Phenolic Composition, Antioxidant, Antimicrobial and Cytotoxic Activites of *Allium porrum* L. (Serbia) Extracts

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Abstract In the present study, we investigated the phenolic composition, antioxidant, antimicrobial and cytotoxic activities of ethanolic extracts from leaves and stems of leek (*Allium porrum* L.), which are growing in Central Serbia (Balkan) and is used in everyday human food. The standard spectroscopic methods were applied to determine total phenols and flavonoids, where present phenolic compounds were estimated by HPLC analysis. The results indicated that the highest content of total phenols and flavonoids were found in total *Allium porrum* plant (93.34 and 25.14 mg/g). The major phenolic compounds were: rosmarinic acid (33.26%), quercetin (33.48%) and rutin (22.32%) in *Allium porrum* L. stem extracts and quercetin (33.59%) and apigenin (17.56%) in *Allium porrum* L. leaf extracts. The total antioxidant (128.01 µgAA/g) and DPPH radical scavenging, IC50 (10.28 µg/mL) activities of total plant *Allium porrum* L. were in correlation with their phenolic and flavonoid contents. The ethanolic extracts of the *Allium porrum* L. showed a favourable antimicrobial activity against eight gram-positive and gram-negative pathogenic bacteria and fungi, especially against *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger*. The cytotoxic studies indicate the *Allium porrum* L. extracts inhibit Hep2c, L2OB and RD cells in a dose dependent manner. The IC50 values of *Allium porrum* L. extracts were 27.18 ± 0.88 and 25.89 ± 0.67 µg/mL against Hep2c and L2OB cells, correspondently and 76.95 ± 11.45 µg/mL against RD tumor cell after 48 h treatment period. These present results confirmed that *Allium porrum* L. possesses potentially important beneficial properties for human health.

Keywords: *Allium porrum* L., phenolic compounds, antioxidant, antimicrobial, cytotoxic activity


1. Introduction

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity [1,2]. The screening of plant species to identify new antioxidants for health improvement have become very important in recent years [3,4,5,6]. Antioxidants are defined as compounds present at low concentration compared to the oxidizable substrate that can significantly delay or prevent oxidation of that substrate [7]. Phytochemical components, especially polyphenols are known to reduce oxidative stress. Phenolic compounds are secondary metabolites are known to be responsible for the antioxidant activity of plants. These compounds are suggested to contribute to the health-promoting properties. In addition to nutritive dietary components plants are a good source of different classes of polyphenolic components as well as flavan-3-ols, hydroxybenzoic and hydroxycinnamic acids, anthocyanins, stilbenoids and other flavonoids. At the present time, the *Allium* family has over 500 members, each differing in taste, form and color, but close in phytochemical, biochemical and nutraceutical content. Leek is consumed throughout Europe, North America, Asia and other parts of the world. *Allium* species contain a wide array of bioactive compounds, which include organo-sulphur compounds, phenolic compounds, non-structural and soluble carbohydrates, various amino acids and organic acids. The volatile sulphur compounds are components that provide both flavor and other quality properties for leeks, while phenolics and other bioactive components may have health promoting properties as well as antimicrobial, antiatherosclerotic, antitumorigenetic and immuno-modulatory properties [8-22]. During the last 20 years, *Allium* plants were among the most studied vegetables and aroused great interest for food and pharmacy industries. Leeks have a relatively long shelf-life and they contain a wide array of bioactive compounds. For these reasons it is an attractive crop plant for industrial agriculture and for providing health-promoting compounds through selective breeding antitumorigenetic and immuno-modulatory properties [23]. The antimicrobial activity of some onion species has been frequently associated with the content of both organosulphur compounds and phenolic compounds [12]. Recent our research suggests potential use of
phenolic compounds in food processing for improving quality, safety and stability of food products [16]. Special attention has been focused on their extraction from inexpensive sources from the agricultural production. Leek (*Allium porrum* L.) was used during centuries as a food and traditional medicine in Balkan region. In this study we investigated phenolic profile and antioxidant, antimicrobial and cytotoxic activities of *Allium porrum* L. cultivars grown in Central Serbia during 2010 and 2011 season. Separation and quantitative determination of individual phenolic compounds was performed using high-performance liquid chromatography (HPLC). The antioxidant activity of the extracts was determined as the free radical scavenging ability. All extracts were screened by the broth microdilution test for antimicrobial activity against eight gram-positive and gram-negative pathogenic bacteria and fungi. Additionally, the cytotoxic activity was determined against Hep2c, L2OB and RD cells. This study was conducted with the aim of evaluating the biological potential of leek (*Allium porrum* L.), which are growing in Serbia.

