing. First of all, it is due to the gradual intensification of agriculture. Accordingly, there are fewer and fewer areas of natural, wild growth of this plant. Nevertheless, it is pretty widespread in many Chinese provinces, such as Anhui, Hebei, Henan, Jilin, Liaoning, Nei Mongol, Ningxia, Shaanxi, Shandong and Shanxi [3].

The following example illustrates the fact that the potential of traditional medicine is far from being fully used. Within TCM, the role of *Speranskia tuberculata* is well known for treating several diseases. For example, this plant is believed to be effective in treating various types of cancer [4, 5, 6]. In addition, it is used as an antioxidant [7, 8]. Preparations based on this plant have an antibacterial or antimicrobial effect [9, 10]. The means also act as analgesics, anti-inflammatories, and antipyretics [11]. In addition to those listed above, it is known that preparations from *Speranskia tuberculata* treat various arthritis diseases. Still, the topic of the article does not refer to this area.

At the same time, with a profound experience of practical application, little is known about the mechanisms of medicinal action of the plant, including active pharmaceutical ingredients [12]. Except for separate works that prove the presence of various types of flavonoids in preparations from *Speranskia tuberculata* [13, 14], the plant is practically unexplored at the level of modern pharmaceutical science.

Thus, on the one hand, there are examples of the effectiveness of using preparations from this plant in treat-

ing a wide range of diseases. On the other hand, very little is known about the bioactive compounds in the plant, which means the possible mechanisms of the plant's action on diseases.

This study **aimed** to identify the most considerable bioactive compounds in the *Speranskia tuberculata* extracts and study their effect on the viability of cancer cells of five lines.

Materials and Methods. The work examines extracts of the herb *Speranskia tuberculata* (Bunge) Baill. In the People's Republic of China, many companies specialise in supplying traditional Chinese medicinal plants and distribute *Speranskia tuberculata* under the Chinese name Tou Gu Cao. Plant samples are usually obtained using the principles of Good Manufacturing Practices and Quality Testing. They are cleaned of foreign impurities and have confirmation of belonging to a biological species. Dried *Speranskia tuberculata* (Bunge) Baill herbs were purchased from Tongrentang Pharmacy Ltd. (Beijing, China). In particular, the genus/species were confirmed by Dr. Liwen Han, a botanist and expert in traditional Chinese herbal medicine. A sample (voucher# 2017-012-TGC) of the Tou Gu Cao used in this study has been kept at the Qilu University of Technology for further analysis/verification.

At the same time, the herb has not yet been entered into the Pharmacopoeia of the People's Republic of China. Accordingly, the number of indicators (for example, available biologically active compounds and their content) that can affect the quality of herbal medicines is limited.

The Chinese herbal medicine Tou Gu Cao was crushed to 20 mesh by a crusher, and the experiment was carried out according to the liquid-solid ratio of 1 to 10. Two solvents were tested for extract preparation. The Soxhlet extractor was used to extract herbs using analytically pure ethyl acetate (EA) and petroleum ether (PE) produced by Tianjin Fuyu Fine Chemical Co., Ltd.

The powder was weighed 50 g, and the filter paper was wrapped in the bottom of the Soxhlet extractor. 50 mL ethyl acetate or petroleum ether was added into the Soxhlet extractor flask and extracted at 72 °C for four hours. After cooling to room temperature, the ethyl acetate (or petroleum ether) extract was centrifuged at 6000 RPM for 5 min, and the supernatant was collected.

Tou Gu Cao extracts were characterized by liquid chromatography-mass spectrometry using a tandem QTOF-LC/MS (Agilent Technologies, USA). In this tandem instrument, a high-performance liquid chromatograph column is used as a separation system, and a quadrupole time-of-flight mass spectrometer (QTOF-MS) is used as a detection and identification system for individual ions and molecules. Thus, the instrument generally has high separation in liquid chromatography and high sensitivity in mass spectrometry.

The separation of compounds was achieved on Waters Cortecs C18 2.1*50mm 1.7 µm column in gradient

mode. Mobile phase A (water with 0.1 % formic acid) and mobile phase B (methanol) were set as follows: 70%A–30%B (0–7min), 60%A–40%B (7–17 min), 20%A–80%B (17–26 min), 10%A–90%B (26–31 min), with 4 min balance back to 90%A–10% B. The injection volume was 20 µL, and the flow rate was 0.3 mL/min.

The mass spectra were acquired in ESI negative mode (100–1500 m/z). The parameters were as follows: drying gas (nitrogen) with a flow rate of 15 L/min; sheath gas temperature 350°C, flow rate 12 L/min; voltage 3200 V.

