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Bioecological and Pharmacognostic Study of Ajara and Ajara-Lazica Endemic Plant Species

(Submitted for the degree of Doctor of Biology)

Specialty: **Plant Biodiversity**

A N N O T A T I O N

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General Description

The theme topicality: Standards of living have certain negative influence on human body and health. A wide range of diseases and current state of treatment requires the necessity of elaborating and producing new medicines. Majority of synthetic medicines used in medical practice are characterized by bad side effects. Therefore, consumption of drugs originated from plant sources has been significantly increased in global pharmaceutical market due to their high efficiency, easy digestibility and less side effects. Developing the nomenclature of plant-based healing remedies is a topical issue for modern pharmaceutical technology, pharmacology and medicine in general.

Nowadays, one of widespread diseases over the world is malignant tumor and its lethal outcomes reach unprecedented scales day by day. It is obvious, that cancer is the second-leading disease after cardiovascular diseases and 70% of cancer death occurs in low and middle developing countries, where diagnosing and treatment of oncologic diseases at their earliest stages still stay as a problem.

According to experts of World Health Organization (WHO), the death rate caused by cancer will be increased by 2030 and it will reach more than 13 million. From this point of view, Georgia is not an exception. Since 2014, the number of cancer patients has been significantly increased among them are children too.

It is a fact, that in parallel with chemotherapy, radiotherapy, hormone therapy and immunotherapy for the treatment of oncologic diseases, plant-based drugs are also applied. Organic, plant derived anti-cancer drugs have an important place, taking approximately 28% of numerous chemotherapeutic agents in oncological practice. Medicinal plants play an important role in disease prevention and treatment. Hence, seeking new, more efficient medicinal plants is always a topical issue.

Inventarization of plant species all around Georgia, included in large taxonomic groups of floristic region of Ajara, among them endemics, has always been a priority for Georgian and foreign scientists and practitioners, which is reflected in their works. Ajara (South Colchis) floristic region is well represented by Caucasian, Georgian and Colchis endemic species. Most of them are studied for medicinal purposes and used in officinal medicine. Regarding 25 species of Ajara and Ajara-Lazica endemics spread locally, there is a lack of information about them in scientific literature and no information at all about their bioecological peculiarities and content of biologically active substances except the following species: *Galanthus krasnovii*, *Galanthus rizechensis*, *Cyclamen adzharicum* and partially, *Rhododendron smirnovii*, *Rhododendron ungernii*, *Osmanthus decorus*. However, systematic-botanic topics about these species are clarified and studied by local botanist scientists (Memiadze, Varshanidze, Kharazishvili, Manvelidze, etc.)

Strong anthropogenic factors has threatened endemics and some of them are endangered. Therefore, their timely study, identification of their biologically active content, reveal of prospective species for medicinal usage and implementation of relative activities for their preservation is one of topical issues for botanists, biologists and pharmacognostists.

Investigation of Ajara and Ajara-Lazica endemic plant species turned out more interesting after the border was opened with neighboring Turkey and Georgia has become a corridor between Europe and Asia. After that, the plants easily surmounted natural-geographical barriers. Thus, studying the plants at the border of Ajara and neighboring historical Lazeti – Georgia and Turkey border zone, is very topical.

The research deals with the studies of bioecological peculiarities of those Ajara and Ajara-Lazica endemics, which have not been studied so far, their research on the content of biologically active substances, pharmacognostic description and determination of prospective usage in medicine.

Research goal and objectives: Research goal is bioecollogical and pharmacognostic study of Ajara and Ajara-Lazica endemic plant species spread in Ajara (South Colchis) floristic region, Southwest of Georgia. To accomplish these goals we set the following objectives**:**

1. Identifying geographical coverage of Ajara and Ajara-Lazica endemics;
2. Botanical description of Ajara and Ajara-Lazica endemics;
3. Investigating growth and development characteristics of Ajara and Ajara-Lazica endemics;
4. Screening on basic kind of content of biologically active substances of Ajara and Ajara-Lazica endemics;
5. Determining the content of biologically active substances applying tandem chromate mass spectrometry GC-MS/MS method of Ajara and Ajara-Lazica endemics;
6. Studying cytotoxic activities of Ajara and Ajara-Lazica endemics containing biologically active compounds.

Innovation and practical values: Growth and development characteristics of some Ajara and Ajara-Lazica endemics in nature during seasonal dynamics are studied. Vegetation beginning date, growth, flowering and fruiting phases are discussed on the base of vital forms of research species. It is confirmed, that vegetation development period includes 3-8 months, all the species are characterized by full generative development. Geographical coverage is analyzed and rythmological and biomorphological descriptions are given as well.

At first, we studied 6 of Ajara and 15 of Ajara-Lazica endemics on the content of biologically active substances. There were detected compounds of various classes – flavonoids, coumarins, alkaloids, tannis, terpenoids and other contents.

For the purpose of their medicinal usage, on the base of phytochemical and pharmacological researches, there are revealed some Ajara and Ajara-Lazica endemics for further investigation: *Angelica adzharica* M.Pop, *Centaurea adzharica* Sosn. *Erysimum contractum* Somm. et Levier., *Astragalus adzharicus* M.Pop., *Astragalus sommieri* Freyn., *Hypericum nordmanni* Khokhr., *Hypericum ptarmicifolium* var.adzharicum, *Scutellaria pontica* C. Koch., *Seseli foliosum* (Somm. et Lev.) Mand.

Cytotoxic activity of methanol extracts of some Ajara and Ajara-Lazica endemics is studied in vitro testing with the help of Resazorin and Hoechst methods on A-549 (lung carcinoma) and DLD-1 (colon carcinoma). It was also revealed that they do not damage healthy cells (normal skin fibroblast cell WS1). According to experimental research, *Erysimum contractum* Somm. et Levier is considered to be a prospective species for further investigation.