2. Materials and Methods

2.1. Chemical Reagents

All standards for spectrophotometric and High Performance Liquid Chromatography (HPLC) analysis, 2,2'-diphenyl -1 - picyrylhydrazyl (DPPH) free radical were of analytical grade and were purchased from Sigma Chemical Co. (Milwaukee, WI, USA) and Extrasynthese (Genay, France). Ethanol, methanol, dimethyl sulfoxide (DMSO), ascorbic acid (AA), butylated hydroxytoluene (BHT), HEPES solution, fetal bovine serum (FBS), cis-diamminedichloroplatinum (Cis-DDP) and 1,3,5-triphenyltetrazolium formazan, were of analytical grade (Sigma-Aldrich Chemical Co., Steinheim, Germany).

2.2. Plant Samples

The Dutch leek (*Allium porrum* L.) cultivar Varna characterized by medium early maturation, long white stem of uniform length and short green leaves that can be preserved for a long period of time was used as the plant material in this study. The plant was cultivated under open field conditions during the autumn growing cycle in 2010 and 2011 on an experimental field near Čačak (Central Serbia). During the stage of leek harvest maturity, samples of edible parts (stem and leaf) were collected for extraction, and cut longitudinally into thin strips.

2.3. Ultrasonic Extraction of Phenolic Compounds

The phenolic compounds were extracted from homogenized plant sample (10.0 g) using ethanol/water (70/30) solvent. Extraction process was carried out using Brason B-220 Ultrasonic Bath (Smith-Kline Company, USA) at the room temperature for 1 hour [16]. After filtration, 5 mL of liquid extract was used for extraction yield determination. Solvent was removed by rotary evaporator under vacuum (Devarot, Elektromedicina, Ljubljana, Slovenia), and was dried at 60°C to the constant mass. Dry extracts were stored in the glass bottles at 4°C to prevent oxidative damage until analysis.

2.4. Determination of Total Phenols

Total phenols were determined with Folin-Ciocalteu method according to the procedure described previously [24]. The dried plant extract was dissolved in the appropriate solvent to make a final concentration of 1 mg/mL and aliquots of 0.5 mL were mixed with 2.5 mL of Folin-Ciocalteu’s reagent and 2 mL of NaHCO₃. The absorbance was measured at 765 nm after 15 min at 45 °C, using a MA9523-Spekol 211 UV-VIS spectrophotometer (Iskra, Horjul, Slovenia) against a blank sample. Total phenols were expressed as gallic acid equivalents (mg GAE/g of dry extract), and the values are presented as means of triplicate analyses.

2.5. Determination of Total Flavonoids

Total flavonoids were determined according to known procedure [25]. A total of 0.5 mL of 2% AlCl₃ in methanol was mixed with the same volume of methanol solution of plant extract. After 1 hour of staying at room temperature, the absorbance was measured at 415 nm in a spectrophotometer against the blank sample. Total flavonoids were expressed as rutin equivalents (mg RU/g of dry extract), and the values are presented as means of triplicate analyses.

2.6. HPLC Analysis

Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using an Agilent 1200 HPLC chromatographic system equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser, an autosampler and a thermostated column compartment, on an Agilent, Zorbax Eclipse Plus-C18, 1.8 μm, 600 bar, 2.1×50 mm column. The gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed, as follows: initial 0-2 min, 100% B; 2-4 min, 100-98% B; 4-6 min, 98-95% B; 6-7 min, 95-73% B; 7-10 min, 75-48% B; 10-12 min 48% B; 12-20 min, 48-40% B; 20-22 min, 40% B. The total running time and post-running time were 21 and 5 min, respectively. The column temperature was 30°C and flow-rate of 0.8 mL/min. The injected volume of samples and standards was 5 μL and it was done automatically using autosampler. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280, 320 and 360 nm with a bandwidth of 4 nm, and with reference wavelength/bandwidth of 500/100 nm [26].