Five commercially available cell lines A549 (human lung adenocarcinoma), HEPG2 (human hepatocellular liver carcinoma), A375 (human malignant melanoma), HELA (Human pancreatic carcinoma) and RAW264.7 (a macrophage-like, Abelson leukemia virus-transformed cell line derived from BALB/c mice) cells were used in this study. Cell culture media contains different concentrations of buffers, inorganic salts, glucose, amino acids, vitamins, and numerous bioactive compounds. All cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) + 10 % FBS (Fetal Bovine Serum) medium.

Cells were cultivated in dishes for cell cultivation with a diameter of 10 cm. The box for cultivation was kept at a constant temperature of 37°C and CO₂ concentration of 5 %; according to cell growth conditions, fluid exchange or passage was applied every two or every other day. Cultured cells were passaged in different ways according to various cells. Adherent cells were passaged by digestion. Part of the adherent growth but not firm cells can also be passed by direct blowing. Suspended cells can be separated and passaged by direct blowing, centrifugal precipitation, or natural sedimentation to remove the supernatant and then blow and passaged. The cell counting method was used to understand the growth state of cultured cells and determine the biological effects of medium, serum and drugs if necessary.

The extraction and concentration of 100 g *Speranskia tuberculata* (Bunge) Baill allowed one to obtain EA-based crude extract weighing 1.53 g and PE-based crude extract weighing 3.63 g. The concentrated extracts were dissolved in dimethyl sulfoxide to prepare a 50 mg/mL solution for the cell model experiment.

MTT cytotoxicity test was used to detect whether the extracts of different concentrations have killing effects on five types of tumour cells. MTT assay (3-(4,5-dimethylthiazol-2yl)-2,4-diphenyltetrazolium bromide, manufacturer - Sigma-Aldrich) is a simple and reliable method for preliminary evaluation of anticancer agents [15]. Five tumour cells were stimulated with different extract concentrations – 12.5 to 75 µg/mL.

The experimental principle was as follows. MTT can react with succinate dehydrogenase in living cells' mitochondria to form blue-purple crystalline formazan, which is insoluble in water deposited in living cells. The blue-violet formazan has a particular absorption peak at 492 nm after being dissolved in dimethylsulfoxide (DMSO).

When the number of collected cells was within a specific range, the number of living cells was proportional to the absorbance after the dissolution of formazan. Cell viability was calculated according to the following formula:

$$\text{Cell viability, \%} = \frac{A_x}{A_0} \times 100 \% \quad (1)$$

where A_x is the absorbance of samples of the experimental group with concentrations x , and A_0 is the absorbance of the blank control.

The experimental steps in MTT tests are as follows. Each of the five studied cells was spread in a 96-cell plate, diluted to 0.5–1×10⁴ well with DMEM + 10 % FBS. They cultured for 24 h (37°C, 5% CO₂). The extract sample was added to stimulate the cells when the cells were entirely adherent. The extracts were added 12.5, 25, 37.5, 50, 62.5 and 75 µg/mL, and the culture system was 100 µL per well.

Three replicate wells were set in each group, with the control wells (only cells and growth medium). 100 µL of a buffer solution of phosphate-buffered saline (PBS) was added to the outermost hole of the plate.

Remove the medium containing the sample, add the appropriate amount of PBS to clean two times, then add 100 µL medium containing 0.5 % MTT (5 mg/mL) per well, and continue to culture for 3–4 h. Remove the medium containing MTT and buckle the 96-well plate on clean paper three times. 100 µL DMSO was added to each well for 10 min, and the cell lysate's absorbance was measured at 492 nm. At this stage, the viability of cells is calculated by equation (1).

All experiments were repeated three times for each extract. The t-test was used to compare groups. It was shown that for all cell lines except RAW264.7, the level of viability after treatment with *Speranskia tuberculata* extracts of all concentrations showed a statistically significant difference at the level of at least $p < 0.05$ compared to the control (untreated) samples.

Results and Discussion. As a result of the LC/MS research, the peaks of almost 300 organic compounds detected in the extracts were identified. The distribution of compounds' mass spectra found in PE extracts is shown according to their peaks' decreasing intensity in Fig. 1a.

If we take 100 % of the intensity of the spectrum of the compound with the largest amplitude, then the 40th spectrum in a row is only 10 % of the maximum. The intensity of the peak of the 56th-ranked compound is 6.5 % of the maximum. An overview of the mass spectrum of compounds with the 56 most intense peaks is shown in Fig. 1b. The mass range for these compounds is from 136 to 737 Da. The compounds are most densely located in the 180–220 Da mass range.