Liposome nanoparticles with new galenic forms received form Erysimum contractum Somm. et Levier grass have selective activity, in particular, high cytotoxic activity on A-549 (lung carcinoma) and DLD-1 (colon carcinoma) and at the same time they don’t damage normal skin fibroblast cell culture WS1.

Methanol extracts received from Erysimum contractum Somm. et Levier grass and cardiac glycoside-enriched fraction have no cytotoxicity unlike curcumin to MCF-7 (human breast adenocarcinoma cell line) and they are also characterized by high specific cytotoxicity toward keratinocytes.

Selective, anti-cancer activity of liposome nanoparticles containing new galenic formation received form Erysimum contractum Somm. et Levier grass will become a base for new drugs elaboration.

Approbation of research outcomes: Thesis materials are presented as seminars and colloquiums at the Faculty of Natural Sciences and Health Care of Department of Biology of Batumi Shota Rustaveli State University (2014-2017). The work successfully got approbation at the faculty council, in 2018.

Research outcomes were introduced in a written and oral way at international scientific forums; the research outcomes have been published in three-impact factor and one high rating international magazines; two works are published in local conference materials and two of them in international conference materials.

Thesis volume and structure. The thesis includes 175 printed pages and combines introduction, literature overview, experimental part, conclusions and bibliography. The text includes 67 diagrams and 82 pictures

Literature overview

The first chapters of the thesis analyze results of literature review. It includes the description of Ajara floristic district and its endemic biodiversity; analyzes content of bioactive substances and importance of its study; deals with general description of soil and climatic conditions of geographical coverage of Ajara and Ajara-Lazica endemics.

Research outcomes are given in the experimental part:

1. Research objects, location and methodology

Research objects are:

1. Ajara endemic plant species: *Angelica adzharica* M.Pop. – *Umbelliferae* Juss., Apiaceae Lindl.
2. *Centaurea adzharica* Sosn. – *Asteraceae* Dumort. (*Compositae* Giseke)
3. *Erysimum contractum* Somm. et Levier. - *Cruciferae* Juss.(=*Brassicaceae* Burnett.)
4. *Psoralea acaulis* var.adzharica - *Fabaceae* Lindl.
5. *Ranunculus ampellophylus* var.adzharica - *Ranunculaceae* Juss.
6. *Rubus adzharicus* Sanadze - *Rosaceae* Juss. .

Ajara-Lazica endemic plant species:

1. Amaracus rotundifolius(Boiss.) Briq. (=*Origanum rotundifolium*) - *Lamiaceae* Juss(=Labiaceae)
2. *Astragalus adzharicus* M.Pop. - *Fabaceae* Lindl.
3. *Astragalus sommieri* Freyn. - *Fabaceae* Lindl.
4. *Hypericum nordmanni* Khokhr. - *Hypericaceae* Juss.
5. *Hypericum ptarmicifolium* var.adzharicum - *Hypericaceae* Juss.
6. *Linaria adzharica* Kem.-Nath.(=L.syspirensis C. Koch.) - *Scrophulariaceae* Juss.
7. *Osmanthus decorus* (Boiss. et Bal.) - *Oleaceae* Hoffm. et Link.
8. *Primula megasaefolia* boiss. Et Bal. - *Primulaceae* Vent. 9
9. *Quercus petra* var. dshorochensis c. Koch. - *Fabaceae* Lindl.
10. *Rhododendron smirnovii* Trautv.- *Ericaceae* DC.
11. *Rhododendron ungernii* Trautv. – *Ericaceae* DC.
12. *Rhynchospora caucasica* Vahl. - *Cyperaceae* Juss.
13. *Scrophularia chloranta* Ky et Boiss. - *Scrophulariaceae* Juss.
14. *Scutellaria pontica* C. Koch. - *Labiateae* L.
15. *Seseli foliosum* (Somm. et Lev.) Mand. - *Umbelliferae* Juss., *Apiaceae*.

Ajara and Ajara-Lazica research endemics are found in Acharistskali River gorge, in general, they are spread from the coastal lowlands and hills to subalpine zones. Their geographical coverage includes biotypes of Colchis-type mixed deciduous forest, broadleaf forest, coniferous forest, rocks and sandstone rubble soil, subalpine forests and fields, coastal wetlands and hills.

The nature of Georgia is rich in terms of biodiversity and its certain regions are characterized by specific soil and climatic conditions. Climatic diversity in Georgia is conditioned on the one hand, by its location being in the middle of the Black and the Caspian Seas, at the North border of subtropical zone and on the other hand, Georgia's very complex terrain – mountain ranges with different directions and heights. Atmospheric processes developed in subtropical and temperature zones take part in the formation of climate in Georgia. Ajara is the Southwestern part of Georgia and is distinguished with special physical-geographical characteristics conditioned by plenty of reasons. Some of important reasons are the followings: Ajara's location in the South latitudes, which is advantageous for getting much solar energy; the Black Sea climatic influence, which is more revealed at the Black Sea littoral; mountain ranges all around the three sides, which are barriers for colder air masses and create humid subtropical climate in Ajara littoral regions, while the Black Sea influence is weakened in the mid-highlands and mountain relief influence is exceeded. Ajara mountain relief is very complex, fractional and the height of mountain ranges is 2000-2500 mm and up above the sea level. The basic characteristic for Ajara orography is three high ranges: Meskheti from the North, Shavsheti from the South and Arsiani from the East. Between Meskheti (West part of Meskheti range in Ajara borders is called Ajara-Guria mountains) and Shavsheti ranges, there is a deep gorge of Acharistkali river – main river of this region and ancient valley (Берошвили, 1972; Sarishvili, Beridze 1973* Колесников, 1974; Гутиев, Мосияш, 1977; Нижарадзе, Джибути, 1978; Манджавидзе, 1982; Metreveli, 2008, and others)

According to long-term observations by local meteorological stations, the Black Sea littoral is characterized by excessive humid and warm subtropical climate, high level of precipitation (2400-2700mm and above annually) is mainly conditioned by strong condensation from the interaction of the Black sea humid air masses and high coastal mountainous relief. Average annual air temperature fluctuates between14-15 °C; average temperature in January is 4-6 °C and in August reaches 23-25°C. Absolute maximum temperature is 38-40°C and absolute minimum temperature can fall down to -9-10 °C that is very rare (one in half-century). The snow may melt too soon, although it can damage some plants, average air relative humidity is 80-82%.

