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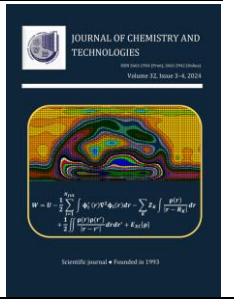

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BIOACTIVE COMPOUNDS EXTRACTED FROM THE HERB *SPERANSKIA TUBERCULATA (BUNGE) BAILL*

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Abstract

Aim. Identification of bioactive compounds in preparations extracted from the herb *Speranskia tuberculata* using two different solvents and comparison of the effects of the extracts on human cancer cells of four different lines. Methods. Crude extracts were extracted using ethyl acetate or petroleum ether, followed by distillation, drying and preparation of working solutions based on dimethyl sulfoxide in the 12.5–75 μg/mL concentration range. MTT tests were used to determine the effect of drug concentrations on the viability of cancer cells of four commercially available lines: A549 (lung adenocarcinoma), HEPG2 (hepatocellular carcinoma of the liver), A375 (malignant melanoma) and HELA (pancreatic carcinoma). The tandem liquid chromatography-mass spectroscopy (LC/MS) method was used to identify the main compounds and compare the composition of both extracts. Results. The developed preparations reduced the viability of all 4 types of cancer cells. Ethyl acetate extracts are always more effective than petroleum ether extracts. IC_{50} of ethyl acetate extract varied between 49–53 μg/mL for all cell lines. Approximately 100–200 compounds have been identified, and their number in petroleum ether extracts is roughly twice as high. Concentrations of 11 compounds significantly increased when switching from extraction with petroleum ether to ethyl acetate. The potential biological activity of identified compounds was analysed based on literature data. Conclusions. The studied preparations reduced the viability of various human cancer cells. Preparations extracted with ethyl acetate were more effective against all types of cells. Hundreds of compounds were identified in the extracts; some anticancer effects were discussed.

Keywords: Speranskia tuberculata (Bunge) Baill; ethyl acetate extract; petroleum ether extract; LC/MS; commercially available cancer cell lines; MTT cytotoxicity test.

БІОАКТИВНІ СПОЛУКИ ЕКСТРАГОВАНІ ІЗ ТРАВИ *SPERANSKIA TUBERCULATA (BUNGE) BAILL*

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Abstract

Мета. Ідентифікація біоактивних сполук в препаратах, екстрагованих із трави *Speranskia tuberculata* із застосуванням двох різних розчинників, та порівняння впливу екстрактів на ракові клітини людини чотирьох різних ліній. Методи. Сирі екстракти готували шляхом екстрагування із застосуванням етилацетату або петролейного ефіру з наступною відгонкою, сушкою і приготуванням робочих розчинів на основі диметилсульфоксиду в діапазоні концентрацій 12.5–75 мкг/мл. MTT тести використані для визначення впливу коцентрації препаратів на життєздатність ракових клітин чотирьох комерційно доступних ліній: A549 (аденокарцинома легень), HEPG2 (гепатоцелюлярна карцинома печінки), A375 (злоякісна меланома), HELA (карцинома підшлункової залози). Використано тандемний метод рідинної хроматографії-мас-спектроскопії (LC/MS) для ідентифікації основних сполук та порівняння складу обох екстрактів. Результати. Обробка розробленими препаратами знижувала життєздатність всіх 4 типів ракових клітин. Етилацетатні екстракти завжди є більш ефективніими. IC_{50} етилацетатного екстракту для всіх клітинних ліній варіювався в межах 49–53 мкг/мл. У препаратах ідентифіковано приблизно 100–200 сполук, причому їх кількість в екстрактах петролейного ефіру була приблизно вдвічі вища. Концентрації 11 сполук суттєво зростають під час переходу від екстрагування петролейним ефіром до етилацетату. На ґрунті літературних даних проаналізовано потенціал біологічної активності ідентифікованих сполук. Висновки. Досліджувані препарати знижували життєздатність різних ракових клітин людини. Препарати, екстраговані етилацетатом, були ефективнішими відносно всіх типів клітин. Сотні сполук ідентифіковані в екстрактах; обговорена протиракова дія деяких.

Ключові слова: Speranskia tuberculata (Bunge) Baill; екстракти на основі етилацетату та петролейного ефіру; LC/MS; комерційно доступні лінії ракових клітин; тест на цитотоксичність MTT.