Since there are no other reasonable assumptions about the importance of certain compounds as APIs, the analysis started with the compounds with the most in-

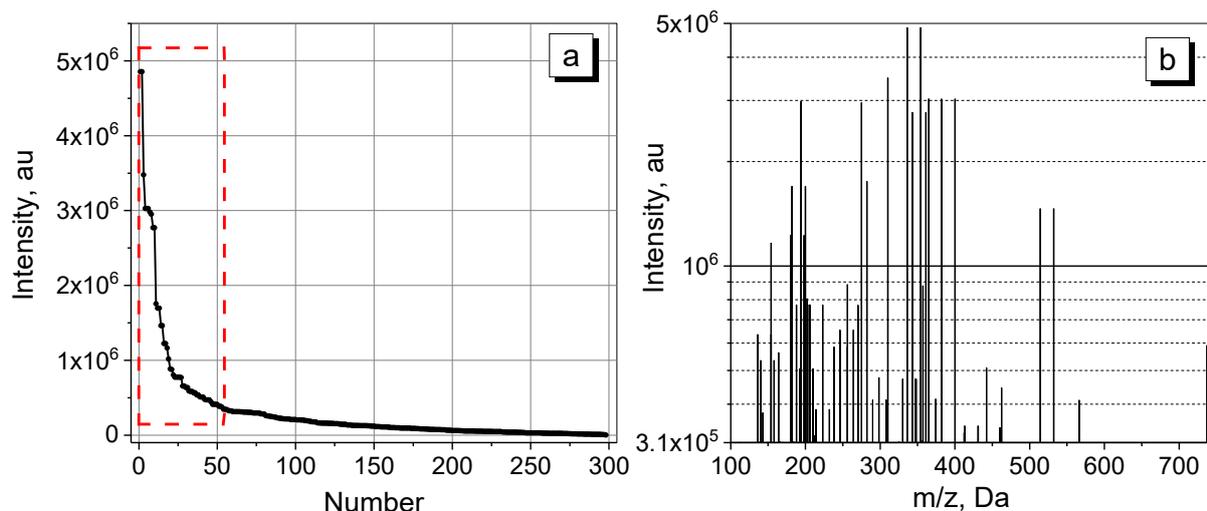


Fig. 1. Detected in PE extracts 300 mass-spectra arranged following their peak intensities in arbitrary units (a) and the intensities of 56 most intense peaks (b) as a function of m/z (in Da).

tense mass spectra. As a rule, the intensity of the peaks correlates with the concentration of the substance. Therefore, it can be hoped that the analysis of the most intense peaks is identical to the study of compounds with the highest concentrations in the extract. It gives reason to expect that those with significant pharmacological effects may exist among the studied compounds. This approach is only the first step in learning a little-studied plant. In the future, it is necessary to include less and less intense peaks in the analysis.

The first 56 compounds with the most intense peaks were classified by chemical classes, as is customary in pharmacognosy. Based on the analysis of literature data, diseases were determined for which each identified compound demonstrated the potential for pharmaceutical influence. Among many diseases, only a few of the most relevant, similar in nature, manifestations and treatment methods have been selected. They were grouped according to 4 directions, and known examples of the action of compounds are divided into anticancer (further on in the tables marked as 1), antioxidant (2), antitumour (3), and anti-inflammatory (4). The literature did not refer to *Speranskia tuberculata*, for which practically no documented observations exist. Compounds affecting other diseases (arthritis, antibacterial, antimicrobial and neuroprotective effects) were out of the article's focus. Such cases will be referred to as other activities and will not be discussed further. Table 1 illustrates part of the analysis and contains information only for the first twenty of the 56 compounds, as the publisher requires to reduce the number of literature references as much as possible.

Looking at the complete list of compounds analysed, 15 out of 56 have API potential to treat diseases outside the paper's focus. For the other 6 out of 56 compounds,

no signs of their action as APIs were found. Accordingly, they do not contain bioactivity records. Other 35 compounds have documented information about their positive effect on the four groups of diseases mentioned above.

Table 2 illustrates the chemical classification of identified compounds by classes based on the presence of pharmacologically active compounds in raw medicinal plant materials. This classification is quite conditional since there are always several groups of biologically active compounds in the raw materials, and it is not always known which of them has a therapeutic effect in a particular herb. Nevertheless, it helps to determine the dominant classes of compounds in *Speranskia tuberculata*. In addition, the analysis made it possible to create, based on [33, 34, 35, 36], a list of generally recognised phytochemical screening methods relevant to *Speranskia tuberculata* extracts. Their application should be a helpful next step to confirm the main classes of compounds in the extracts and identify active APIs discovered by LC/MS.

