myopia. The purpose of the study is to measure the density of retinal layers and choriocapillaris, as well as evaluation of the thickness of these tissues through optical-coherence tomography-angiography and determine its relationship with the anterior-posterior axis of different eye sizes in myopic children.

96 eyes of 48 myopic subjects and 40 eyes of 20 emmetropic volunteers were examined. The spherical equivalent of myopes was greater than -1.0 D. For emmetropes, from +0.5 to 0.5 D; The length of the axial axis is 24.58mm (SD±1.22) and 22.88mm (SD±0.65). Patients aged 7-16, who were also involved in the study, underwent a complete ophthalmological examination. Retinal and choriocapillaris density were examined using SS-OCTA DRI Triton.

According to the results of the study, the density of superficial retinal blood vessels is lower in myopic eyes than in emmetropic eyes and correlates with the axial axis.

In patients with medium and high myopia, the choroid is significantly thinner than in patients with low-grade myopia; Also, there is a decrease in the density of choriocapillaris in patients with moderate and high myopia in the upper and lower segments, but not in the nasal and temporal regions. Obviously, it is very important to carry out long-term observations of such patients in terms of determining microvascular changes in the future.

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BIOSYNTHESIS OF SILVER NANOPARTICLES USING EXTRACT OF CENTAUREA ADZHARICA SOSN. AND EVALUATION OF THEIR BIOACTIVITY

TSMU, DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY[1]; UNIVERSITÉ DU QUÉBEC À CHICOUTIMI, CANADA[2]

Introduction

Silver has been considered as a special metal for a long time and its antibacterial properties are well known but while discussing silver nanoparticles, completely new characteristics appear. Due to their distinct chemical, thermal, mechanical, and electrical capabilities compared to bulk material in nowadays, nanoparticle applications are subject to a wide spectrum of study. Increased biological activity is caused by the small size, high surface-to-volume ratio /1/. Various physicochemical techniques are currently employed to create nanoparticles for use in a variety of industries, including the medical, biological sensor, solar cell, textile, and agricultural sectors /2/. Though, it has to be mentioned that their integration in the biological and medical fields is limited due to the use of toxic chemicals, the difficulty of the synthesis process, the high cost, and the involvement of hazardous products in the process /3/.

One of the most attractive processes for making nanoparticles is so-called green synthesis, which involves the use of bio sources as reducing agents without the need for any chemicals. Among described ways of biosynthesis process involving plant extracts is the most widely used. Plant extracts contain coating and reducing agents that can reduce metal ions and create nanoparticles with high stability and a variety of sizes and shapes. These compounds include polysaccharides, amino acids, flavonoids, alkaloids, terpenes, enzymes, proteins and etc. /4, 5/.

Implementation of silver nanoparticles as antibacterial and anticancer agents in medicine is one of the tasks that scientists are trying to solve. The antibacterial effects of these particles have been proved in multiple research. Scientific works has demonstrated that AgNPs exert their antibacterial activity by degrading enzymes, damaging DNA, increasing membrane permeability, and inactivating cellular proteins /6,7/. What about cytotoxicity, silver nanoparticles have been shown to have anticancer properties. Mainly the generation of ROS and the release of silver ions are two important factors that cause cytotoxic effects /8/ but some parameters may influence this process and change the characteristics of nanoparticles. Data available regarding it is huge yet not sufficient, therefore, it is crucial to continue the research around AgNPs' anticancer effects.

In the given research silver nanoparticles were biosynthesized using watery extract of Centaurea adzharica Sosn., an endemic plant of the Adjara region. The synthesis of nanoparticles was observed and proved by Uv-vis spectroscopy, dynamic light scattering method and was used to characterize them. Antibacterial activity was evaluated against Gram-negative Escherichia coli, Gram-positive Staphylococcus aureus and antifungal activity on Candida albicans. Their cytotoxic effects were tested on human lung carcinoma A-549 (ATCC #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221) and healthy human skin fibroblasts WS1 (ATCC CRL-1502) cell lines.