2.7. Antioxidant Activity

2.7.1. Determination of Total Antioxidant Activity

The total antioxidant activity of the vegetable extracts was evaluated by the phosphomolybdenum method [24]. The assay is based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at pH below 7. A total of 0.3 mL of sample extract was combined with 3 mL of reagent solution (0.6 sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes
containing the reaction solution were incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm against the blank after cooling to room temperature. Methanol (0.3 mL) in place of extract was used as blank. Ascorbic acid (AA) was used as the standard and total antioxidant capacity was expressed as ascorbic acid equivalents (mg AA/g dry extract).

2.7.2. Determination of DPPH Free Radical Scavenging Activity

The method described previously [27,28] was adapted with suitable modifications: DPPH (2,2-diphenyl-1-picryl-hydrazyl free radical (8 mg) was dissolved in methanol (100 mL) to obtain a concentration of 80 µg/mL. Serial dilutions were carried out with the stock solution (1 mg/mL) of the extract. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur, and the absorbance was measured at 517 nm. The DPPH-scavenging activity (\% / 100) = \left(\frac{A_0 - A_t}{A_0}\right) \times 100\]

Where: \( A_0 \) is the absorbance of DPPH of the stock solution and methanol, \( A_t \) is the absorbance of the sample (or standard) with the stock concentration of DPPH free radical. Ascorbic acid (AA) and butylatedhydroxytoluene (BHT) were used as reference standards and dissolved in methanol to make the stock solution with the same concentration (1 mg/mL). Control sample was prepared containing the same volume without test compounds or reference antioxidants. 95% methanol was used as a vlank.

2.8. Antimicrobial activity

2.8.1. Bacterial Strains, Culture Media, and Growth Condition

The antimicrobial activity of the plant extract was tested in vitro against the following bacteria: Staphylococcus aureus ATCC 25923, Klebsiella pneumoniae ATCC 13883, Escherichia coli ATCC 25922, Proteus vulgaris ATCC 13315, Proteus mirabilis ATCC 14153, Bacillus subtilis ATCC 6633, and fungi: Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404. Bacterial culture S. aureus was grown in 5 mL of Müller Hinton broth (MHB, Oxoid, Hampshire, UK) aerobically for 20 h, continuously shaken at 100 rpm at 37°C. E. coli was incubated in Triptone soya broth (TSB) or Triptone soya agar (TSA) (Oxoid, Hampshire, UK). Bacterial cultures for antimicrobial testing were incubated for 20 h in MHB and for antibacterial activity assays 1 mL of each culture was diluted with MHB medium to ca. 10^6 CFU/mL. The fungi were cultured on potato-glucose agar for 7 days at room temperature of 20°C under alternating light and dark conditions. The culturing procedure was performed four times until pure culture was obtained. The identification of the test microorganisms was confirmed by the Laboratory of Mycology, Department of Microbiology, Torlak Institute, Belgrade (Serbia).

2.8.2. Minimum Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentration (MIC) of the extract and cirsimarin against the test bacteria were determined by microdilution method in 96 multi-well microtiter plates [29]. The tests were performed in Muller–Hinton broth (MHB) with the exception of the yeast, in which case Sabouraud dextrose broth was used. A volume of 100 µL stock solutions of oil (in ethanol, 200 µL/mL) and cirsimarin (in 10 % DMSO) was pipetted into the first row of the plate. Fifty µL of Mueller Hinton or Sabouraud dextrose broth supplemented with Tween 80 at a final concentration of 0.5 % (v/v) for analysis of oil) was added to the other wells. A volume of 50 µL from the first test wells was pipetted into the second well of each microtiter line, and then 50 µL of scalar dilution was transferred from the second to the twelfth well. Ten µL of resazurin indicator solution (prepared by dissolution of a 270-mg tablet in 40 mL of sterile distilled water) and 30 µL of nutrient broth were added to each well. Finally, 10 µL of bacterial suspension (10^5 CFU/mL) and yeast spore suspension (3×10^5 CFU/mL) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic amracin was used to control the sensitivity of the tested bacteria, where a ketoconazole was used as control against the tested yeast. Plates were wrapped loosely with cling film to ensure that bacteria did not become dehydrated and prepared in triplicate, and then they were placed in an incubator at 37 °C for 24 h for the bacteria and at 28 °C for 48 h for the yeast. Subsequently, color change was assessed visually. Any color change from purple to pink or colorless was recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. All measurements of MIC values were repeated in triplicate and the most representative values were used.