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Introduction

Methods and means of folk traditional medicine are widely distributed throughout the world. Traditional medicine, as a form of alternative medicine, has developed exceptionally in large Asian countries, such as China and India, facilitated by a thousand-year-old culture and rich natural resources for producing plant and animal remedies [1; 2]. Modern pharmaceutical science coexists with healing practices, which mutually enriches both approaches. Thus, herbal medicinal products from traditional medicine have long become an essential source of the creation of new active pharmaceutical ingredients (APIs). On the other hand, traditional medicine increasingly uses data from modern scientific studies.

However, the cooperation of traditional and scientific medicines is far from ideal. The development of alternative medicine dates back millennia, while the path of modern pharmaceuticals is much shorter. As a result, pharmaceuticals often cannot master the many years of experience of traditional medicine on time. Pharmacopoeias of countries frequently do not contain a description of plants that have long proven themselves in the best way as a means of alternative medicine. The only reason is the poor scientific study of such plants. The herb *Speranskia tuberculata (Bunge) Baill* is a typical example.

This herb (also known in China as Tou Gu Cao) is a perennial drought-resistant herbaceous plant endemic to Northeast China. It grows on grassy slopes, meadows or shrubs, usually in dry places, at an altitude of 300–1900 m [3]. On the one hand, this plant is well-known in traditional Chinese medicine (TCM). It is effective in treating various types of cancer [4–6] and has antioxidant, antibacterial and antimicrobial properties [7; 8]. Pain-relieving, anti-inflammatory, antipyretic and antiarthritic drugs are known to be based on *Speranskia tuberculata* [9]. On the other hand, the plant is still not included in the pharmacopoeia of the People's Republic of China and other countries.

In contrast to significant experience in the practical use of *Speranskia tuberculata* in TCM, little is known about the plant at the level of modern pharmaceutical science, namely about the available APIs. The exception is several works that prove the presence of different types of flavonoids in the plant [10–12].

Thus, many examples of effective remedies based on *Speranskia tuberculata* in TCM are known for treating many diseases. Simultaneously, almost nothing is known about the biologically active compounds in the plant. The work aimed to identify bioactive compounds in *Speranskia tuberculata* extracts based on ethyl acetate and petroleum ether and compare the extracts' effects on cancer cells of different lines.

Experimental

Dried and cleaned specimens of *Speranskia tuberculata (Bunge) Baill* were purchased from Tongrentang Pharmacy Ltd. (Beijing, China). The samples were crushed with a crusher to 20 mesh to prepare crude extracts. Weighed 50 g of powder; 500 mL of analytically pure ethyl acetate (EA) or petroleum ether (PE), produced by Tianjin Fuyu Fine Chemical Co Ltd, was added to a Soxhlet extraction flask. Extraction was performed at 72 °C for EA and 85°C for PE for four hours in each case. After cooling to room temperature, the crude ethyl acetate (or petroleum ether) extract was centrifuged at 6000 rpm for 5 min. The resulting liquid crude extracts were dried; dried extracts were weighed. A stock solution with a 50 mg/mL concentration was prepared from these extracts (solvent – dimethyl sulfoxide). Later, this solution was used to prepare working preparations with concentrations from 12.5 to 75 μg/mL for experimentation with cells.

Four commercially available cell lines of different types of human cancer, namely A549 (lung adenocarcinoma), HEPG2 (hepatocellular carcinoma of the liver), A375 (malignant melanoma) and HELA (pancreatic carcinoma), were used to test the effectiveness of working solutions against cancer cells. All cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) + 10 % FBS (Fetal Bovine Serum) in culture dishes with a diameter of 10 cm. Other details of cell culture are given in [13].

The generally recognised MTT test (manufacturer – Sigma-Aldrich) was used to assess the effect of working drugs on the viability of cancer cells of various origins. It is based on the ability of a mitochondrial membrane enzyme to reduce the yellow salt of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide to purple formazan crystals. The colour intensity of the accumulated formazan (determined using a spectrophotometer) is proportional to the viability of the cell.

All MTT concentration-dependent cell viability experiments were repeated three times for each cell and extract type. The obtained data were expressed as means with standard deviations. If it was necessary to determine the significance of the

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difference between the average indicators of individual experiments, the Student's t-test was used (p = 0.05 was taken as the threshold value). The experimental curves in all cases had a quasi-linear character. Concentration curves of viability obtained from MTT tests allowed one to calculate the value of the half-maximum inhibitory concentration IC_{50} (concentration of the solution required to inhibit the biological process by half) for each cell type and both solutions. For this, linear approximations of the experimental viability-concentration curves were used. Calculations were made using OriginPro 2017 (Origin Lab Co, USA).

