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Antioxidant and antiproliferative activity of *Juniperus* L. species of Bulgarian and foreign origin and their anticancer metabolite identification

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The genus *Juniperus* L. (Cupressaceae) comprises more than 50 species in the world. Widely distributed junipers are evergreen plants that are easy for cultivation, produce a considerable amount of biomass all the year round and are rich of biologically active compounds, including antioxidants and anticancer substances. This study is pointed out at a systematic investigation of Bulgarian *Juniperus* species in comparison with foreign representatives with the aim to select these having high antiproliferative and antioxidant activity. In the group of studied plant extracts, the best antiproliferative activity in NB4 acute promyelocytic leukemia cells was determined for *J. sabina* L., *J. virginiana* L. and *J. virginiana* 'Grey Owl' extracts. The best antioxidant activity was exhibited by *J. sibirica* and *J. excelsa* leaves extracts in the group of analyzed species. While efficient antioxidant activity is desired for cancer prevention, efficient antiproliferative agents are required in the anticancer chemotherapy. Using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS) we identified podophyllotoxin, a known anticancer drug precursor, in selected juniper extracts with antiproliferative activity. Identification of other plant metabolites (lignans, phenolic compounds etc.) is in progress. The identification of juniper extracts with efficient antiproliferative and antioxidant activity has potential application in clinical trials about combination therapy with other anticancer agents for treatment of resistance to conventional chemotherapy, disease relapse and reduction of the therapeutical doses of cytostatic drugs.

Keywords: *Juniperus* L., Antiproliferative activity, Antioxidant activity, Podophyllotoxin, HPLC/HRMS analysis

INTRODUCTION

Junipers are exquisite evergreen plants that appear in the nature as magnificent trees or small shrubs with trailing branches. The genus *Juniperus* L. (Cupressaceae) includes more than 50 juniper species, widely spread throughout the world [1]. Juniper representatives are currently under investigation as alternative sources of podophyllotoxin derivatives, known as precurors of antiviral and anticancer agents (etoposide, teniposide etc.) [2]. Podophyllotoxin is an aryltetralin lignan, currently isolated from the *Podophyllum peltatum* L. and *Podophyllum hexandrum* Royle, which are considered already as endangered species because of their intensive industrial exploitation and difficult cultivation. On the other side, junipers are widely distributed evergreen plants, easy for cultivation and producing big amount of biomass all the year.

A great diversity of cytotoxic compounds (podophyllotoxin derivatives, thuriferic acid, savinin, yatein, isocupressic acid, communic acid, arctiol; widdrol, etc. [3]) were identified in *J. communis* L., *J. chinensis* L., *J. sabina* L., *J. excelsa* M. Bieb., *J. taxifolia* Hook. et Arn., *J. brevifolia* (Seub.) Antoine, *J. phoenicea* L., *J. thurifera* L. etc. [4]. In addition, plenty of polyphenol compounds (quercetin, rutin, apigenin, luteolin, amentoflavone, chlorogenic acid, tannins, catechin, proanthocyanidin, etc.) with antioxidant properties were also detected in juniper extracts [5]. Efficient antioxidant activity in correlation with high total polyphenol content was found for extracts of *J. excelsa, J. sibirica, J. communis, J. sabina* and other juniper species of different origin [6]. Extracts with antioxidant properties were proposed to enhance the therapeutic potential of cytotoxic drugs (Vincristine) in combination with *J. excelsa* extract by treatment of leukemia cells [7]. Combination of cytostatic agents with antioxidants in future clinical trials is aimed at reduction of the therapeutic doses and side effects of the cytostatic drugs.

Cancer is a life-threatening disease, involving abnormal division of genetically modified malignant cells with potential to metastasise in

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different organs of the living beings. Activation of oncogenes is associated with complicated, including genetically inheritable, factors. In this respect, conversion of normal cells into cancer cells is often an oxidative-stress associated process, involving carcinogenic action of free radicals, produced by many metabolic pathways or by environmental pollution substances, penetrating through the living tissues.

We targeted our study at determination of the growth inhibitory activity of juniper extracts on NB4 APL t(15;17) (acute promyelocytic leukemia) cell line. APL t(15;17) is characterized by a balanced reciprocal chromosomal translocation that fuses the retinoic acid receptor alpha gene (RARα) with the promyelocytic leukemia gene (PML), leading to formation of a fusion oncogene. After therapy with all-*trans*-retinoic acid (ATRA) and anthracycline antibiotics, up to 80-90% of the PML-RARα positive patients achieve remission. However, about one quarter of the patients relapse, become resistant to ATRA and/or develop a life-threatening retinoic acid syndrome (fever, dyspnea, pulmonary infiltrates etc.).