2.9. Cytotoxic Activity

The human melanoma FemX and human colon carcinoma LS174 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Both cancer cell lines were maintained in the recommended RPMI-1640 medium supplemented with 10% heat-inactivated (56°C) fetal bovine serum, l-glutamine (3 mM), streptomycin (100 µg/mL), penicillin (100 IU), and 25 mM HEPES solution and adjusted to pH 7.2 by bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Stock solutions (100 mg/mL) of extracts, made in DMSO, were dissolved in corresponding medium to the required working concentrations. Neoplastic FemX cells (5000 cells per well) and neoplastic LS174 cells (7000 cells per well) were seeded in 96-well microtiter plates, and 24 h later, after the cell adherence, five different, double diluted, concentrations of investigated compounds, were added to the wells. Final concentrations applied to target cells were 200, 100, 50, 25 and 12.5 µg/mL. Nutrient medium was RPMI 1640 medium, supplemented with l-glutamine (3 mM), streptomycin (100 µg/mL), and penicillin (100 IU/mL), 10% heat inactivated (56°C) fetal.
bovine serum (FBS) and 25 mM HEPES, and was adjusted to pH 7.2 by bicarbonate solution. The cultures were incubated for 72 hrs. The effect of extracts on cancer cell survival was determined by MTT assay (microculture tetrazolium test) 72 h upon addition of the compounds, as it was described earlier [30]. Briefly, 20 μl of MTT solution (5 mg/mL PBS) were added to each well. Samples were incubated for further 4 h at 37 C in 5% CO₂ and humidified air atmosphere. Then, 100 μL of 10% SDS were added to extract the insoluble product 1,3,5-triphenyltetrazolium formazan, resulting from the conversion of the MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was then read in an ELISA plate reader at 570 nm. Absorbance (A) at 570 nm was measured 24 h later. To get cell survival (%), A of a sample with cells grown in the presence of various concentrations of the investigated extracts was divided with control optical density (the A of control cells grown only in nutrient medium), and multiplied by 100. It was implied that A of the blank was always subtracted from A of the corresponding sample with target cells. IC₅₀ concentration was defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. As a positive control was used cis-diaminedichloroplatinum (II) (Cis-DDP) [31]. All experiments were done in triplicate.

2.10. Statistical Analysis

The results are presented as mean ± standard deviations of three determinations. Statistical analyses were performed using Student's t-test and one way analysis of variance. Multiple comparisons of means were done by LSD (least significant difference) test. A probability value of 0.05 was considered significant. All computations were made by employing the statistical software (SPSS, version 11.0).

3. Results and Discussion

3.1. Phenolic Composition

The plants produce phenols as a response to the negative impacts from the environment as well as UV radiation, various pathogens, fungi etc. The phenolic composition in the vegetables widely and is usually determined by several factors, such as: the variety of vegetable and conditions under which they were grown (soil, geographical location, light exposure, temperature, sun exposure of the clusters) and other agrochemical polyphenol-rich extracts with strong antimicrobial and antioxidant activity. Results of the spectrophotometric factors. Understanding of the chemical composition and potential biological properties of plant extracts is of importance for their use in the food and pharmacy industries. A range of spectrophotometric and chromatographic assays was applied to create a data base that could be used to evaluate the potential of *Allium porrum* L. plant as an inexpensive and accessible natural resource for the production of investigated of the total phenols and flavonoids of ethanolic extracts of *Allium porrum* L leaves and stems, as well as *Allium porrum* L total plant, which growing on Central Serbia are presented in Table 1:

<table>
<thead>
<tr>
<th>Extrait</th>
<th>Total phenols (mg/g of dry extract)</th>
<th>Total flavonoids (mg/g of dry extract)</th>
<th>Total antioxidant activity, IC₅₀ (µg/mL)</th>
<th>DPPH radical scavenging activity, IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>45.39±2.52</td>
<td>10.24±2.84</td>
<td>121.37±3.18</td>
<td>98.91±0.18</td>
</tr>
<tr>
<td>Stem</td>
<td>69.46±1.65</td>
<td>33.51±2.51</td>
<td>123.80±4.03</td>
<td>61.05±0.12</td>
</tr>
<tr>
<td>Plant</td>
<td>93.34±0.34</td>
<td>35.14±0.32</td>
<td>128.01±0.72</td>
<td>10.28±0.08</td>
</tr>
</tbody>
</table>

The presence of total phenols expressed as gallic acid equivalent varied from 45.39 to 93.34 mg/g dry extract for tested samples. In this experiment, the highest phenolic content was found in total plant extract. Then, the concentrations of total flavonoids expressed as rutin equivalent varied from 10.24 to 33.53 mg/g dry extracts. The results indicate that the highest content of total phenols and total flavonoids were found in total *Allium porrum* plant. It is clear that *Allium porrum* L stem ethanolic extracts possessed higher concentration of total phenols (for 34.50%) and flavonoids (for 68.98%) than the *Allium porrum* L leaf extracts. Phenols content in *Allium* plants varies considerably particularly with cultivar. The amount of total phenols varied widely in the *Allium* species and ranged from 845 to 2075 mg/kg of different onions and garlics, respectively [13,23].