The compounds in the obtained extracts were studied using a quadrupole time-of-flight tandem liquid chromatograph/mass spectrometer (QTOF-LC/MS, Agilent Technologies, USA). Compounds were separated on a Waters Cortecs C18 2.1·50 mm 1.7 μm column in gradient mode. The mobile phase A (water with 0.1 % formic acid) and mobile phase B (methanol) were set as follows: 70 % A–30 % B (0–7 min), 60% A–40 % B (7–17 min), 20 % A–80 %B (17–26 min), 10 % A–90 % B (26–31 min), with a 4 min balance to 90 % A–10 % B. The injection volume was 20 μL, with a 0.3 mL/min flow rate. Mass spectra were obtained in negative ESI 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; capillary voltage 3200 V.

Results

Using MTT tests to study the influence of the concentration of extracts based on ethyl acetate (from now on referred to as EA extracts) and petroleum ether (PE extracts) on the viability of cancer cells allowed one to determine the IC_{50} values for cells of different origins. The efficiency of the solutions increased with increasing concentration ($p < 0.05$). The results of IC_{50} calculations are shown in Fig. 1. Both extracts suppress all investigated cancer cells.

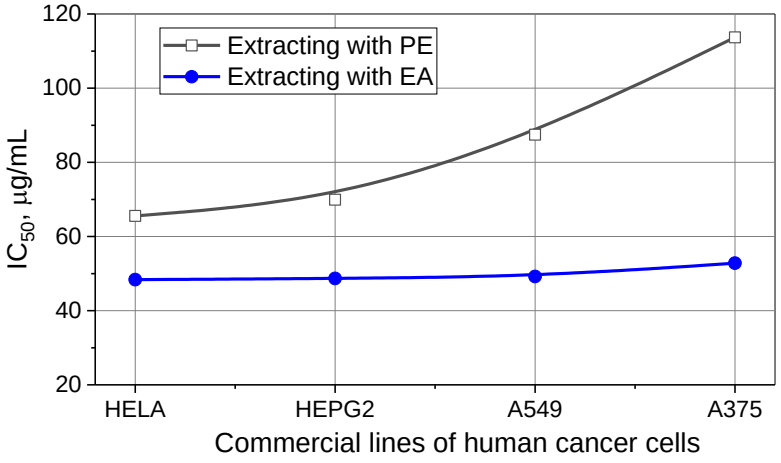

Fig. 1. The IC_{50} values for different cancer cells suppressed by preparations extracted from the *Speranskia tuberculata* herb with EA and PE

Preparations obtained with the use of EA extract demonstrate better effectiveness. Thus, the IC_{50} indicator for them only slightly increases from 48 to 53 μg/mL when switching from HELA cells to A375. A similar indicator for PE extracts increases more noticeably from 65 to 114 μg/mL (the last value is approximated by calculation, as it exceeds the studied experimental concentration range). As a result, the difference in the effectiveness of both preparations remains relatively moderate for HELA and HEPG2 cells (35–45 % in favour of EA extract). For A549 and A375 cells, the advantage increases significantly (up to 80–100 %).

The results indicate that the extracts based on the herb *Speranskia tuberculata* reduce the viability of human cancer cells of all investigated types. The extracts obtained using ethyl acetate are more effective in the fight against human cancer cells than PE extracts.

Analysis of the mass spectra of samples of both extracts allows one to compare their composition and determine the extract inequality. Both mass spectra were recorded using the same instrument under as close experimental conditions as possible. The total ion currents of both samples were quite close to each other. The amplitude of the maximum peaks of EA and PE extracts was about 10^6 arbitrary units (au). The most minor peaks in intensity vary at the level of 10^3 au. Since the composition of the compounds is entirely unknown, and at this stage, it is not clear which

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compounds will be of most significant interest, it is advisable to remove the peaks with the smallest amplitude from further analysis. The probability of misidentification of compounds grows with peak amplitude decay due to the increased influence of noise and masking smaller satellite peaks with interferences. Therefore, in the following, we consider only peaks with amplitudes exceeding 10^4 au, that is, approximately 1% of the amplitude of the maximum peak. Figure 2 illustrates the number of compounds with peaks of 10^4–10^6 au identified either in one of the preparations or both at once.