Materials and Methods

Biosynthesis of Silver Nanoparticles

Plant material was washed with distilled water. After drying, they were cut into 1 cm long pieces. To obtain the extract, 10 g of finely chopped raw material was placed in a beaker. 200 ml of double distilled water was added. Beaker was later placed in a Hyundai microwave for 10 minutes. The mass was heated by the dielectric heat and then was left cool down at room temperature for one hour. In the next step the material was drained onto cotton to remove the finely chopped plant waste. Finally, to obtain the pure extract, the liquid was filtered into the filter paper produced by MELIOR XXI Ltd. (ashless filter d = 150 mm).

Silver nitrate was purchased from Sigma-Aldrich Chemie GmbH and the powder was dissolved in double distilled water and solutions were prepared with 1mM, 2mM, 4mM, 7mM concentrations. For each prepared sample silver nitrate solution was placed in a 400 ml volume flask and placed on a magnetic stirrer. The plant extract was added slowly under continuous stirring in different ratios (plant extract: silver nitrate solution ratios are shown in Table 1) and the flasks were left on the stirrer for 1h. For the further synthesis of silver nanoparticles (AgNPs) the mixtures were then left in a dark place, at 21^{O}C for 24 hours. The samples were afterward centrifuged at 14,000 rpm for 7 min to remove watery extracts together with the supernatant. The precipitate containing nanoparticles was diluted in distilled water and the process was repeated two times to obtain purified AgNPs. To analyze the characteristics and biological activities of synthesized nanoparticles further assays were performed.

Characterization of silver nanoparticles

The biosynthesis of silver nanoparticles was detected using i9 UV-VIS spectrophotometer (Hanon Instruments). This method is based on the detection of plasmon resonance which is characteristic for AgNPs in the range of specific wavelength, which mostly varies between 380-450nm / 9/. The absorbance of prepared samples was scanned in the wavelength range of 300-600 nm. NPs were characterized by measuring zeta-potential (ZP) and size by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, U.K.) at 25°C. The result is presented as an average of three individual measurements ± standard deviation (SD). Transmission electron microscopy (TEM) was used to study the morphology and distribution of formed silver nanoparticles. Microscopy was performed using a JEOL JEM-100SX transmitting electron microscope. Images were obtained at 120,000 magnification. Particle sizes were measured following it.

Study of biological activity

The antibacterial and antifungal activity assays of biosynthesized silver nanoparticles were performed using the previously described method /12/. The cellular density of the inoculum was measured via optical density, measured at 600 nm for E. coli /10/, 660 nm for S. aureus /11/, using a Multiskan™ GO Spectrophotometer (Thermo Fisher Scientific). MIC90 for the lowest concentration of AgNPs resulting in 90% inhibition of bacterial and fungal growth was determined.

For cytotoxicity assay the human lung carcinoma A-549 (ATCC #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221) and healthy human WS1 (ATCC CRL-1502) cell lines were used, obtained from the American Type Culture Collection (ATCC, Manassas, USA). Cytotoxicity was assessed using the resazurin reduction test as described by O'Brien /13/ and Hoechst method. Fluorescence was measured on an automated 96-well Fluoroskan Ascent FlTM plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxicity was expressed as the concentration of extract or compound inhibiting cell growth by 50% (IC50). Etoposide was used as a control in the cytotoxicity assay.

Results and discussion

The biosynthesis of silver nanoparticles is visible by color change after adding the extract to a silver salt solution, often yellow to brown, which occurs within a few minutes. The color change is caused by their optical properties. In accordance with the available data /14/ the first sign of biosynthesis of silver nanoparticles in this case was the rapid change of color after mixing the AgNO3 solution with the extract. Obtained light yellowish color became darker after passing 24 hours. The change in coloration proves that the silver ions present in the solution were reduced. Among the prepared twelve objects color was changed in each but precipitate or tendency to strong agglomeration was visible in some of them.