As can be seen, 35 compounds identified in *Speranskia tuberculata* extracts and have the potential to be used as APIs are classified into ten classes. The largest number of compounds is the class of alkaloids (10 compounds or 28.6 % of the total number of potential APIs). Next, regarding the number of compounds, the classes of lipids and polyphenols have 6 and 4 compounds, respectively.

The three classes mentioned contain 20 compounds or more than 57 % of the total. At the opposite pole are the classes of lactones and coumarins. Each of them is represented by only one compound.

Figure 2 illustrates the concentration-dependent effect of *Speranskia tuberculata* extracts on the viability of

Table 1

Identified 20 compounds with the most intense peaks and bioactivity cited according to the literature data

Identified compound	Compound class	Intensity, au	Molecular mass	PubChem CID	Bioactivity from the literature
(+)-Sesamin	Polyphenol	4854176	354.1	72307	2, 3 from [16]
Glabrone	Flavonoid	4854176	336.3	5317652	2, 4 from [17]
11-Eicosenoic acid (or gondoic acid)	Lipid	3477396	310.3	5282768	1, 4 from [18]
Tylophorinidine	Alkaloid	3027625	365.4	161749	1 from [19]
Corynoxine	Alkaloid	3027625	382.2	44568160	1 from [20]
Anti-isorhynchophylline N-oxide	Alkaloid	3027625	400.2		2, 4 from [21]
(+)-Myrtenyl acetate	Volatile oil	2979411	194.1	61262	2 from [22]
Annotine	Alkaloid	2950868	275.3	442468	4 from [23]
Codamine	Alkaloid	2770196	343.4	20056510	Other activity
Cephalofortuneine	Alkaloid	2770196	361.4	625285	1, 2, 4 from [24]
4-Prenyldihgdropinosylvin (miltirone)	Polyphenol	1753168	282.2	160142	1 from [25]
Jacaranone	Quinone	1694648	182.1	73307	1 from [26]
Rehmaglutin C	Glycoside	1694648	200.1	21637649	2, 3 from [27]
9-Deacetyl-9-benzoyl-10-debenzoyl brevifoliol	Volatile oil	1461323	514.6		1 from [28]
Calactin	Carbohydrate	1461323	532.6	441849	1 from [29]
Paeonilactone A	Lactone	1222949	198.2	10081437	1 from [30]
3,5-Dimethyl-4-methoxybenzoic acid	Phenylcarboxylic acid	1222949	180.2	88944	Other activity
3,5-Dihydroxybenzoic acid	Phenylcarboxylic acid	1163814	154.1	7424	Other activity
1-Ethyl-4,8-dimethoxy-beta-carboline	Alkaloid	884227	256.1	5317243	1, 4 from [31]
Protostephanine	Alkaloid	876922	357.4	632119	2, 4 from [32]

Table 2

Distribution by classes of the number of identified compounds with API potential and responses of phytochemical screening reactions to identify these classes

Name of test	Observed changes
1	2
The class of alkaloids covers 10 of 35 compounds (28.6 %); reactions for phytochemical screening are:	
Tests by: Hager	Hager's reagents form a colour (yellow or creamy white) complex.
Dragendorff/Kraut	With Dragendorff's reagents, a reddish-brown precipitate falls out.
Mayer/Bertrand/Valsler	With Mayer's reagent, a creamy white/yellow precipitate is formed.
Wagner	A reddish-brown precipitate is formed with Wagner's reagent.
Hager	Hager's reagents form a colour (yellow or creamy white) complex.
Ehrlich	With Ehrlich's reagent, two separate yellow and brown coloured layers are formed.
The class of lipids includes 6 of 35 compounds (17.1 %); reactions for phytochemical screening are:	
Sudan's test	The sample is treated with Sudan III or Sudan IV solution. A red colour is formed.
Emulsion test	Take the sample and mix it with equal volumes of ethanol and water, followed by shaking it. A cloudy white emulsion will form if lipids are present.
The class of polyphenols includes 4 of 35 compounds (11.4 %); reactions for phytochemical screening are:	
Iodine test	A few drops of dilute Iodine solution form a transient red colour.