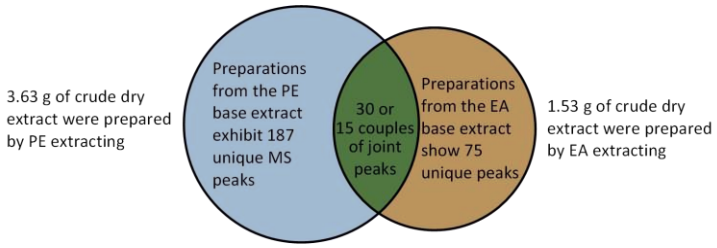

Fig. 2. The number of unique peaks with an amplitude of at least 10^4 au identified either in only one of the extracts or present in both extracts simultaneously

Even though samples of the same plant were studied, the recorded spectra were surprisingly different regarding the number of peaks and the identified compounds (Fig. 3).

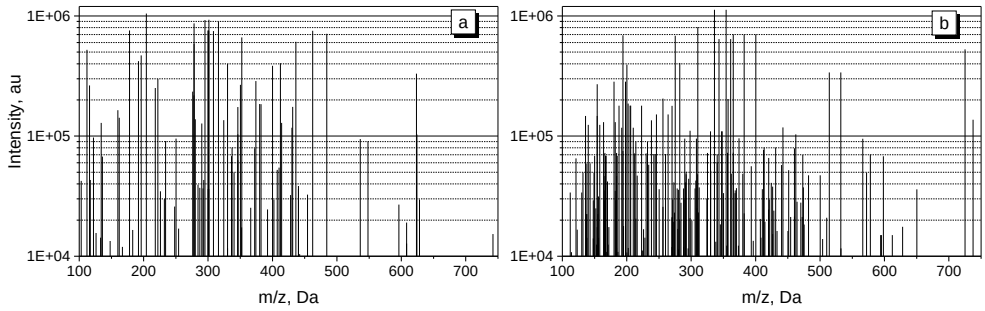

Fig. 3. Peaks of unique compounds with intensities 10^4-10^6 au as a function of m/z (Da) in *Speranskia tuberculata* preparations extracted with EA (a) and PE (b)

The number of unique peaks in the sample extracted using PE was almost 2.5 times more than in EA (187 versus 75 peaks). This observation correlates with PE extraction yielding approximately 3.63 g of crude dried extract from 100 g of herb powder, while EA extraction yields only 1.53 g (Fig. 2). At first glance, the relatively small number of compounds simultaneously present in both samples is unexpected. For peaks with 10^4-10^6 au, only 15 compounds present in both samples were identified; their peaks are shown in Fig. 4.

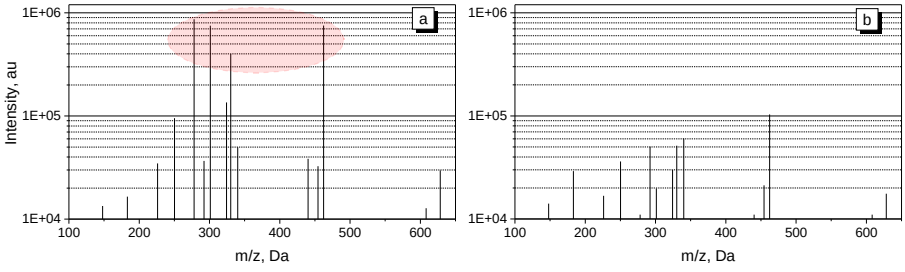

Fig. 4. Peaks of joint compounds with intensities 10^4-10^6 (au) as a function of m/z (Da) in *Speranskia tuberculata* preparations extracted with EA (a) and PE (b) crude extracts

Only 20 compounds were simultaneously found in both samples. Among them, the peaks' amplitudes significantly increased from PE to EA extract only in 10 cases. For others, the changes were either close to zero or negligible in favour of PE extracts. The compounds identified only in the EA extract sample with better medicinal

properties should be added to the compounds with intense peaks in both extracts.

It is advisable to start the analysis with the most intense peaks, which are easier to register and identify. Let us take 2×10^5 au as the lower threshold. Then, in the range of 2×10^5–1.1×10^6 au, there were detected 54 compounds. Among them, 26 compounds showed significant peaks in PE and minor peaks in EA extracts. For this reason, they are not attractive enough to be considered further. Among the other 28 compounds, 24 are characteristic of only EA preparations. Four more compounds sharply increased their concentration in EA but were also present in small amounts in PE. These compounds are the first candidates for ensuring the biological activity of preparations with anticancer properties.