Hence, in response to demands of the clinical practice in identification of new therapeutic agents for prevention and therapy of cancer we focused our research on the first systematic investigation of the antioxidant and antiproliferative properties of the extracts of Bulgarian juniper species in comparison with foreign juniper representatives.

EXPERIMENTAL

Materials

Chemicals and reagents: Podophyllotoxin, DPPH (2,2-diphenyl-1-picrylhydrazyl), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium bromide], Folin-Ciocalteu's reagent (2N), gallic acid, formic acid, RPMI 1640 medium were purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA). Fetal calf serum for cell culture was delivered from Biochrom GmbH (Berlin, Germany), DMSO was from Fluka Chemie AG (Buchs, Switzerland). LC grade ultra-pure water was prepared by a Millipore Direct-Q3 system (Bedford, MA, USA). LC-MS grade solvents were purchased from Ficher Scientific (USA) and Sigma-Aldrich (USA).

Plant material: J. virginiana 'Grey Owl' was received from the Arnold Arboretum, Harvard University, USA (specimen №00175599, accession №1136-61*A, 15.06.2017). *J. communis* L. was from the village Ognyanovo, Blagoevgrad Province, Rhodope Mountains (41°37'47.3" N; 23°47'14.5" E, 700 m a.s.l., 11.04.2017, SOM 174400); *J. sibirica* Burgsd. - from the Vitosha mountain, on the outskirts of Sofia (42°34'59.6" N; 23°17'28.6" E, 1803 m a.s.l., 13.04.2017, SOM 174401); *J. pigmaea* C. Koch - from the Smolyan Province, Mursalitsa region of the Rhodope Mountains (41°38'40.8" N; 24°29'58.5" E, 1898 m a.s.l., 13.05.2017, SOM 174402); *J. deltoides* R. P. Adams - from the village Ognyanovo, Blagoevgrad Province, Rhodope Mountains (41°37'46.6" N; 23°47'15.4" E, 695 m a.s.l., 11.04.2017, SOM 174403); *J. excelsa* M. Bieb. - from the reserve Tisata, on the riverside of Struma (41°44'01.6" N; 23°09'22.5" E, 199 m a.s.l., 09.04.2017, SOM 174404). *J. sabina* L. was collected from the resort Borovets, Sofia Province, Rila mountain (42°14'19.8" N; 23°32'33.6" E, 1182 m a.s.l., 14.05.2017, SOM 174405); *J. virginiana* L. was collected from the University of Forestry Arboretum, Sofia (42°39'08.7" N; 23°21'30.1" E, 612 m a.s.l., 13.04.2017, SOM 174406). Voucher specimens were deposited in the Herbarium of Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. *Juniperus* species were authenticated by A. Tashev (University of Forestry, Sofia) and according to R. P. Adams [1].

Extraction procedure

Fresh plant material was stored in a freezer (at -20ºC) in vacuum plastic bags until extraction. Then, the plant material (5 g) was ground and 80% (v/v) methanol (50 ml) was added. The suspension was placed in an Erlenmeyer flask with stopper and was stirred for 1.5 h in a shaker water bath at 20ºC (ambient temperature). The mixture was filtered and the extract was collected. The remaining solid material was subjected to a second extraction for 1.5 h with a new portion of 80% methanol (50 ml). After filtration, the solid mass was stirred again for 1.5 h in 80% methanol (25 ml). The combined extracts were concentrated by a vacuum evaporator. The remaining residue was freeze-dried (24 h, -50ºC, 0.2 mbar) and kept at -20ºC until analyses**.**

Folin-Ciocalteu method for determination of total phenol content

The total polyphenol content (TPC) of the corresponding juniper extract was determined by Folin-Ciocalteu method with minor modifications [8]. In brief, 20 µl of the extract [5 mg/ml in 80% (v/v) methanol] were mixed with distilled water (1.58 ml) and FC-reagent (100 µl) was added. The control sample contained the same reagents but without plant extract. After 3-5 min 300 µl of sodium carbonate (20% w/v) were added and the samples were kept at room temperature for 2 h. The

absorbance at 765 nm was registered on a spectrophotometer.