Objects prepared from 2mM silver nitrate solution appeared to be more stable and for further research was chosen sample N4 (1:10 ratio of extract : 2mM silver nitrate solution). As an instrumental analytical method for observing biosynthesis UV-vis absorption spectra is usually recorded. Received UV-Vis spectra for our research object proved the formation of silver nanoparticles. For silver nanoparticles biosynthesized C. adzharica watery extract absorption maximum was recorded at 434 nm (Fig. N1) which comes in accordance to characteristic plasmonic resonance for AgNPs which is detected in a specific range, mostly between 380-450nm like it was mentioned above.

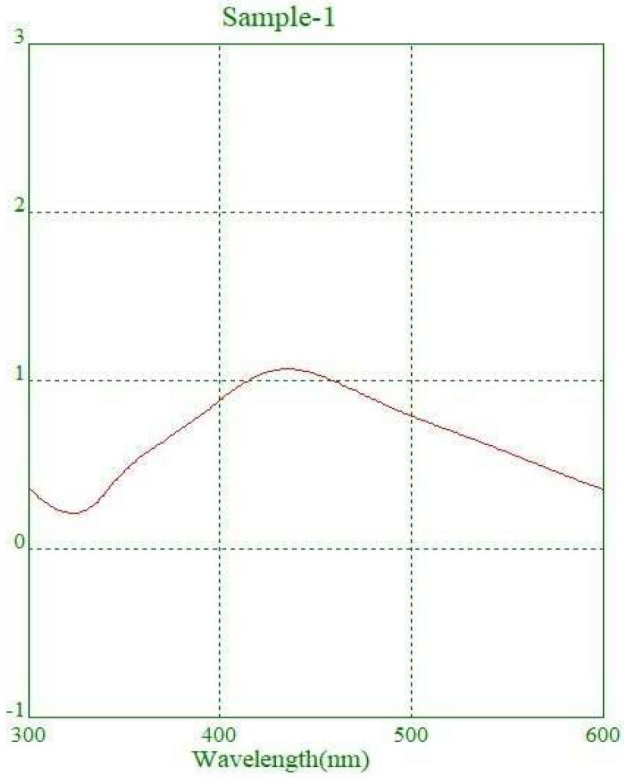

Fig. 1. Characteristic absorption peak to silver nanoparticles detected with UV-vis spectroscopy

The Zeta potential value for the prepared silver nanoparticles equals to: - 21,4±4.5 mV (Fig.N2). Zeta potential of NPs is considered as an important characteristic. It provides useful details about the electric charges close to the particle surfaces and can help to predict the stability. According to the scientific data the negative value indicates the stability of the nanoparticles and it can avoid the agglomeration of nanoparticles /15/. The negative potential value can be caused by the capping action of biomolecules present in the watery extract of C. adzharica. The average size was equal to 309,5 ± 31.54 nm and PDI 0.453 ± 0.024.

Table N1

Plant extract: silver nitrate solution ratios in prepared objects

Conc.	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12
1 mM	1:10	1:20	1:40									
2 mM				1:10	1:20	1:40						
4 mM							1:10	1:20	1:40			
7 mM										1:10	1:20	1:40