Average annual temperature is 10,4 ^{0} C in highlands, absolute maximum reaches 39°C, absolute minimum falls down to -18 ^{0} C, average relative humidity is 70%, annual precipitation is 1250 – 1300 mm.

The following methods were applied during research period:

Experimental and herbarium materials for research objects were processed according to Скворцов (1977) method; taxonomic and nomenclature analysis were carried out with "Plant Guide of Ajara" (Дмитриева,1959, 1990 а,б)," "Plant Guide of Georgia", "Georgian Flora", "Summary of Georgian Flora: Nomenclature List" (Gagnidze, 2005), "Summary of Nomenclature by Cherepanov (*Черепанов,* 1995); While describing growth and development characteristics of Ajara and Ajara-Lazeti endemics, we applied for "Biomorphological Analysis of Adventure Flora of Ajara" guide (Davitadze ∂., 2002) and the method by Srebriakov (*Серебряков,* 1975).

Screening of Ajara and Ajara-Lazeti endemic plant species on the content of bioactive substances was carried out with qualitative reactions and thin-layer chromatography. For the identification of alkaloids, we used the method (H. Wagners. Bladt, 2nd edition, 2003; Pharmacopoeia, 2013; Vachnadze, 2012) described in scientific literature. Raw materials for analysis were taken in 2014-2015, early spring and late summer periods, when plants are in active growing and flowering phases.

We dried grass and roots for analysis, obtained samples were crushed in accordance with the requirements of the tenth edition of state pharmacopeia.

For the purpose of identification of biologically active substances, the study was conducted with tandem chromate mass spectrometry GC/MS/MS method at the Toxicology and Chemical Expertise Laboratory of Levan Samkharauli Court Expertise National Bureau (Georgia). We used tandem chromate mass spectrometry – device: *Agilent Technologies 7000 GC/MS/MS Tripe Quad;* column - *Elite 5-MS; 30MX250 µm X 0,25 µm;* furnace temperature - *600C-3100C (program regime);* injector temperature - *250^0C;* transfer line temperature - *310^0*; airborne – helium 1ml/m, ionization source - *El-70 ev*; scanning regime - *TIC*. For the purpose of identification of the target substance in the object under study, mass spectrums of the peaks existing on chromatographs were compared with the mass spectrums of the substances existing in the database *(NIST 2011*).

For studying the activities on cancer cells with in vitro testing, we prepared water and methanol extracts form Ajara and Ajara-Lazeti endemic plant species distinguished by high content of biologically active substances. We also obtained cardiac glycoside-enriched fractions and liposome nanoparticles containing dry methanol extracts.

In accordance with the solvent evaporation method, liposome nanoparticles were prepared from dry methanol extracts obtained from *Erysimum contractum* with cytotoxic activity. For the preparation of liposome nanoparticles, we used different kind of solvents, polymers, surfactants and cell membrane-type substance – lecithin. There were prepared 1) chloroform phase containing total mix of alkaloids 0.1gr; chloroform 0.25 ml; polyvinylopirolidin 0.5gr; lecithin 0.25 gr. 2) Water phase containing 0.50 distilled water; 0.5 – polyvinyl alcohol.

We mixed chloroform and water phases on magnetic stirrer during 24 hours. We got milky-type viscous fluid. We made chloroform volatile from the given fluid and treated the remained mass with ultrasonicator. Then, we identified sizes of given nanoparticles with Zetasizer and Scanning Electronic Microscope. The sizes of given liposome nanoparticles fluctuates from 130 to 230nm.

The studies of liposome nanoparticles containing total alkaloids with selective cytotoxic activity were conducted in vitro testing: 1. In accordance with Resazurine and Hoechst methods at Quebec University, Shikutim city, Canada; 2. in Belorussia, at Minsk State University. Testing was done on cancer cells, in particular, breast cancer cells MCF -7 (human breast adenocarcinoma cell) and skin keratinocytes in accordance with *HaCaTand*ა Resazurine and Hoechst methods. Pictures were taken with a microscope equipped with fluorecentic digital camera Axiovert 25 (Ceis, Germany).

Statistical analysis of outcomes was conducted with the help of standard computer program «Excel». Statistical data is given in $M \pm SD$ way, where M is the arithmetic mean, SD – standard deviation.

2. Botanical description and identification of geographical coverage of Ajara and Ajara-Lazica research endemics

For identifying geographical coverage of Ajara and Ajara-Lazica endemics, obtaining herbarium and experiment materials, botanical description and observing growth and development peculiarities, expeditions were conducted throughout Ajara Black Sea littoral and highlands according to vertical belts. Fieldwork researches were carried out by traditional expedition-excursion method.

We used to collect and process herbarium and experiment materials. Scientists of local floristic field – N. Memiadze, N. Varshanidze, Z. Manvelidze gave us professional assistance in organizing expeditions and material processing. Rich photographic materials were prepared for species. GPS coordinates for each species were identified. Botanical description for each species were prepared based on observations on phenologic phases.

We identified the global conservation status of species according to the International Union for Conservation of Nature (Red List IUCN). Moreover, we found out about the responsibilities of two countries, Georgia and Turkey, for protection and conservation on a global scale of some Ajara and Ajara-Lazica trans-border endemic plant species (vh-very high; h-high; m-medium; l-low).

Ajara endemics are spread mainly in biotypes of mixed and broad-leaved forests, rocks, sand and gravel, subalpine fields, high grass, subalpine forests and valleys, coastal swamps and ponds.