As is known, *Speranskia tuberculata (Bunge) Baill* has still not been studied much. Therefore, there is virtually no information on the role of individual compounds in this herb, including 28 identified compounds with the most intense peaks. However, these compounds and their bioactivity were studied in other plans. Under such conditions, the available literature data should be analysed to specify possible clinical practice fields of 28 identified compounds. The most critical information is regarding anticancer activity, as it provides an opportunity for additional understanding of the results of the MTT tests.

Based on the literature data, 15 compounds, including 3 items detected in both extracts, showed anticancer and antitumor activity in various manifestations. Below is a list of compounds with their brief characteristics and information sources summarised in Table 1.

Tetrahydrobungeanool. The compound inhibited nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages, with an IC_{50} value of 27.1±1.15 [14].

Dibutyl phthalate (detected in both extracts). The compound is found in extracts of both types. It displays antibacterial and anticancer properties [15]. The peak intensity of this compound increased more than 98-fold in the EA-based extract compared to the PE extract.

Table 1

Unique and joint compounds with intense peaks (2×10^5–1.1×10^6 au) and expressed anticancer activity were found in preparations extracted with EA

Name	Superclass	Class	Formula	Height, au	Mass, Da
Tetrahydrobungeanool	Lipids and lipid-like molecules	Fatty Acyls	$C_{18}H_{33}NO_2$	923772	295.2511
Dibutyl phthalate	Benzenoid	Benzene and substituted derivatives	$C_{16}H_{22}O_4$	867979	278.1518
Alpha-Dichroine	Organoheterocyclic compounds	Diazanaphthalenes	$C_{16}H_{19}N_3O_3$	752043	301.1426
Magnolignan A	Benzenoid	Benzene and substituted derivatives	$C_{18}H_{20}O_4$	752043	300.1362
10-Gingediol	Benzenoid	Benzene and substituted derivatives	$C_{21}H_{36}O_4$	662337	352.2614
Ugonin B	Phenylpropanoids and polyketides	Flavonoids	$C_{26}H_{28}O_6$	609384	436.1886
Loliolide	Organoheterocyclic compounds	Benzofurans	$C_{11}H_{16}O_3$	470114	196.1099
6-Methoxy-7-hydroxycoumarin	Phenylpropanoids and polyketides	Coumarins and derivatives	$C_{10}H_8O_4$	420933	192.0423
L-(-)-alpha-Monopalmitin	Lipids and lipid-like molecules	Glycerolipids	$C_{19}H_{38}O_4$	403553	330.277
5,6-Dimethoxy-7-hydroxycoumarin	Phenylpropanoids and polyketides	Coumarins and derivatives	$C_{11}H_{10}O_5$	330762	222.0528
Uzarigenin	Lipids and lipid-like molecules	Steroids and steroid derivatives	$C_{23}H_{34}O_4$	299339	374.2457
Andrographolide	Organoheterocyclic compounds	Lactones	$C_{20}H_{30}O_5$	287520	350.2093
Levulinic acid	Organic acids and derivatives	Keto acids and derivatives	$C_5H_8O_3$	266790	116.0473
(-)-Guaia-1(10),11-dien-15-olide	Lipids and lipid-like molecules	Prenol lipids	$C_{15}H_{22}O$	263862	218.1671
Artecanin	Lipids and lipid-like molecules	Prenol lipids	$C_{15}H_{18}O_5$	234569	278.1154

Alpha-Dichroine (detected in both extracts). Alpha-dichroine, known as Febrifugine, effectively inhibits the proliferation growth of bladder cancer cells T24 and SW780; the IC_{50} was 0.02 and 0.018 μM, respectively. Febrifugine suppresses DNA synthesis and induces cell death by reducing steroidogenesis and promoting apoptosis [16]. It was found in both extracts, but the peak was 38 times more intense in the EA-based extract than in the PE.

Magnolignan A. The compound exhibits physiological activity against tumour cells [17].

10-Gingediol. It is the most potent among other gingerols, exhibiting antioxidant activity against superoxide and hydroxyl radicals [18]. It also shows anticancer activity [19].

Ugonin B. Ugonins showed potent melanin reduction, significant neuraminidase inhibition and inhibited lung cancer [20; 21].