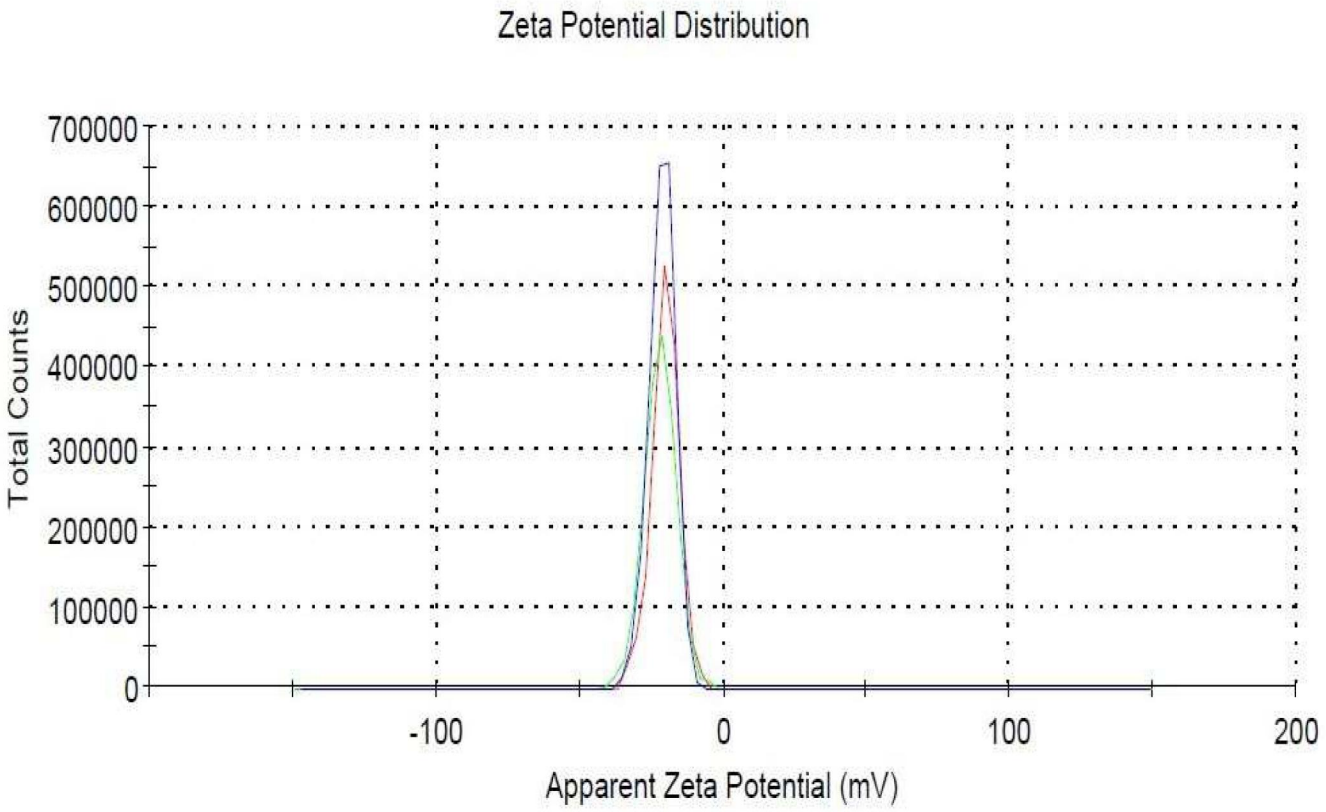

Figure 2. Zeta potential of silver nanoparticles

On obtained images from Transmission electron microscopy, we can see formed silver nanoparticles (Figure N3), with some tendency to agglomerate but still, the shape can be defined and we can say that biosynthesized silver nanoparticles have a pyramid shape.