1	2
Lead acetate test	A white precipitate is formed with a 10 % lead acetate solution.
Boronic-citrate reaction	When reacting with boric acid and citrate, a red or pink colour is formed
Phthalein dye test	The pink colour solution shows the presence of polyphenol.
The class of glycosides includes 3 of 35 compounds (8.6 %); reactions for phytochemical screening are:	
Baljet's test	A drop of Baljet's reagent changes colour into yellow-orange.
Keller-Killani test	After adding methanol, alcoholic KOH, and 1 % alcoholic 3,5-dinitrobenzene to the extract and heating, a disappearing violet colour is observed.
The class of flavonoids includes 3 of 35 compounds (8.6 %); reactions for phytochemical screening are:	
Alkaline reagent test	The addition of 2 % NaOH solution changes colour to intense yellow. It becomes colourless with the addition of drops of dilute HCl acid. The addition of 10 % ammonium hydroxide solution leads to the appearance of yellow fluorescence.
Shinoda's test	Fragments of magnesium ribbon are added to the extract (dissolved in alcohol) with a few drops of concentrated HCl. A pink to crimson coloured solution {flavonols}
Shibata's reaction/ Cyanidin test	After dissolving aq. extract in 50 % methanol and heating, add metal magnesium with conc. HCl, and observe red colour {flavonols}, or orange colour {flavones}.
The class of quinones includes 3 of 35 compounds (8.6 %); reactions for phytochemical screening are:	
Alcoholic KOH test	Adding alcoholic potassium hydroxide changes the extract's colour from red to blue.
Concentrated HCl test	Concentrated HCl changes the extract colour to green.
Sulphuric acid test	Adding the conc. H ₂ SO ₄ to extract dissolved in isopropyl alcohol changes its colour to red.
The class of carbohydrates includes 2 of 35 compounds (5.7 %); reactions for phytochemical screening are:	
Molisch's test	A purple ring is observed after adding alcoholic α -naphthol and concentrated H ₂ SO ₄ .
Barfoed's test	After adding Barfoed reagent & heating, brick-red precipitate {monosaccharides} formed.
Seliwanoff's Test	Adding Seliwanov's reagent & heating the water bath causes a pink-red colour {ketoses}
The class of volatile oils covers 2 of 35 compounds (5.7 %); reactions for phytochemical screening are:	
Reaction with Sudan III solution	A thin slice is placed in Sudan III solution for some minutes, then transferred to water or glycerine. The volatile oil turns green. Screening is hindered by fats (change colour to red)
Fluorescence test	Filtrate saturation extract and expose it to UV light to see bright pinkish fluorescence.
The class of coumarins includes 1 of 35 compounds (2.9 %); reactions for phytochemical screening are:	
NaOH test	Alkalis form a yellow-pink salt of o-coumaric acid when diluting, after which they are restored to their original state when acidified or saturated with carbon dioxide.
NaOH paper test	Observed yellow fluorescence of paper treated with 1N NaOH, which was used to cover a tube of heated extract
The class of lactones includes 1 of 35 compounds (2.9 %); reactions for phytochemical screening are:	
Kedde's reaction	When 3,5-dinitrobenzoic acid is added, a purple-red colour is formed.
Legal's reaction	A red colour occurs when sodium nitroprusside is added.
Ballier's reaction	Under the action of picric acid, cardenolides turn red-orange.
The subtotal is 35 identified compounds (100 %) active in 4 targeted disease groups.	
15 more identified compounds are not considered because they have activities other than those that target diseases.	
No API potential has 6 more identified compounds.	
Total	56 compounds were analysed in EA and PE extracts

cancer cells of different lines. For each of the five cell lines, comparative data on the effects of extracts based on PE and EA are given.

Based on Fig. 2, two main conclusions are suggested. First, in most cases, exposure to extracts significantly decreases the viability index with increasing extract