The calibration curve was obtained using gallic acid standard. The TPC of the extracts was expressed in GAE (Gallic Acid Equivalents) according to the formula: $C = c.V/m$,

where C is concentration of phenolic compounds in mg GAE per gram dry extract; c – gallic acid concentration [mg/ml] from the calibration curve; m – weight of plant extract [g]; V – volume of plant extract [ml]. The TPC of each extract was determined by 2 independent analyses and was given as an average value ± SD.

DPPH radical scavenging method for determination of antioxidant activity

The radical scavenging activity of the extracts was determined by the DPPH-method [9]. Half-maximum DPPH-scavenging concentration (SC_{50}) of the corresponding plant extract was determined as concentration of the extract in the test sample that decreased the DPPH-concentration by 50%. Briefly, stock solutions (10 mg/ml) of the corresponding plant extract in 80% (v/v) methanol were prepared by ultrasonication (2×5 min, 55ºC). Then, 1 ml of the extract (at different concentrations) was mixed with 4 ml of DPPH solution (0.004% w/v) in a test tube. The control sample was prepared with the same reagents but without plant extract. The blank sample contained 80% (v/v) methanol. The solutions were kept at room temperature for 1 h in the dark and then the absorption at 517 nm was measured on a spectrophotometer. The percentage of the DPPH-inhibition was calculated according to the formula:

$$\%\, inhibition = [(Ac - As)/Ac]x100,$$

where A_c is the absorbance of DPPH solution in the control sample without extract and A_s is the absorbance of DPPH in sample containing the corresponding plant extract.

A *UV-1600PC* spectrophotometer (*VWR int.*) was used for Folin-Ciocalteu and DPPH-assays.

Cell culture and MTT-test for determination of antiproliferative activity of plant extracts

NB-4 cells (DSMZ, Germany) were cultured in a humidified incubator (37ºC, 5% CO_2) in RPMI-1640 medium, supplemented with 10% fetal calf serum, glutamine (2 mM) and HEPES buffer (25 mM). Stock solutions of freeze-dried extracts (10 mg/ml in DMSO) were diluted with RPMI-1640 to obtain the desired concentrations. The solvent in the medium was less than 0.5% (v/v). Cells (3×10^5 cells/ml) were seeded into 96-well plates (100 µl/well) and were exposed to various extract concentrations for 72 h. Cell proliferation was determined by MTT-assay as it was described in the literature [10]. MTT-tests were carried out using a microplate reader (Labexim LMR1s).

Data processing and statistics

The MTT-assays were carried out in at least 4 separate experiments. The MTT data were fitted to sigmoidal concentration–response curves and the IC_{50} values were calculated using non-linear regression analysis (GraphPad Prism software). Statistical processing exploited Student's t-test with $p \leq 0.05$ set as the lowest level of statistical significance.

LC-ESI-MS/MS and UHPLC/HRMS for podophyllotoxin identification:

For liquid chromatography - electrospray ionization mass spectrometry analyses (LC-ESI-MS/MS) an Agilent 1200 HPLC system (USA) was connected to 3200 QTRAP Mass spectrometer (AB Sciex, USA). The QTRAP-MS system operated at positive-ion mode, capillary temperature 500°C and source voltage 4.5 kV. Nitrogen was used as curtain and collision gas. The optimum conditions of Multiple Reaction Mode monitoring were determined in the infusion mode. Eclipse XDB-C18 column (4.6 × 50 mm, 1.8 µm particle size; Agilent Technologies, USA) was maintained at 25°C. Mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile with 0.1% formic acid) were used. The gradient program was as follows: 0-0.5 min 35% B, then a linear ramp to 53% B to 1 min, 1-4 min 53% B, next a linear ramp to 90% B to 5 min, and a hold at 90% B until 6 min. The total run time was 9 min, including 2 min equilibration. The injection volume was 5 µL with a mobile phase flow rate of 450 µL/min. Data acquisition and processing were carried out by *Analyst 1.5* software (AB Sciex, USA). Triplicate injections were made for each standard solution and sample. Prior to injection, samples were subjected to solid-phase purification by Sep-Pak C18 Cartridges (Waters, Ireland), prepared for sample loading using 60% (v/v) acetonitrile.