The very center of Ajara and Ajara-Lazica endemics coverage is the gorge of Acharistskali River - broad-leaved forest zone. Most of Colchis, Georgian and Caucasian endemics are developed in highlands of Imereti, Arsiani and Shavsheti ranges that is because the center of their origin is high mountain ecosystems and subalpine zone has all optimal conditions for their existence and the center of origin of Ajara and Ajara-Lazica endemics is the Acharistskali river gorge (Memiadze ., 2005:20).

We identified the coverage areas for each species and most of their GPS coordinates:

Angelica adzharica M. Pimen. *IUCN* status: *CR (vh).* coverage: subalpic zone. Arsiani range, Goderdzi pass, Danisparauli, Ghorjomi, it can be also found out near road and forest edges. According to A. Dimitrieva's observation, they are distinguished with vegetation renewal type – one can find the said plant in much quantities and sizes across roads than in sand and gravel places.

Centaurea adzharica alb.(=c.dmitriewiae D.sosn.) IUCN status : *CR (vh).* coverage: Acharistskali gorge, near dry forests of pine-oak foresrs, in the vicinities of Makhuntseti village, Keda, Shuakhevi, Zamleti, Alme, Khulo. N41^{0}37$^{/}$716$^{//}$, E 041^{0}17$^{/}$812$^{//}$, H 739 m. TLC-

Erysimum contractum Somm.et Lev. IUCN status : *EN (h).* coverage: Acharistskali gorge, Keda, Shuakhevi, Khulo N41$^{0/}$37$^{//}$755 E 042^{0}12$^{/}$624$^{//}$ H464 m. TLC+

Psoralea acaulis Stev. var.adzharica . *IUCN* status : *CR (vh).* coverage: from coastal terraces to subalpine zone, mostly in the middle zone, dry slopes, rocks, bushes, sometimes across roads and in plantations; Kobuleti, Kokhi, Keda, Khulo, Chvana, Danisparauli, Sarbiela Mountain Z. D. 2400 m. N41^{0}71$^{/}$969$^{/}$, E 042018$^{/}$118$^{/}$, H 1800m. TLC +

Ranunculus ampellophylus Somm. et Lev. *(= R.capadocicus Wild.)*. *IUCN* status: *CR (vh).* Coverage: from coastal hills (Sarpi, Tsikhisdziri, Mttirala) to the whole forest zones 2200m above the sea level. They are typical of beech, fir and spruce forests. Sarpi, Tsikhisdziri, Mttirala.

Rubus adzharicus Sanadze. *IUCN* status: *CR (h).* coverage: from coastal terraces to subalpine zone, slopes, rocky places of forests, together with other species of Rubus.

Amaracus rotundifolius(=Origanum rotundifolium). IUCN status:VU (m). coverage: Acharistskali River gorge, on slopes and dry rocks: Lower Chvana, Shuakhevi, Shubani, Khulo-Tago, Khikhadziri, Ghorjomi, Alme. Under protection N41$^{0/}$27$^{//}$296, E 042014$^{/}$799$^{//}$, H 529 m. TLC-

Astragalus adzharicus M.Pop. *IUCN status: EN (h).* coverage: lower and middle forest zones, rocks, dry places of pine and oak forests, mainly in the gorges of Acharistskali River basin. Keda, Chvana, Shuakhevi, Ghorjomi, Alme, Khulo, Khikhadziri, etc. N41$^{0/}$37$^{//}$755, E 042^{0}12$^{/}$624$^{//}$, H 464 m. TLC+

Astragalus sommieri Freyn. *IUCN status: VU (m).* coverage: on the rocks of the Acharistskali River gorge in Keda and Khulo, pine and oak forest zones. N41^{0}32$^{/}$578$^{//}$, E 042^{0}14$^{/}$970$^{//}$, H 544 m. TNC.

Hypericum nordmanni Boiss.(=*H. montbretii Spach.)* coverage: upper forest belt and subalpine zone; forest edges, bushes, fields further from the forests, 2000m above the sea level. $N41^{0}47^{/}199^{//}$, $E\ 042^{0}11^{/}465^{//}$, H 2315 m. TLC +

Hypericum ptarmicifolium var.adzharicum *(=H. Orientale L.)*. Coverage: 2000 – 2400m above the sea level. Dry rocks, sand and gravel slopes; pine and oak forests of Acharistskali gorge; Makhuntseti, Keda, Chvana, Shuakhevi, Khulo, Khikhadziri. It is rare in Kintrishi gorge and subalpine: Chirukhi, Sarichairi, Tbeti, Sarbiela. It is decorative and medicinal plant.

Linaria adzharica Kem. Nath. (=*L.genistifolia ssp. artvinensis Davis.). IUCN status: EN(h).* Coverage: rocky places of Acharistskali gorge: vicinities of Khichauri, Shuakhevi, Zamleti, Alme, Khulo. We recorder the following coordinates: $N41^{0}38^{/}368^{//}$, $E\ 042^{0}09^{/}171^{//}$, H 351 m. TLC +

Osmanthus decorus Boiss. et Bal. Kasapligii.(=*Phillyrea medwedewii Sred.). IUCN* status: VU(I). $N41^{0}32^{/}578^{//}$, $N41^{0}34^{/}319^{//}$, $E\ 041^{0}52^{/}099^{//}$ H 133 m. TNC+ coverage: coastal lowlands and lower belt of mountain; leaved forest rich in chestnut and evergreen sub-forest; forest edges, stone and gravel places; Tiker forest industry, Chakhati, Tsikhisdziri, Khala, Tskavroka, Sarpi, Botanical Garden, Makhuntseti, Agara. The plant is decorative and cultivated in some places.