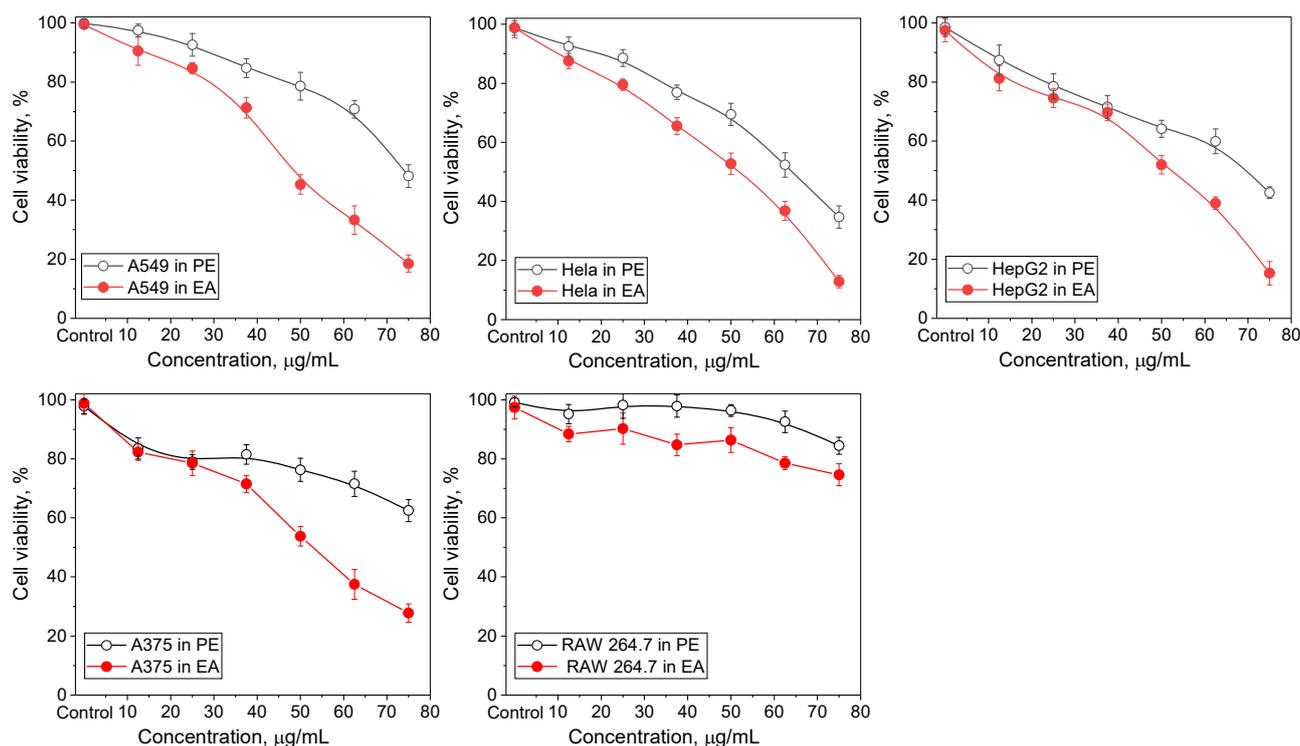


Fig. 2. Dependence of the viability of cancer cells of different lines on the concentration of *Speransky Tuberculata* extracts based on PE and EA.

concentration. The only exception is the effect on RAW264.7 cells, which was considerably less than on other cultures. Unlike other used cell lines, representing various types of human cancer cells, RAW264.7 cells are a semi-adherent macrophage-like cell line derived from BALB/c mice, which was transformed by the Abelson leukemia virus. These cells are commonly used as a model of mouse macrophages for the study of cellular responses to microbes and their products. However, it is impossible to say why RAW264.7 cells were more resistant to the effects of *Speranskia tuberculata* extracts at this research stage.

Second, in all investigated cases, the viability decline rate in EA solutions was higher than in PE. In other words, EA extracts proved to be more effective. It is worth noting that for some cells, the effect of EA is significantly accelerated at certain stages of increasing concentration. For example, for A549, such an acceleration begins after a concentration of 25 µg/mL and becomes evident after 37.5 µg/mL. For A375 and HEPG2, the picture looks similar, with additional acceleration after 37.5 µg/mL. The picture is less apparent for the HELA line since the acceleration is more monotonous. Still, with increasing concentration, the action of the extract always occurs at a higher speed.

For PE, the influence of the concentration of *Speranskia tuberculata* looks much closer to linear. That is, it depends less on the concentration interval. If there is

any acceleration, it occurs only in the last concentration interval (62.5–70 µg/mL).

The enhancement of the extract effect is most probably due to increased concentrations of certain bioactive compounds. Therefore, EA can extract more compounds from *Speranskia tuberculata* than PE. It is known that the extraction of bioactive compounds is influenced by the polarity of the solvent [37], and the effect is the opposite for different classes of compounds. Thus, according to [37], the extraction of flavonoids and phenolic compounds decreases with increasing polarity of the solvent. Conversely, with increasing polarity, there is an increase in total extraction yield, antioxidant activity, and free radical scavenging activity, which can be explained by the high affinity of antioxidant compounds to more polar solvents than non-polar ones. One may suggest that phytochemical compounds extracted in polar solvents are pharmaceutically more critical, at least due to comparatively higher values of antioxidant activity, reducing properties and free radical scavenging activity. The polarity index, a relative measure of the degree of interaction of the solvent with various polar test solutes, known as polarity, is significantly higher for EA than the polarity of PE: 4.4 versus 0.1 [38]. Accordingly, the better extractive capabilities of EA do not contradict the above literature data.