Ultra-high performance liquid chromatography analyses in tandem with high resolution mass spectrometry (UHPLC/HRMS) were performed on a Thermo Scientific Dionex Ultimate 3000 RSLC system (Germany), coupled to Thermo Scientific Q Exactive Plus mass spectrometer (Bremen, Germany) with heated electrospray probe HESI-II. The instrument operated at spray voltage 3.5 kV, while the ion transfer tube and HESI-II vaporizer temperatures were set at 320°C. Data acquisition

and processing were done using *Thermo Scientific Xcalibur* 3.0 software. A AkzoNobel Kromasil Externity XT-1.8-C18 (Bohus, Sweden) narrow-bore column (2.1×100 mm, 1.8 μm) with Phenomenex Security Guard ULTRA UHPLC EVO C18 (Torrance, USA) was used and maintained at 40°C. The mobile phase consisted of systems A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The following gradient was used: the mobile phase was held at 5% B for 0.5 min, gradually turned to 60% B over 22.5 min, kept at 60% B for 2 min, followed by a gradual increase to 85% B over 2.5 min, kept at 85% B over 2 min and the system was turned to the initial condition of 5% B in 0.5 min. The system was conditioned at 5% B for 4.5 min before injection. The flow rate and injection volume were 300 μL/min and 2 μL, resp.

RESULTS AND DISCUSSION

Selection of Juniperus species with best antiproliferative activity

In a preliminary screening we determined that *Juniperus virgiana* 'Grey Owl' extract showed best antiproliferative activity after treatment of a panel of cancer cells [11] with different medicinal plant extracts [12]. Thus, we directed our research at a first systematic analysis of the antiproliferative properties of extracts obtained from *Juniperus* species of Bulgarian origin in comparison with extracts of foreign juniper representatives. Considering the pharmaceutical interest in identification of plant extracts for prevention of the living cells from the carcinogenic action of free radicals, we analyzed also the antioxidant activity of the juniper extracts.

Several *Juniperus* species are distributed in Bulgaria [13]: *J. communis* L., *J. deltoides* R. P. Adams, *J. excelsa* M. Bieb., *J. sabina* L., *J. pigmaea* K. Koch, *J. sibirica* Burgsd., as well as junipers, native to North America, such as *J. virginiana* L. etc.

The study of the antiproliferative activity of extracts of junipers of Bulgarian and foreign origin was focused on NB4 APL cell line, bearing t(15;17) PML-RARA fusion oncogene. Efficient anticancer extracts provide a potential alternative treatment of APL in cases of resistance to conventional therapy, life-threatening ATRA-syndrome and disease relapse. The analysis of the dose-response curves by the MTT- assay revealed that all studied juniper extracts exhibited antiproliferative properties on NB4 cells, however *J. sabina*, *J. virginiana* and *J. virginiana* 'Grey Owl' extracts were selected as the best antiproliferative agents (Table 1).

Selection of Juniperus species with best antioxidant activity

Considering the interest in identification of plant extracts for prevention of the living cells from the carcinogenic action of free radicals, we analyzed also the antioxidant activity of the studied juniper extracts in correspondence with their total polyphenol content (TPC). The DPPH-radical scavenging activity of the extracts was evaluated by their SC_{50} value, calculated as concentration of the extract that decreased the initial DPPH concentration by 50%. Hence, lower SC_{50} values denote higher DPPH-radical scavenging activity. In general, the leaves extracts demonstrated better TPC and DPPH-SC_{50} values in comparison with the galbula extracts (with the exception of the *J. sabina* extract, where both galbula and leaves extracts exhibited similar antioxidant properties). Best antioxidant activity was determined for leaves extracts of *J. sibirica* and *J. excelsa* that corresponded to their highest total polyphenol content values (Table 1).

However, the different mechanism of action of the antioxidant and antiproliferative compounds does not point at a correlation between both activities. This conclusion was confirmed by our study of other plant extracts in order to compare their antioxidant and anticancer properties.

In this connection, we registered a low antiproliferative activity (IC_{50} 131±26 μg/ml on NB4 cells) of an efficient antioxidant *Rhodiola rosea* L. rhizome extract with high TPC value (374±17 mg GAE/g dry extract). *Rh. rosea* extract contained 0.8% of salidroside and 2.2% of rosavin as antioxidant compounds. By contrast, *I. helenium* root extract exhibited low TPC value (21±1 mg GAE/g dry extract), however it possessed efficient anticancer activity (IC_{50} 5±1 μg/ml on NB4 cells). Anticancer compounds alantolactone and isoalantolactone were identified in the *I. helenium* extract. In the case of juniper extracts, their antioxidant properties are feasible to render a preventive effect on the healthy tissues during intense chemotherapies.