Loliolide. It has anticancer, antibacterial, antifungal and antioxidant properties [22]. Plants with loliolide are also used in the treatment of diabetes and depression.

6-Methoxy-7-hydroxycoumarin. This compound, known as scopoletin, shows promise in antioxidant, antimicrobial, anticancer, anti-inflammation, and neuroprotective activities [23].

L-(-)-alpha-Monopalmitin (detected in both extracts). This compound (1-Mono) is found in extracts of both types. The peak intensity is 7.8 times greater in the EA extract than in the PE. It is a natural product in *Sciadopitys verticillata*, *Nelumbo nucifera*, and other organisms. 1-Mono significantly inhibited A549 and SPC-A1 cell proliferation, induced G2/M arrest and caspase-dependent apoptosis [24].

5,6-Dimethoxy-7-hydroxycoumarin. Coumarins have specific antiviral, antimicrobial, antioxidant, anti-inflammatory, antiadipogenic, cytotoxic, apoptosis, antitumor, antitubercular, and cytotoxicity properties [25].

Uzarigenin. Uzarigenin is a potential carbonic anhydrase II (CAII) activity inhibitor and exhibits antiproliferative activity [26].

Andrographolide. The antitumor activity of andrographolide is well-documented due to its reported antiproliferative and antiangiogenic properties. Andrographolide exerted its effects on several cancers like breast, cervical, lung, colon, liver, and prostate [27].

Levulinic acid. The methanolic extract from the *Citrus medica* leaves also showed anticancer activity against MCF7 breast cancer cell lines [28].

(-)-Guaia-1(10),11-dien-15-olide. This compound determined specific agarwood odours and exhibited antioxidant and anticancer properties against breast cancer (MCF-7) [29].

Artecanin. It is active in human tumour cell lines (A2780, A431, HELA and MCF7) [30].

The other 13 compounds were either promising for treating different diseases or information on their medical activity could not be found. Their main characteristics (name, chemical class, formula, mass, peak intensity and information sources are given in Table 2.

Discussion

A comparison of the obtained results allows one to draw a few conclusions. First, they testify to the complex nature of herbal medical preparations. In the most effective EA preparations, no less than 15 compounds claim the role of API, while synthetic drugs usually have only one API. It is still unclear which compounds are vital in forming medicinal properties. One can only assume that such compounds should be sought among those with sufficiently high concentrations, available in effective EA extracts, and are known for their bioactivity. First candidates are listed in Table 1. A sharp increase in peak intensity from PE to EA extracts observed in three compounds (dibutyl phthalate, alpha-dichroine, and L-(-)-alpha-monopalmitin) could be an additional argument for their importance.

Secondly, the mass spectrometric profiles of two preparations from the same plant are radically different. Only 4 compounds were registered simultaneously in both preparations, and their concentrations drastically increased from PE to EA extracts. All other compounds are unique to preparations based on either EA or PE. As the study showed, PE extraction more than doubled the total amount of substance in the dry extract but reduced the amount of extracted APIs. The apparent reason for this difference is the use of different solvents.

In general, the chemical composition of herbal preparations is susceptible to various factors, including plant origin [46]. To the best of the authors' knowledge, no generally accepted universal theory can establish an unambiguous relationship between the type of solvent and extracted compounds. Specific known facts are difficult to extend to other experimental conditions.

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Table 2

Unique and joint compounds with intense peaks (2×10⁵–1.1×10⁶ au) which were found in EA preparations and showed no anticancer activity