In order to determine individual phenolic compounds of investigated extracts HPLC analysis were used. Ten phenolic acids (gallic, protocatechuic, p-dihydroxybenzoic, caffeic, vanillic, chlorogenic, sinapenic, rosmarinic and syringic) and seven flavonols (quercetin, rutin, myricetin, kaempferol glucoside, quercetin glucoside, naringenin and apigenin) were detected from extracted *Allium porrum* L chromatograms and qualified (Table 2). The results of HPLC analysis show that higher content of phenolic acids was found in *Allium porrum* L. stem extracts (for 70.61%) than in *Allium porrum* L. leaf extracts. The obtained results indicated that the rosmarinic acid with 63.67% was the predominant acid in *Allium porrum* L. stem extracts. It is clear that investigated *Allium porrum* L. leaf and stem extracts possessed higher concentrations of flavonoids (for 77.07% and 56.79%, correspondently) than phenolic acids. Quercetin derivatives were the predominant flavonols, which represented the 39.67% and 50.62% of the total flavonoids in leaves and stems *Allium porrum* L. extracts, correspondently. *Allium porrum* L stem ethanolic extracts have higher concentration of flavonoids for 24.85% than the *Allium porrum* L. leaf extracts:

<table>
<thead>
<tr>
<th>HPLC analysis of <em>Allium porrum</em> L. leaf and stem extracts (mg/g dry extract)</th>
<th>Phenolic compound</th>
<th>Leaf extract</th>
<th>Stem extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.02 ± 0.02</td>
<td>0.15 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>0.03 ± 0.01</td>
<td>0.11 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Dihydroxybenzoic acid</td>
<td>0.26 ± 0.01</td>
<td>0.26 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.03 ± 0.02</td>
<td>0.09 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0.02 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.11 ± 0.01</td>
<td>0.08 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.03 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Sinapenic acid</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>0.17 ± 0.07</td>
<td>1.55 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.88 ± 0.06</td>
<td>1.56 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Rutin</td>
<td>0.04 ± 0.02</td>
<td>1.04 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Myricetin</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Kaempferol glucoside</td>
<td>0.07 ± 0.05</td>
<td>0.11 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Quercetin glucoside</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Naringenin</td>
<td>0.23 ± 0.05</td>
<td>0.03 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Apigenin</td>
<td>0.36 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Total phenolic acids</td>
<td>0.71 ± 0.12</td>
<td>2.44 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>2.42 ± 0.09</td>
<td>3.22 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Total polyphenols</td>
<td>3.14 ± 0.11</td>
<td>5.66 ± 0.12</td>
<td></td>
</tr>
</tbody>
</table>
3.2. Antioxidant Activity

Phenolic compounds have been reported to be associated with the antioxidant activity in investigated Allium porrum L. extracts, mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [4,5,9,11,22,23]. The results for total antioxidant capacity, expressed as ascorbic acid (AA) equivalent per gram of dry extract, as well as DPPH free radical scavenging activity, expressed in IC50 values, are given in Table 1. The results show that the highest total antioxidant capacity (128.01 µgAA/g dry extract) and free radical scavenging activity (IC50 was 10.28 µg/mL extract) were found of extracts of Allium porrum L. total plant. The standard compound ascorbic acid and butylated hydroxytoluene, at same conditions showed IC50 was 10.61 and 39.25 µg/mL, respectively. The high total antioxidant and free radical scavenging activities of Alliums were reported by numerous investigators [7-16,22,23].

It is observed that concentrations of phenolic compounds, determine by spectroscopic methods and HPLC analysis to correlate with antioxidant and free radical scavenging activities investigated Allium porrum L. extracts.