Primula megaseifolia Boiss. et Bal.ex Boiss. *IUCN* status: EN(h). Coverage: mostly in beach forests with evergreen sub-forests. Coastal slopes 1200 above the sea level. Chakvistskali and Korolistskali slopes, Dagva, Kinkisha, gorges of affluences of Acharistskali and Chorokhi vicinities. The plant is decorative, green in winter and under protection.

Quercus dschorochensis (C.Coch.) Menitsky.(= Q. Petraea Liebl.ssp dschorochensis (C.Koch.)Menitsky. IUCN status: VU(I). Coverage: widespread on dry stony slopes of Acharistskali gorge frequently together with pines; several of them can appear in fir forests 1300 – 1400 m. above the sea level. Its hard wood has industrial importance and the forest itself is anti-erosive. The plant is under protection and frequently deformed into bushes due to massive usage of its stems.

Rhododendron ungernii Trautv. IUCN status: VU(I). Coverage: from middle, sometimes lower belts of forests (Chikuneti vicinities 450m above the sea level) to upper zones of forest (1900m above the sea level), mainly in the gorges of Ajara-Imereti and Ajara-Shavsheti in forms of dense rhododendrons well developed in warm humid coastal gorges of Korolistskali and Chakvistskali, 1000m above the sea level, where trees are found more than 8 meters high and 30cm diameters growing horizontally on slopes of narrow gorges in forms of pure rhododendrons as well as mixed with cherry laurel, rhododendron ponticum, Betula medwedewii, etc. The plant is decorative, poisonous and requires protection.

Rhododendron smirnowii Trautv. *IUCN status: EN(h).* coverage: middle belt of forest, 900-1600 m above the sea level, Keda region, in the vicinities of Namonastrevi and Satevzio villages , on the slopes of two gorges covered with beech and beech-fir forests, dense sub-forest of ungerni and rhododendron ponticum; rocks with forests; together with oriental fir trees. The plant is highly decorative and all exemplars from five places require protection.

Rhynchospora caucasica Vahl. IUCN status: EN(h). coverage: Coastal peat wetlands in the vicinities of Ochkhamuri and Kobuleti.

Scrophularia chloranta Ky et Boiss. Coverage: upper and subalpine zones of forest; across deformed tress, in bushy places, frequently near cattle housing, fields further from forests. The plant is a weedy type. The whole Ajara highlands. $N41^{0}35^{/}523^{//}$, $E\ 042^{0}35^{/}172^{//}$, H 2181∂TLC+ Beshumi border line, stone-gravel slopes, subalpine slopes.

Skutelaria pontika C.Koch**.** Coverage: subalpine rocks and stone-gravel places; widespread in Khino, Taginauri, Sarbiela and Tbeti mountains. N41$^{0/}$45$^{//}$526, E 42^{0}11$^{/}$736$^{//}$, H 2186 m. North exposition, stone-gravel slopes.

*Seseli foliosum (*Somm. et Lev.) Mand. Coverage: lower belt of forest. Slopes of Ajaristskali gorge in the vicinities of Ajaristskali, Makhuntseti, Keda, Zesopeli villages. The plant is decorative and very rare. It requires protection. N41^{0}34$^{/}$319$^{//}$, E 041^{0}52$^{/}$099$^{//}$, H 133 m.TLC+

The thesis includes botanical descriptions of each species, analyzes vital forms of plants, their height, morphological descriptions of vegetative (root, stem, leaf) and generative organs (flower, fruit, seed), different types of flowers, flower clusters, leaves, sticking process, etc.

Table №1 shows only vital form and height of research species and its rythmology group.

Table №1

Ajara and Ajara-Lazica research Endemics Plants Species vital form and rythmology group and height

№	Species	Vital form and rythmology group	Height (m)
1	*Angelica adzarica*	Perennial grass, summer green	0,5-2,5
2	*Centaurea adzarica*	Perennial grass, summer - wintergreen	0,15-0,30
3	*Erysimum contractum*	Perennial grass, summer - wintergreen	0,15-0,30
4	*Psoralea acaulis*	Perennial grass, summergreen	0,70-1,30
5	*Ranunculus ampellophylus*	Perennial grass, summergreen	0,20-0,60
6	*Rubus adzaricus*	Perennial half-evergreen creeping bush	2 -…
7	*Amaracus rotundifolius*	Perennial grass, summergreen	0,15 - 0,30
8	*Astragalus adzaricus*	Perennial grass, summergreen	0,10- 0,25
9	*Astragalus sommieri*	Perennial grass, summergreen	0, 20
10	*Hypericum nordmanni*	Perennial grass, summergreen	0,60
11	*Hypericum ptarmicifolium*	Perennial grass, summergreen	0,10-0,35
12	*Linaria adzarica*	Perennial grass, summergreen	0,20-0,40
13	*Osmanthus decorus*	evergreen small bush or tree	2 - 4
14	*Primula megaseifolia*	Perennial grass, summer - wintergreen	0,25
15	*Quercus dschorochensis*	Deciduous tree	15-20
16	*Rhododendron ungernii*	Evergreen bush	2-6 მ
17	*Rhododendron smirnowii*	Evergreen bush	3-4 მ

18	*Rhynchospora caucasica*	Perennial grass, summergreen	0,30-0,80 ∂
19	*Scrophularia chloranta*	Perennial grass, summergreen	0,80-1 ∂
20	*Scutelaria pontica*	Perennial grass, summergreen	0,15-0,30 ∂
21	*Seseli foliosum*	Perennial grass, summergreen	0,40-0,75 ∂

3. Growth and development characteristics of Ajara and Ajara-Lazica endemics

We have studied characteristics of growth and development of 11 species of Ajara and Ajara-Lazica endemics in nature. These species are the followings: *Rubus adzharicus; Osmanthus decorus*; *Centaurea adzharica; Erysimum contractum; Psoralea acaulis; Ranunculus ampellophylus; Amaracus rotundifolius; Astragalus adzharicus; Linaria adzharica; Primula megasaefolia; Seseli foliosum* (based on our investigation, these species are especially distinguished by the content of bioactive substances and have perspective for further usage. See below). Our objective was to identify the characteristics of phenologic development of research species, which is a way to reveal the dynamics of phenologic phases.