Name	Superclass	Formula	Height, au	Mass, Da	Ref
(Z)-5-Hydroxy-3-butylidene-phthalide	Benzenoid	$C_{12}H_{12}O_3$	1047841	204.0786	[31]
(E,E,E)-N-(2-Methylpropyl)-hexadeca-2,6,8-trien-10-ynamide	Lipids and lipid-like molecules	$C_{20}H_{31}NO$	930408	301.2406	[32]
16-Carboxytotarol	Lipids and lipid-like molecules	$C_{20}H_{28}O_3$	897243	316.2038	[33]
Abieta-8,12-dien-11,14-dione	Lipids and lipid-like molecules	$C_{20}H_{28}O_2$	758604	300.2089	[34]
1-Allyl-2,4-dimethoxybenzene	Benzenoid	$C_{11}H_{14}O_2$	756989	178.0994	[35]
Irisoquin F	Organic oxygen compounds	$C_{29}H_{50}O_4$	753111	462.3709	[36; 37]
Linoleyl acetate	Lipids and lipid-like molecules	$C_{20}H_{36}O_2$	749170	308.2715	[38]
Dehydroxy-15-O-methylcimigenol	Lipids and lipid-like molecules	$C_{31}H_{48}O_4$	709758	484.3553	[39]
(Z,Z,Z)-9,12,15-Octadecatrienoic acid	Lipids and lipid-like molecules	$C_{18}H_{30}O_2$	587819	278.2246	[40; 41]
2-4'-Imidazolylethanol	Organoheterocyclic compounds	$C_5H_8N_2O$	522680	112.0637	[42]
Aschantin	Lignans, neolignans and related items	$C_{22}H_{24}O_7$	399790	400.1522	[43]
Pingbeidinoside	Lipids and lipid-like molecules	$C_{34}H_{57}NO_9$	384980	623.4033	[44]
(S)-Murpanidin	Phenylpropanoids and polyketides	$C_{15}H_{16}O_5$	251700	276.0998	[45]

For example, the effect of solvent polarity on extraction is known. However, in general, the impact of polarity on extraction is complex. It depends on both extraction conditions and extracted compounds. The degree of interaction of EA with various polar solutions is significantly higher (polarity index is 4.4) than PE (0.1) [47]. The extraction of flavonoids and phenolic compounds decreased with the solvent's increasing polarity [48].

In contrast, the total extraction yield of antioxidant reagents and free radical scavenging activity increased. Similar findings were reported when studying *Thymelaea hirsuta (L.) Endl*. In [49], polar solvents' suitability was suggested for extracting phytochemical compounds from *T. hirsuta* areal parts and their antioxidant activity against several radicals and ions. The lipids yield extracted by polar solvents were higher than non-polar ones [50]. The polarity of solvents also affected the composition of fatty acid methyl esters. Under such conditions, experiments to optimise solvents and extraction conditions become a priority for further research.

Figure 5 qualitatively illustrates the difference between the actions of solvents. The range of the studied masses was divided into segments with a step of 50 Da. For each range, the total ion current from all peaks was calculated and plotted as a function of ranges. The maximum total peak for EA extracts falls in the range of 300–350 Da, and for PE extracts – 350–400 Da.

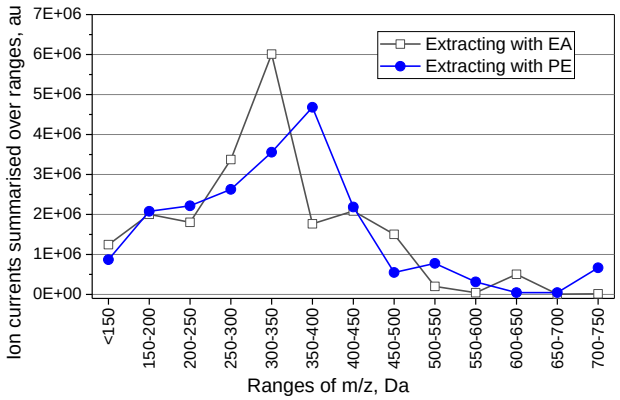

Fig. 5. The total intensity of all peaks in each 50 m/z range as a function of m/z (Da) in *Speranskia tuberculata* preparations extracted with EA and PE

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The picture is simplified by dividing all 54 compounds with the most intense peaks into the chemical superclasses they belong to. The classification of detected compounds using the approach and software from work [51] is shown in Table 3.

Table 3

The numbers of compounds by various chemical classes exhibiting peaks with an intensity of more than 2×10^5 au in multiple extracts

Chemical superclasses	Numbers in EA extracts	Numbers in PE extracts	Numbers in both EA and PE extracts
Lipids and lipid-like molecules	14	4	1
Benzenoid	4	5	1
Phenylpropanoids and polyketides	4	3	
Organoheterocyclic compounds	3	6	1
Lignans, neolignans and related compounds	2	1	
Organic acids and derivatives	1		
Alkaloids and derivatives		2	
Organic oxygen compounds		1	1
Total	**28**	**22**	**4**

Generally, the number of different superclasses is higher in the PE extracts than in the EA - 7 against 6. Among 22 compounds found in PE extracts, only 3 of 7 classes dominate over others: organoheterocyclic compounds, benzenoids, and lipids and lipid-like molecules, amounting to 68 %. Three leading superclasses in EA extracts, lipids and lipid-like molecules, benzenoids, and phenylpropanoids and polyketides, cover almost 79 % of the total.