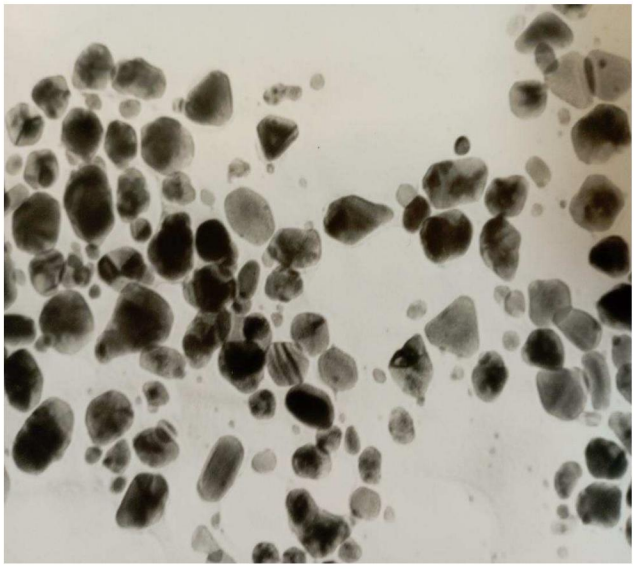

Figure 3. TEM image of silver nanoparticles

As a result of performed in vitro assays to assess the bioactivity of our object, we can say that it shows antibacterial and antifungal activity against tested E.coli, S. aureus and C. Albicans. The cytotoxicity assay of biosynthesized silver nanoparticles against human lung carcinoma A-549 (ATCC #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221) and healthy human WS1 (ATCC CRL-1502) cell lines showed that obtained AgNPs exhibit cytotoxic activity against all of the tested cells, but the strongest effect was shown on colon adenocarcinoma cells. According to available data nanoparticles small in size show stronger cytotoxicity /16/. In our case we should mention that obtained nanoparticles are not close to the desirable size, their average diameter according to the results of DLS is 309,5 ± 31.54 nm. If we manage to obtain nanoparticles of smaller size with variating conditions of biosynthesis we can predict that the bioactivity will be increased.

Table N2
Results of antibacterial and antifungal activity assay of silver nanoparticles

	E. coli IC90	**S. aureus IC90**	**C.albicans IC90**
AgNPs	68±8 µg/ml	57±2 µg/ml	89±8µg/ml
Gentamycin	0,045±0,004	0,07±0,01	
AmphotericinB			0,55±0,08

Table N3

Results of cytotoxicity assay of silver nanoparticles

Sample	Resazurine A-549	Resazurine DLD-1	Resazurine WS-1	Hoechst A-549	Hoechst DLD-1	Hoechst WS-1
AgNPs	91±11 μg/ml	58±10 μg/ml	82±10 μg/ml	83±2 μg/ml	63±3 μg/ml	61±4 μg/ml
Etoposide	27±14μM	14±2μM	3,7±0,8 μM	2,0±0,3 μM	1,7±0,3 μM	0,5±0,1 μM

Conclusion

The results of conducted work enable us to conclude that the watery extract of C. adzharica, can be used as reducing agent in the synthesis of silver nanoparticles. Compounds represented in it aid not only in reducing silver ions, but also contribute to the stability of synthesized nanoparticles by serving as a capping agent and formed a coating with a negative surface charge that can avoid agglomeration. The Biosynthesis of silver nanoparticles involving extract of the endemic plant to Adjara region - C. adzharica is easy and cost-effective. Results show that obtained silver nanoparticles show antibacterial and antifungal activities. They have cytotoxic effects on human lung carcinoma A-549, colon adenocarcinoma DLD-1 and healthy human skin fibroblasts WS1 cell lines. Further work is planned to obtain silver nanoparticles with smaller sizes. Synthesis of nanoparticles with appropriate size will be achieved by changing the main parameters of biosynthesis, concentrations, extract: silver nitrate solution ratio, the temperature during the process. Based on available scientific literature the formation of silver nanoparticles with a small diameter will likely increase the cytotoxicity.

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SUMMARY

Mosidze E.[1], Legault J.[2], Mshvildadze V.[2], Ebralidze L.[1], Bakuridze L.[1]

BIOSYNTHESIS OF SILVER NANOPARTICLES USING EXTRACT OF CENTAUREA ADZHARICA AND EVALUATION OF THEIR BIOACTIVITY

TSMU, DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY[1]; UNIVERSITÉ DU QUÉBEC À CHICOUTIMI, CANADA[2]

Silver nanoparticles have high biological activity due to their small size and big surface. They show antibacterial activity and cytotoxicity on cancer cells. Both the properties and the biological activity of silver nanoparticles depend to a significant extent on the technologies of their preparation.