Podophyllotoxin identification by LC-ESI-MS/MS and UHPLC/HRMS

Podophyllotoxin (PPT) acts as a suppressor of the mitotic-spindle microtubule assembly [14]. However, its derivatives (etoposide, teniposide) were supposed to have different mechanisms of action (inhibition of DNA topoisomerase II, DNA unwinding and replication), while other PPT derivatives have as yet unknown mechanisms of action [15].

Table 1. Comparison of the total polyphenol content (TPC), DPPH-radical scavenging (SC_{50}) and NB4-growth inhibitory half-maximum (IC_{50}) concentrations of the studied juniper extracts.

№	Name of *Juniperus* species	TPC [GAE mg/g]	DPPH-SC_{50} [μg/ml]	NB4-IC_{50} [μg/ml]
1A	*J. sabina* L.	79±1	394	0.5 ± 0.2
1B	*J. sabina* L.	87±2	246	0.5 ± 0.0
2A	*J. virginiana* L.	55±1	561	0.5 ± 0.1
3A	*J. virginiana* 'Grey Owl'	97±0	251	0.7 ± 0.1
3B	*J. virginiana* 'Grey Owl'	45±0.2	352	0.5 ± 0.2
4A	*J. communis* L.	132±4	154	1 ± 0.4
4B	*J. communis* L.	90±2	207	4 ± 2
5A	*J. sibirica* Burgsd.	182±18	104	3 ± 1
5B	*J. sibirica* Burgsd.	68±0.4	530	15 ± 3
6A	*J. pigmaea* K. Koch	138±4	140	5 ± 1
6B	*J. pigmaea* K. Koch	113±6	145	29 ± 7
7A	*J. deltoides* R. P. Adams	135±7	154	66 ± 8
7B	*J. deltoides* R. P. Adams	47±4	411	70 ± 5
8A	*J. excelsa* M. Bieb.	169±7	103	137 ± 12
8B	*J. excelsa* M. Bieb.	119±9	152	188 ± 55

Abbreviations: TPC - total polyphenol content in milligrams Gallic acid equivalents per gram dry extract; DPPH-SC_{50} – half-maximum DPPH-scavenging concentration of the plant extracts; NB4-IC_{50} – half-maximum growth-inhibitory concentration of the extracts on NB4 cells. MTT-test positive control: podophyllotoxin with NB4-IC_{50} 0.005±0.001 μg/ml. IC_{50} and SC_{50} values were given in micrograms dry extract per milliliter of solvent. Lower IC_{50} and SC_{50} values denote higher activity. All experiments were performed using leaves (A) and galbula (B) extracts;

Podophyllotoxin identification in *J. sabina*, *J. virginiana* and *J. virginiana* 'Grey Owl' extracts with best antiproliferative activities was performed with both LC-ESI-MS/MS and UHPLC/HRMS methods. This anticancer plant metabolite was identified by its protonated molecular ion at 415 $[M+H]^+$ *m/z* and fragment ions at 397 *m/z* and 247 *m/z* (mass-to-charge ratios), comparing their retention times (RT) and transitions with the corresponding values and transitions of the PPT standard tested under the same conditions (Table 2).

Table 2. LC-ESI-MS/MS parameters for podophyllotoxin identification.

Compound	RT (min)	Mol. weight	$[M+H]^+$ (*m/z*)	Fragment ions (*m/z*)	Collision energy (eV)
Podophyllotoxin	3.60	414	415	397	-46
				247	-60

Abbreviations: RT - retention time, *m/z* – mass-to-charge ratio, M- molecular mass.

Using UHPLC/HRMS we detected the exact mass of the protonated molecular ion of PPT at 415.1385 *m/z* (calculated for $C_{22}H_{23}O_8$ 415.1387 *m/z*). Identification of other anticancer metabolites in the studied extracts is in progress.