The consequence of the greater effectiveness of EA is the level to which it was possible to reduce the viabi-

ty of cancer cells for a given concentration interval of the extracts. Thus, for the HELA and HEPG2 lines, viability dropped from 100 % to 13–15%, and for the A549 and A375 lines, it fell to 19–28 %. There is always a more significant decrease in viability than PE, for which the residual level of viability is always at the level of 35–62 %, i.e., 22–35 % higher than in EA.

Usually, the half-maximal inhibitory concentration IC_{50} value is used to evaluate the effectiveness of a particular drug. IC_{50} indicates how much of a drug is needed to inhibit a biological process by half, thus providing a measure of the potency of an antagonist drug in pharmacological research. IC_{50} values (Fig. 3) were calculated by approximating the experimental curves from Fig. 2.

For Raw 246.7 cells, the value of IC_{50} seems unattainable for the studied concentration interval. Therefore, it

is not shown in the graph. As we can see, for EA, IC_{50} values are relatively weakly dependent on the extract concentration, varying for different cell lines between 49 and 53 $\mu\text{g/mL}$. Nevertheless, a slightly lower value of IC_{50} is observed for HELA and the highest value for A375. Therefore, HELA cells are the most sensitive among these five cells to stimulating crude extracts with ethyl acetate.

For PE-based extracts, IC_{50} values for all cells are higher than for EA extracts, and they markedly increase when going from HELA to A549 and further to A375. In the case of cells of the A375 line, the value of IC_{50} was conditionally calculated by linear approximation beyond the studied concentration interval of 12.5–75 $\mu\text{g/mL}$. But, we can confidently say that the IC_{50} of PE-based extracts for such cells is significantly inferior to other cases.

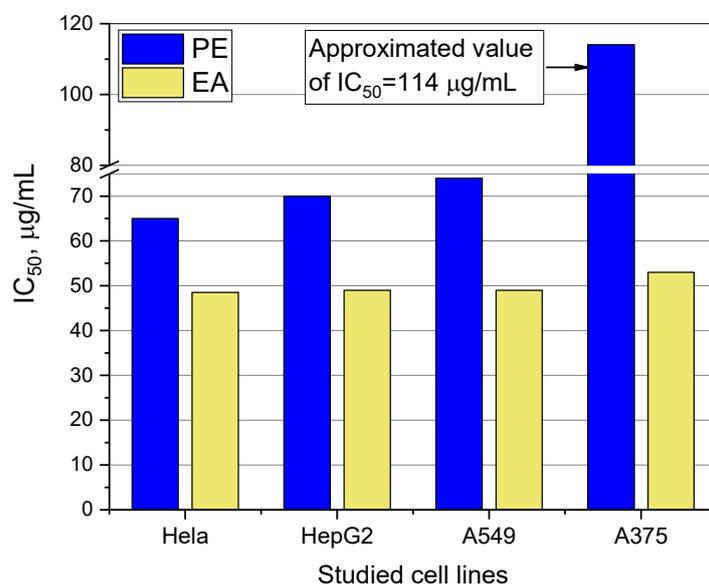


Fig. 3. Experimental IC_{50} values for evaluating the effectiveness of EA and PE extracts in relation to cells of different lines (the IC_{50} value is approximated beyond the studied concentration range for A375 in PE).

Conclusions. 1. Two series of extracts from the medicinal plant *Speranskia tuberculata* were prepared using petroleum ether and ethyl acetate in the concentration range from 12.5 to 75 $\mu\text{g/mL}$ and then studied by the high-performance liquid chromatography-tandem mass spectroscopy method.

2. About 300 compounds in the extracts were identified by high-performance liquid chromatography-tandem mass spectroscopy, but the focus was on the 56 most intense peaks. According to the scientific literature data, 35 out of 56 compounds studied have anticancer, antitumour, antioxidant and anti-inflammatory effects.

3. These bioactive compounds are divided into several chemical classes, with alkaloids, lipids and polyphenols being the most dominant. The total number of these compounds exceeded 57 % of the total number of iden-

tified substances. Furthermore, a directory of chemical reactions optimal for phytochemical screening of *Speranskia tuberculata* extracts has been developed, which can serve as an essential tool for further research and study of the plant's chemical composition.