In the given research silver nanoparticles were biosynthesized using a watery extract of Centaurea adzharica Sosn. The approach was chosen based on its safety, low cost, and simplicity of the technology. The synthesis of nanoparticles was observed and proved by Uv-vis spectroscopy. Dynam-

ic light scattering method and Transmission electron microscopy were used to characterize them. Antibacterial activity was evaluated against Gram-negative Escherichia coli, Gram-positive Staphylococcus aureus and antifungal activity on Candida albicans. Their cytotoxic effects were tested on human lung carcinoma A-549, colon adenocarcinoma DLD-1 and healthy human skin fibroblasts WS1 cell lines. Results show that biosynthesized silver nanoparticles have antibacterial, antifungal action and are cytotoxic against tested cancer cell lines.

ნერსეზაშვილი მ[1]., ბერაშვილი დ[1]., ჯოხაძე მ[1]., გოქაძე ს.[1], კორონა-გლოვნიაკი ი.[2]

ხევსურის დიყის *(Heracleum sosnowskyi Manden.)* ფესვების მეთანოლიანი ექსტრაქტის ფიტოქიმიური და ბიოლოგიური შეფასება

თსსუ, ფარმაცევტული ბოტანიკის დეპარტამენტი[1]; ლუბლინის სამედიცინო უნივერსიტეტი, ფარმაცევტული მიკრობიოლოგიის დეპარტამენტი[2]

დიყი - *Heracleum* ქოლგოსანთა (Aapiaceae) ოჯახის ერთ-ერთი ყველაზე დიდი გვარია. მისი სახეობები მაღალი, ორი ან მრავალწლოვანი ბალახოვანი მცენარეებია. აქვს დიდი ზომის დანაკვთული და მთლიანი ფოთლები. ყვავილები თეთრი, მომწვანო-ყვითელი ან ვარდისფერი, რთულ ქოლგა ყვავილედად შეკრებილი. ამ გვარის 120-ზე მეტი სახეობიდან კავკასიაში გავრცელებულია 25 სახეობა, ხოლო საქართველოში - 23, მათ შორის 5 ენდემია [1]. იზრდება უმეტესად ტყისა და სუბალპურ სარტყელში. საქართველოს სუბალპურ მაღალბალახეულში ფართოდაა გავრცელებული სოსნოვსკის დიყი (*Heracleum sosnowskyi*) და მანტეგაცის დიყი (*Heracleum mantegazzianum*). ხორკლიანი დიყის (*Heracleum asperum M.Bieb.*) და თეთრი დიყის (*H. leskovii Grossh.*) ნორჩ ყლორტებს მთის მოსახლეობა საჭმელად იყენებს.

დიყის ფოტოალერგიული თვისებები დაკავშირებულია მცენარის ყველა ნაწილში ფუროკუმარინების შემცველობასთან. ფუროკუმარინები აღმოჩნდა მცენარის ბუსუსებშიც, რომელიც ფარავს მის ფოთლებსა და ღეროს. ეპითელური შრის მეშვეობით მათ შეუძლიათ კანში შეღწევა, რაც შესაძლებელია საფრთხის შემცველიც იყოს ადამიანის ჯანმრთელობისთვის. მცენარესთან კონტაქტის შემდეგ, კანზე მზის სხივების ზემოქმედებამ, შეიძლება გამოიწვიოს დიდი ზომის ბუშტუკების წარმოქმნა და დამწვრობის სიმპტომების განვითარება. სიმპტომები ვითარდება მზის სხივების ზემოქმედებიდან რამდენიმე საათში. ერითემა და სეროზული სითხით სავსე ბუშტუკები შეინიშნება კანის კონტაქტურ უბანზე. ყველაზე ხშირად ზიანდება კანის დაუცველი ადგილები, მაგალითად სახე, მკლავები და ქვედა კიდურები. კუმარინების ალერგიული თვისება ძლიერდება მზის სხივების პირდაპირი ზემოქმედებით, მაღალი ტემპერატურითა და ტენიანობით, ასევე, დიყის ყვავილობისა და სიმწიფის პერიოდში. ასეთ პირობებში ალერგიული რეაქცია შესაძლებელია განპირობებული იყოს მცენარის სიახლოვეს ყოფნითაც და არამარტო მასთან პირდაპირი კონტაქტით, შეიძლება განვითარდეს კონიუქტივიტიც [12].