Table 1 lists 15 compounds with the most intense peaks recorded in EA extracts (including 3 compounds present in large quantities in extracts of both types). According to literature data, all these compounds showed anticancer activity in other plants. When classifying into superclasses, 4 compounds out of 15 belong to the share of lipids; another 3 items belong to phenylpropanoids and polyketides. A total of 5 superclasses are mentioned.

A more detailed classification (Table 4) significantly clarifies compounds' belongingness. Thus, 15 registered compounds belong to 11 classes and, simultaneously, to 14 subclasses (Table 4). All this testifies to the broad nomenclature of the discovered bioactive compounds and the absence of pronounced preferences favouring a particular class or subclass.

Table 4

The number of compounds with intense peaks (> 2×10^5 au) and anticancer properties identified in EA and both EA and PE extracts are classified by superclasses, classes and subclasses according to [51]

Supeкclasses	Classes	Subclasses	EA	PE-EA
Benzenoid	Benzene and derivatives	Benzoic acids and derivatives		1
		Biphenyls and derivatives	1	
		Methoxybenzenes	1	
Lipids and lipid-like molecules	Fatty Acyls	Fatty amides	1	
	Glycerolipids	Monoradylglycerols		1
	Prenol lipids	Sesquiterpenoids	1	
		Terpene lactones	1	
	Steroids and steroid derivatives	Steroid lactones	1	
Organic acids and derivatives	Keto acids and derivatives	Gamma-keto acids and derivatives	1	
Organoheterocyclic compounds	Benzofurans	No available	1	
	Diazanaphthalenes	Benzodiazines		1
	Lactones	Gamma butyrolactones	1	
Phenylpropanoids and polyketides	Coumarins and derivatives	Hydroxycoumarins	2	
	Flavonoids	Flavones	1	
Total	11	14	12	3

One may preliminarily suppose that the properties of the studied plant are determined not by the action of one potent component but rather by the synergistic effect of many substances. The data denote that up to 15 compounds can form the value of *Speranskia tuberculata* as an anticancer remedy. In any case, further research is needed to get clearer and more accurate conclusions.

Conclusions

The influence of the concentration of the medicinal plant *Speranskia tuberculata (Bunge) Baill* in the 12.5–75 μg/mL range on the viability of cancer cells of 4 different lines was studied. Two preparations were obtained by extracting crude extracts with petroleum ether or ethyl acetate. Values of half-maximum inhibitory concentration IC_{50} in MTT tests were determined. In both extracts, cell viability known as A549, A375, HELA and HEPG2 significantly decreased with increasing concentration.

The most significant effect of *Speranskia tuberculata* preparations, revealed during the experiment, was observed for HELA cells and the least – for A375. The magnitude of the effect of ethyl acetate-based preparations is significantly superior to that of petroleum ether-based preparations. In particular, the IC_{50} value for the ethyl acetate extracts was relatively stable at 49-53 μg/mL. For petroleum ether, the IC_{50} gradually increased from 65 to 74 μg/mL, from HELA to A549. The IC_{50} for A375 was outside the concentration range studied and estimated at 114 μg/mL.

About 100 and 200 compounds were identified by LC/MS in extracts based on EA and PE. Special attention was paid to the first 28 compounds in EA extracts with the most intense peaks (>2×10^5 au). Of them, 24 compounds are observed only in EA extracts. The other 4 (alpha-dichroine, dibutyl phthalate**,** L-(-)-alpha-monopalmitin and irisoquin F) were found in both preparations, with the first three having anticancer activity. In general, the analysis of literature data allowed one to identify 15 compounds with high biological activity against cancer cells of various origins. The other 13 compounds did not exhibit such properties.

Extraction using different solvents significantly changed the chemical composition of the obtained extracts. For the compounds with the most intense peaks (> 2×10^5 au), three superclasses, organoheterocyclic compounds, benzenoids, and lipids and lipid-like molecules, dominate PE extracts. They account for 68 % of all identified compounds (15 out of 22). Lipids and lipid-like molecules, benzenoids, phenylpropanoids and polyketides play similar roles in EA extracts - together, 22 among 28 compounds (almost 79 %). The results of the MTT tests suggest that despite PE extracting a greater total mass of substances from the herb, the solvent EA was more effective concerning the extraction of API.

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