CONCLUSION

To our knowledge, we performed the first systematic investigation of the antiproliferative and antioxidant properties of total extracts of *Juniperus* species of Bulgarian origin and compared their activity with extracts of foreign juniper representatives. While efficient antioxidant activity is desired in the prevention of cancer, the powerful antiproliferative agents are required in the anticancer chemotherapy. We determined that in the group of studied plant species the leaves extracts of *J. sibirica* and *J. excelsa* exhibited best antioxidant activity in accordance with their best TPC values (Table 1). However, efficient antioxidants might not have high antiproliferative activity, while weak antioxidants might possess excellent antiproliferative properties due to the different mechanisms of both actions. Our study pointed out that most juniper representatives exhibited antiproliferative activity, however in the

group of studied junipers the extracts of *J. sabina*, *J. virginiana* and *J. virginiana* 'Grey Owl' were selected as the best antiproliferative extracts on NB4 cells (Table 1). These species possessed also antioxidant activity that is perspective in studies of prevention of the healthy cells from the harmful cytostatic drugs during anticancer chemotherapy.

Using liquid chromatography in tandem with electrospray ionization-mass spectrometry (LC-ESI-MS) we identified podophyllotoxin in *J. sabina*, *J. virginiana* and *J. virginiana* 'Grey Owl' extracts with best antiproliferative activities.

Our study set the pattern for further identification of other plant metabolites (lignans or polyphenolic compounds) in the analyzed juniper extracts, as well as it pointed out at a future comparative bioactivity-guided study of *Juniperus* species distributed in different habitats.

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12. *Inula helenium* L., *Betula pendula* Roth., *Buxus sempervirens* L., *Ruta graveolens* L., *Inula britannica* L., *Juniperus virginiana* L. 'Grey Owl', *Rhodiola rosea* L., *Clinopodium vulgare* L., *Achillea collina* (Becker ex. Rchb.f.) Heimerl, *Achillea thracica* Velen., *Sideritis scardica* Griseb., *Paeonia peregrina* Mill., *Thalictrum aquilegifolium* L., *Ononis arvensis* L., *Tribulus terrestris* L., *Smilax excelsa* L., *Physalis alkekengi* L., *Marrubium peregrinum* L., *Eryngium campestre* L., *Anemone pavonina* Lam., *Stachys recta* L., *Centaurea trinervia* Willd, *Centaurea finazzeri* Adamovic, *Cynara cardunculus var. scolimus* L. etc.
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АНТИОКСИДАНТНА И АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ НА ВИДОВЕ ОТ РОД *JUNIPERUS* L. ОТ БЪЛГАРСКИ И ЧУЖДОЗЕМЕН ПРОИЗХОД И ИДЕНТИФИКАЦИЯ НА ТЕХНИ АНТИТУМОРНИ МЕТАБОЛИТИ

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(Резюме)

Род *Juniperus* L. (Cupressaceae) включва над 50 вида хвойна. Широко разпространените видове хвойна са вечнозелени растения, лесни за култивиране, които целогодишно произвеждат значително количество биомаса, богата на биологично-активни съединения, включително антиоксиданти и потенциални антитуморни вещества. В настоящето изследване е проведен систематичен сравнителен анализ на български видове хвойна по отношение на чуждоземни представители от този род с цел да се изберат тези, които притежават висока антипролиферативна и антиоксидантна активност. В групата на изследваните растителни екстракти най-висока антипролиферативна активност при NB4 APL t(15;17) клетки (остра промиелоцитна левквмия) е установена за екстракти на *J. sabina* L., *J. virginiana*' L. и *J. virginiana* 'Grey Owl'. Най-висока антиоксидантна активност в групата на изследваните видове проявяват екстрактите от листа на *J. sibirica* и *J. excelsa.* Ефективната антиоксидантна активност е необходима за превенция на рака, докато ефективни антипролиферативни вещества се изискват при хемотерапията на рака. Чрез метода на течна хроматография в тандем с масспектрометрия (LC-ESI-MS/MS) в екстрактите от хвойна с антипролиферативна активност е идентифициран подофилотоксин, който е известен прекурсор за синтез на антитуморни лекарства. Предстои идентификация и на други растителни метаболити (лигнани, фенолни съединения и др.) във видове *Juniperus* с различен произход. Идентифицирането на екстракти от хвойна с ефективна антиоксидантна и антипролиферативна активност има потенциално приложение при бъдещи клинични изследвания относно комбинирана терапия с други антитуморни средства в случаи на резистентност към конвенционалната химиотерапевтика, рецидиви на онкологични заболявания, както и за понижаване на терапевтичните дози на цитостатици при лечение на рака.