დიყის სხვადასხვა სახეობაში იდენტიფიცირებულია ფუროკუმარინები: ბერგაპტენი, ბიაკანგელიკოლი, ფელოპტერინი, ქსანტოტოქსინი, იზოპიმპინელინი, იმპერატორინი. თითოეული მათგანი ხასიათდება ფართო სპექტრის ბიოლოგიური აქტივობით, როგორიცაა: ანტიბაქტერიული, ანტიმიკრობული, ანტიოქსიდანტური, ფუნგიციდური, ანთების, სიმსივნის საწინააღმდეგო მოქმედება [17]. გამოიყენება ვიტილიგოს, ალოპეციის და ფსორიაზის სამკურნალოდ [17,4].

დიყის სახეობები შეიცავს ეთერზეთებს, ფლავონოიდებს, ფენოლკარბონმჟავებს, მთრიმლავ ნივთიერებებს, ანტრაქინონებს. თუმცა უნდა აღინიშნოს, რომ კუმარინები და ფუროკუმარინები ყველაზე დიდი რაოდენობით არის აღმოჩენილი [7].

ხევსურის დიყი (*Heracleum sosnowskyi Manden.*) 1772 წელს აღმოაჩინეს. ხოლო 1944 წელს საქართველოში მოზარდი ხევსურის დიყი ი.პ. მანდენოვამ აღწერა როგორც ცალკე სახეობა [12]. ხევსურის დიყის საერთაშორისო დასახელება - *Heracleum sosnowskyi* მომდინარეობს კავკასიის ფლორის მკვლევარი ბოტანიკოსის, პროფესორ დ.ი. სოსნოვსკის გვარიდან, ხოლო *Heracleum* - ანტიკური გმირის, ჰერაკლეს (Heracles) სახელიდან [16].

ველურად მოზარდი დიყის ენდემური სახეობები ძირითადად გავრცელებულია აღმოსავლეთ და დასავლეთ ამიერკავკასიასა და დაღესტნის მთისწინეთში [8,10,18],

ხევსურის დიყი გავრცელებულია კახეთის, ქვემო ქართლის, მცხეთა-მთიანეთის, სამეგრელო-ზემო სვანეთისა და სამცხე-ჯავახეთის ტერიტორიებზე [19]. გავრცელებულია თურქეთშიც. განხორციელდა მისი ინტროდუცია ბულგარეთში, რუსეთის ცენტრალურ, აღმოსავლეთ და ჩრდილოეთ - ევროპულ ნაწილში, პოლონეთში, უკრაინაში, სახალინსა და დასავლეთ ციმბირში [13].

ხევსურის დიყის შემადგენლობაში აღმოჩენილია ანგელიცინის, ბერგაპტენის, მეთოქსალენის, იმპერატორინის, იზოიმპერატორინის, მარმეზინის, (+)-პანგელინის, ოქსიპეუცედანინის, ასევე, არაბინოგალაქტინისა და პექტინური პოლისაქარიდების არსებობა [11,15,9]. მიუხედავად იმისა, რომ ამ ნაერთების უმეტესობა ქიმიურად იდენტიფიცირებულია, მათი ბიოლოგიური აქტივობა ჯერ კიდევ არ არის ბოლომდე შესწავლილი.

დერმატოფიტოზი მსოფლიოში ერთ-ერთი ყველაზე ფართოდ გავრცელებული ინფექციური დაავადებაა. დერმატოფიტებს გააჩნიათ კერატინზებულ ქსოვილებში (კანი, თმა და ფრჩხილები) შეღწევის უნარი, რის შედეგადაც ვითარდება დერმატოფიტოზი [3]. *Trichophyton rubrum* და *Trichophyton mentagrophytes,*