4. The effects of varying concentrations in both types of extracts on the viability of five different cancer cell lines, known as A549, A375, HELA, HEPG2 and RAW264.7, were investigated using MTT tests. Cell viability in four out of five lines significantly decreased with increasing concentration in both extract types but more intensely in ethyl acetate-based extracts than in petroleum ether extracts.

5. The IC_{50} value for ethyl acetate extracts was relatively stable at 49–53 $\mu\text{g/mL}$. The IC_{50} gradually increased from 65 to 74 $\mu\text{g/mL}$ from HELA to A549 in pe-

troleum ether extracts. The IC_{50} for A375 was not found within the studied concentration range but was approximately estimated at 114 $\mu\text{g}/\text{mL}$. The most significant impact found during the experiment was observed for the HELA line. The RAW264.7 line was the most resistant to

the effects of *Speranskia tuberculata* extracts, with the IC_{50} not reached in both extract types.

Conflicts of interest: authors have no conflict of interest to declare.

Конфлікт інтересів: відсутній.

БІОЛОГІЧНО АКТИВНІ РЕЧОВИНИ В ЕКСТРАКТАХ ТРАВИ *SPERANSKIA TUBERCULATA* (BUNGE) VAILL ТА ЇХНІЙ ВПЛИВ НА ЖИТТЄЗДАТНІСТЬ РАКОВИХ КЛІТИН П'ЯТИ РІЗНИХ ЛІНІЙ

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Мета роботи. Ідентифікація основних біоактивних речовин в екстрактах трави *Speranskia tuberculata* та дослідження їхнього впливу на життєздатність ракових клітин різного походження.

Матеріали і методи. Екстракти трави *Speranskia tuberculata*, виготовлені з використанням етилацетату та петролейного ефіру в інтервалі концентрацій 12,5–75 $\text{мкг}/\text{мл}$. Ідентифікацію сполук в отриманих екстрактах проведено за допомогою тандемного методу високоефективної рідинної хроматографії із мас-спектрометрією. МТТ-тест на цитотоксичність використано для оцінки ефективності дії екстрактів на п'ять комерційно доступних клітинних ліній: A549 (аденокарцинома легенів людини), HEPG2 (гепатоцелюлярна карцинома печінки людини), A375 (злоякісна меланома людини), Hela (карцинома підшлункової залози людини) та RAW264.7 (макрофагоподібна лінія клітин від мишей, трансформована вірусом лейкемії Абельсона).

Результати та обговорення. У мас-спектрах ідентифіковано піки 300 речовин, серед яких 56 сполук з найбільшими піками було досліджено з точки зору відомих із літератури проявів протиракової, протипухлинної, антиоксидантної та протизапальної активностей. Лише 35 з 56 сполук мають задокументований вплив на перераховані захворювання, ще 15 речовин активні щодо інших хвороб, і ще 6 не мають доведеної біологічної активності. Виявлені 35 речовин з доведеною терапевтичною активністю належать до 10 різних хімічних класів, серед яких домінують алкалоїди, ліпіди та поліфеноли. Наведено хімічні реакції для фітохімічного скринінгу сполук із тих хімічних класів, які були виявлені в екстрактах. В МТТ-тестах при обробці 4 із 5 клітинних ліній суттєво знижувалась життєздатність при підвищенні концентрації екстрактів. Лише клітини лінії RAW264.7 виявилися стійкими, оскільки їхня життєздатність не падала нижче за 75–85 %. Етилацетатні екстракти виявилися найбільш ефективними, оскільки вони максимально знижували життєздатність (до 18 %), а IC_{50} цього екстракту для усіх клітинних ліній варіював у межах 49–53 $\text{мкг}/\text{мл}$. Для петролейного ефіру IC_{50} трьох ліній становив 65–74 $\text{мкг}/\text{мл}$ (для A375 апроксимована величина сягнула 114 $\text{мкг}/\text{мл}$).

Висновки. За допомогою методу ВЕРХ-МС в екстрактах трави *Speranskia tuberculata* було ідентифіковано кілька сотень сполук. Серед 56 найбільш інтенсивних піків виявлено 35 сполук, які, за даними наукової літератури, мають протиракову, протипухлинну, антиоксидантну та протизапальну дії. МТТ-тести показали, що вивчені екстракти суттєво зменшують життєздатність ракових клітин чотирьох із п'яти вивчених ліній.

Ключові слова: *Speranskia tuberculata* (Bunge) Baill; етилацетатний екстракт; петролейно-ефірний екстракт; високоефективна рідинна хроматографія-тандемна мас-спектрометрія; лінії ракових клітин; тест МТТ на цитотоксичність.

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