We carried out phenologic observations in 2015-2017, from the beginning of March until November.

As shown in the table №1, all research objects are perennial grasses except *Osmanthus decorus, Rhododendron ungernii, Rhododendron smirnowii, Quercus dschorochensis, Rubus adzaricus.* We studied the characteristics of phenologic development through observing virgin, generative and death periods of their life cycle. We analyzed basic stages of morphogenesis. New buds of perennial grass species are formed from renewed shoots locating on the root or rootstock, at the bottom of stem. Aboveground organs mostly die or disappear at the end of vegetation, but Erysimum is an exception.

The observation was carried out on the following phenophases of research perennial grass species: 1) plant awakening, vegetation – beginning of growth of stem, leaf and vegetative organs; 2) buds, butonization period; 3) beginning of flowering, massive blooming, end of blooming; 4) fruit and seed formation; 5) fruit and seed ripening; 6) end of vegetation – death of aboveground organs (or hibernation).

We determined basic phenol-intervals in research grasses: 1) the length of vegetation (the number of days and months) – from the activation of shoots and the development of aboveground organs until their death; 2) fruit-bearing period – from the end of blooming until fruit and seed ripening period.

The vegetation process of most of research plants starts when the average air temperature is not less than 2-7^{0}C. However, best conditions for their vegetation is 20-25 ^{0}C. We had an opportunity to observe shoot morphogenesis in nature - the development of stem, leaves, shoots, flowers, etc. formed in shoot apical meristem; sequential formation of leaves; development of primordiums, formation of leaf flanks until their full growth.

We carried out the observation of hardwood species - *Osmanthus decorus* on the following phenol-phases: 1) plant awakening – vegetation; 2) shoot development, leaf formation, the end of growth; 3) the beginning and the end of blooming; 4)fruit formation, ripening, the end of fruit bearing; 5) leaf color change; 6)the beginning and the end of leaf fall.

We identified the phenointervals: 1) the length of vegetation (the number of days from the beginning of vegetation until its end); 2) the length of blooming – (the number of days from the beginning of blooming until its end; 3) fruit-bearing period (the number of days from the end of blooming until fruit ripening); 4) the length of leaf fall (the number of days from the beginning of leaf fall until the end).

The phenophases of growth, vegetative organs development, blooming and fruit bearing depend on air temperature, humidity and precipitation. The dissertation includes climatic data - air temperature, precipitation and relative humidity according to basic coverage centers of endemics. Phenophases of research species, especially blooming depends on air temperature and precipitation – blooming period is shorter in warm and dry weather according to years, while the said phenophase is longer in wet and cold weather.

In full doctors work based on vegetative and generative development analysis, we gave theoretical descriptions of each phenophase for each species. The tables represent phenodates in dynamics for each phenophase according to years as well as phenointervals according to annual growings of hardwood species. We created phenospectrum of 11 species. We provided general average records for each species based on our observations and conditions and dates of growth and development phenophases of research objects. In the annotation, there are given tables and diagrams about phenodates (according to months) and short descriptions of the beginning of vegetation, the beginning and the end of blooming, the beginning and the end of fruit-bearing and the end of vegetation.

Basic phenophases and phenointervals (according to months) of research species growth and development are shown in the table №2.

Table №2

Basic phenophases and phenointervals of research species of
Ajara and Ajara –Lazica endemics

№	species	Vegetation duration (months)	Blossom duration (month)	Fruit and seed ripening period
1	Osmanthus decorus	April-October	August-September	October
2	Centaurea adzharica	March-September	May-June	October
3	Erysimum contractum	March-August	March- May	May-June
4	Psoralea acaulis	April-November	April-June	June-July
5	Ranunculus ampelophyllus	March-July	April-June	June-July
6	Rubus adzharicus	April-November	May-August	July-October
7	Astragalus adzharicus	March-August	April-May	May-June

8	Linaria adzharica	April-October	April-September	August-September
9	Primula megaseifolia	January-March	January-February	March
10	Amaracus rotundifolius	April-November	July-September	September- October
11	Seseli foliosum	March-October	May-August	September

As shown in the table, *Psoralea acaulis, Rubus adzharicus, Amaracus rotundifolius; Seseli foliosum* are distinguished by long period of vegetation, approximately 8 months and *Primula megaseifolia* is distinguished with short period of vegetation, approximately 3 months. The length of vegetation for the rest of species is 5-7 months. Two of research species - *Rubus adzharicus* and Erysimum contractum preserve aboveground organs and they are classified as wintergreen plants.

Linaria adzharica, Rubus adzharicus and *Seseli foliosum* are characterized with the longest blooming period, approximately 6 months. The blooming of the rest of species last 2-3 months.

Fruits and seed are ripened generally in summer and autumn except *Primula megaseifolia,* which seed are ripen in spring.

Osmanthus decorus is an evergreen hardwood plant. It is characterized with the change of its leather-like leaf color in May, spring and leaf fall occurs in June-August.

As we mentioned above, Ajara and Ajara-Lazica endemics extremely depend on air temperature. When winter is mild and the temperature is higher than normal, they sometimes start vegetation processes one month earlier or more. We carried out observation during the expedition in 2018 and it was interesting that due to high temperature of spring 2018, the vegetation of grass species began 20-30 days earlier than in 2015-2017 conditioned by high temperature in spring and too much precipitation.

4. The study of Ajara and Ajara-Lazica endemics on the content of bioactive substances

4.1. Screening of Ajara and Ajara-Lazica endemics on the content of basic classes of bioactive substances (alkaloids, flavonoids, coumarins).