3.3. Antimicrobial Activity

The results of the analysis of the antimicrobial activity of investigated Allium porrum L. extracts, obtained by the dilution method are given in Table 3:

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Leaf extract</th>
<th>Stem extract</th>
<th>Ambracin</th>
<th>Ketoko-nazol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>156.25</td>
<td>39.10</td>
<td>0.97</td>
<td>/</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>156.25</td>
<td>39.10</td>
<td>0.24</td>
<td>/</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>312.51</td>
<td>78.12</td>
<td>0.49</td>
<td>/</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>312.50</td>
<td>78.12</td>
<td>0.97</td>
<td>/</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>78.12</td>
<td>156.25</td>
<td>0.49</td>
<td>/</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>78.12</td>
<td>78.12</td>
<td>0.49</td>
<td>/</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>156.25</td>
<td>78.12</td>
<td>/</td>
<td>1.95</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>39.10</td>
<td>19.53</td>
<td>/</td>
<td>0/97</td>
</tr>
</tbody>
</table>

Minimum inhibitory concentrations (MICs) were determined for eight selected indicator strains: Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633 gram-positive and Klebsiella pneumoniae ATCC 13883, Escherichia coli ATCC 25922, Proteus vulgaris ATCC 13315 and Proteus mirabilis ATCC 14153 gram-negative bacteria strains, and fungi: Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404.

The obtained results showed that the tested extracts possessed different antimicrobial activity within the concentration range from 39.10 to 312.50 µg/mL. Increased concentrations of extracts caused decrease in survival of bacterial cells. Very strong reduction of gram-positive Staphylococcus aureus and Bacillus subtilis growth were observed during incubation of bacteria in Allium porrum L. stem extracts (MIC was 39.10 µg/mL). Lower antimicrobial effect was demonstrated against gram-negative strains Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis and fungus Candida albicans (MIC was 78.12 µg/mL) in Allium porrum L stem extracts. The results of antimicrobial activity of the extracts of Allium porrum L. stems showed the strongest antimicrobial effect on the fungus Aspergillus niger with MIC was 19.53 µg/mL. Antimicrobial effect of Allium porrum L. leaf extracts was slightly less efficient in comparison with Allium porrum L stem extracts, which is in accordance with certain concentrations of phenolic compounds in tested samples.

3.4. Cytotoxic Activity

There are a large number of plant species that are a source of antitumor compounds used in the treatment or prevention of cancer [24]. For this purpose anticancer effect of Allium porrum L. extract was investigated using tumor lines: Hep2 (surface: Eagle MEM/5% FCS) human cell lines - human laryngeal carcinoma, RD (surface: Eagle MEM/10% FCS) - a human cell line miosarkoma, L2OB (surface: MEM Eagle/10% FCS) - murine fibroblastic tumor lines. The cytotoxic activity of Allium porrum L. extract on inhibit Hep2c, L2OB and RD cells in a dose dependent manner. As controls were used the non-stimulated cells, the cells in the culture which grew to 100%. The cytotoxic effect of the extract was expressed as an IC50 value (the concentration which inhibits 50% of cell growth). In our study used reagent MTT [3-(4,5-dimethylthiazol-2-ol)-2,5-diphenyltetrazolium bromide] which is in the presence of viable cells reduced to a blue colored formamazan product. Based on the results ethanol extract of Allium porrum L. meets the NCI (<30 µg/mL ) criteria. The IC50 values of Allium porrum L. extract were 27.18 ± 0.88 and 25.89 ± 0.67 µg/mL against Hep2c and L2OB cells, correspondently and 76.95 ± 11.45 µg/mL against RD tumor cell after 48 h treatment period (Figure 1):
accordance with the NCI criteria. It is known that this extract has the highest content of phenolic compounds, especially quercetin.

4. Conclusion

On the basis of the obtained results, it can be concluded that the Allium porrum L. leaves and stems displays a pronounced antioxidant and free radical scavenging activity owing to the presence of the phenolic acids and flavonols, especially of rosmarinic acid and quercetin. The ethanolic extracts of the Allium porrum L. showed a favourable antimicrobial activity when applied in vitro against different gram-positive and gram-negative bacteria and fungi, especially against Staphylococcus aureus, Bacillus subtilis and Aspergillus niger. The Allium porrum L. extracts inhibit Hep2c, L2OB and RD cells in a dose dependent manner. The IC50 values calculated were <30 µg/mL for 48 h of action against Hep2c and L2OB cells. These results confirmed that Allium porrum L. might indeed be a potential source of biologically compounds.

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References