The aim of this stage of research was studying chemical content of 21 Ajara and Ajara-Lazica endemics and their screening on the content of basic classes of bioactive substances such as alkaloids, flavonoids, coumarins with qualitative reactions and thin-layer chromatography methods. The outcomes of the said study is given in the table №1.

Table №3

Screening outcomes of Ajara and Ajara-Lazica endemics on the content of bioactive substances

№	Species	Biologically active compounds		
		alkaloids	flavonoids	coumarins
1	*Angelica adzharica*	+		+

2	*Astragalus adzharicus*	+	+	+
3	*Astragalus sommieri*	+	+	+
4	*Amaracus rotundifolius,*			
5	*Centaurea adzharica*	+	+	+
6	*Erysimum contractum*	+	+	
7	*Hypericum nordmanni*	+	+	
8	*Hypericum ptarmicifolium*		+	
9	*Linaria adzharica*	+		
10	*Osmanthus decorus*	+		
11	*Psoralea acaulis var.adzharica*	+		
12	*Primula megasaefolia*			
13	*Rhynchospora caucasica*			
14	*Rhododendron smirnovii*	+	+	
15	*Rhododendron ungernii*		+	
16	*Ranunculus ampellophylus*			
17	*Rubus adzharicus*		+	
18	*Scutellaria pontica*	+	+	
19	*Scrophularia chloranta*	+		
20	*Seseli foliosum*	+		+
21	*Quercus petra var. Dshorochensis*	+	+	

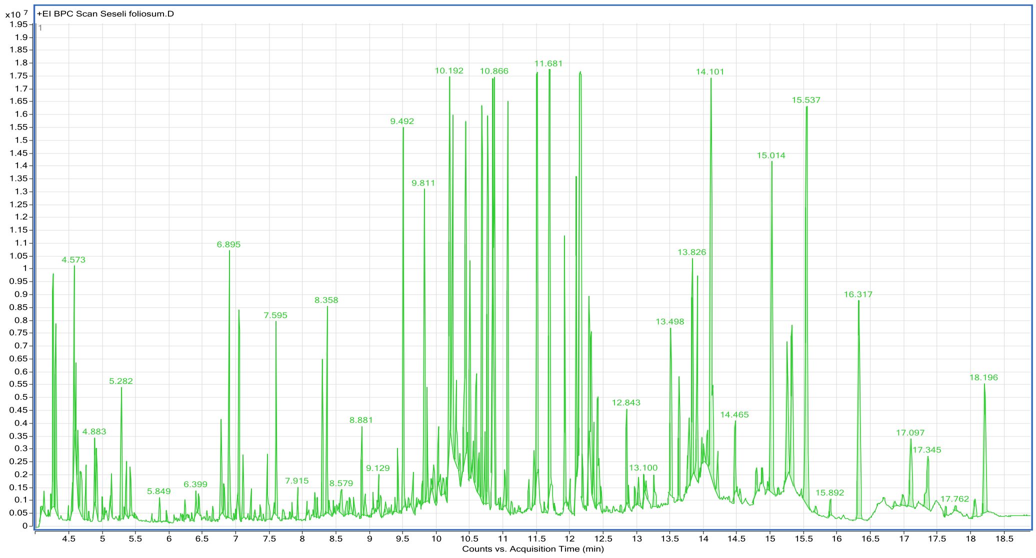
The second stage of the research is studying the content of bioactive substances according to gas chromate mass spectrometry (GC-MS-MS) method.

4.2. Studies of Ajara and Ajara-Lazica endemics on the content of bioactive substances according to gas chromate mass spectrometry (GC-MS/MS) method

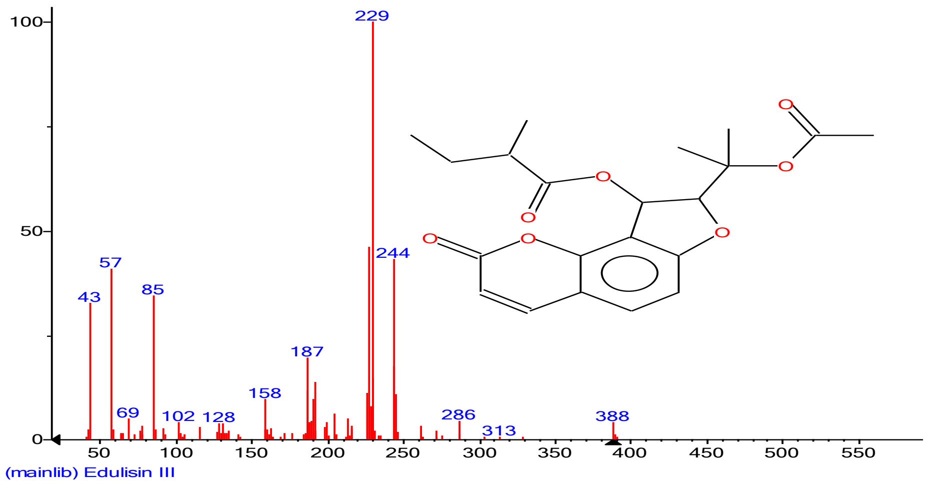
Ajara and Ajara-Lazica hardwood and grass endemic plant species were investigated with the help of gas chromate mass spectrometry (GC-MS/MS) method. Most of primary and secondary compounds of biosynthesis revealed by the investigation coincide with the library database of the National Institute of Standards and Technology (NIST). The thesis presents tables and diagrams of GC-MS-MS research outcomes of 21 species. Some compounds found out in the content of endemics are used in food industry, medicine, perfumery and pesticides manufacturing. They have antioxidant, anti-pneumonic, antimicrobial and anticancer activities.

GC-MS-MS research outcomes are shown with tables, diagrams, chromatograms. Although we have set only two main examples of Ajara-Lazica endemics - ***Seseli foliosum*** and Ajara endemics - ***Erysimum contractum*** in the annotation, total outcomes of the said subject about all the species are totally illustrated on 60 pages in the thesis and it’s impossible to present all of them in the annotation.

Ajara-Lazica endemics, ***Seseli foliosum*** is quiet rich in primary and secondary compounds of biosynthesis (pic.1). There were identified 53 compounds (table 8) in total. The shortest retaining period has alpha.-Phelland… [RT 5.08], while the longest has gamma.-Sitoste [RT 20.901]. The following bioactive substances were detected: a-pinene from terpenoids, carene a-murolene, cadinene; criptone, b myrcene – from monoterpenic compounds; kesane, junenol, y-atlantol from sesquiterpenic compounds; Falcarinol from fatty alcohols; Edulisin III, Z-cridimine, E-cridimine from coumarines; malic acids from organic acids; oleic acid from fatty acids; benzoic acid from phenol acids; stigmasterol from steroid compounds; trans-verbenol, a-bergamot, bicyclogermacrene from bicyclic monoterpenids; spathulenol, a-copaen, b-copaen, a-ilangene, b-ilangene, kesane from tricyclic sesquiterpenids; pseudolimonene, 3-carenine from cyclic terpenoids; Benzofuran products; coumarines, archangelicine from polyphenol compounds, naphthalene products from fatty alcohols, etc. Dominant substances from monoterpenic compounds is krypton, junenol from sesquiterpenic compounds (pic 4, table 4), falcarinol from fatty alcohols (pic.5, table 5), edulisin III from coumarines (pic.2, table 2), Z-crimidine (pic.6, table 6), E-Cridimine (pic.7, table 7).



Pic.1. *Seseli foliosum* chromatographic-mass spectrometry

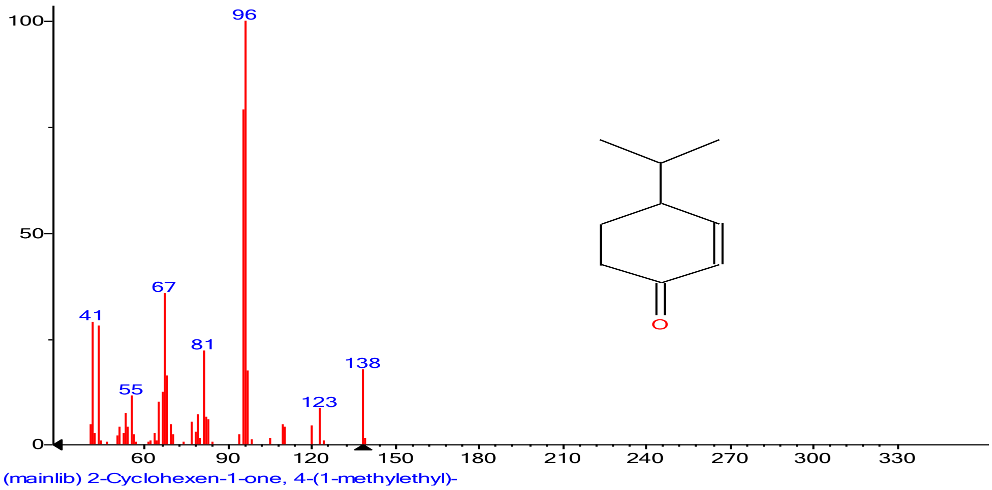


Pic. 2. *Seseli foliosum edulisin III chromatograph*

Table 4

Seseli foliosum GC /MS/MS characteristics of *edulisin III*

Name	Formula	MW	Exact Mass	CAS	NIST	ID
Edulisin III	$C_{21}H_{24}O_7$	388	388.152203	158515-39-0	412439	200515

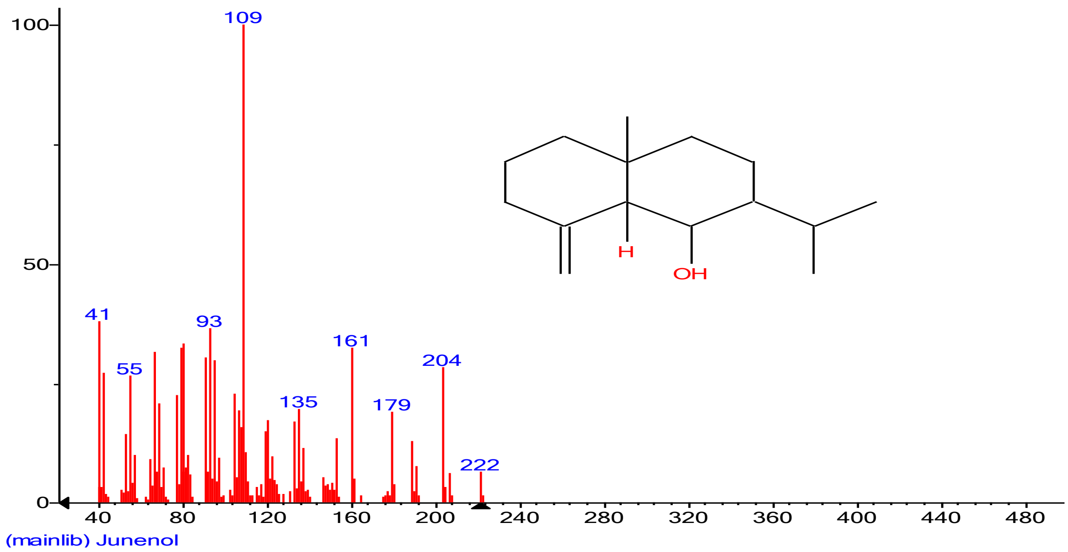


Pic.3. *Seseli foliosum cyclohexane* -1 *4-(1-methylethyl*)- chromatograph

Table 5

Seseli foliosum GC /MS/MS characteristics of cyclohexane-1, *4(1-methylethyl)*

Name	Formula	MW	Exact Mass	CAS#	7 NIST:	ID
2-Cyclohexen-1-one, 4-(1-methylethyl)- Synonyms: 1.4-Isopropylcyclohex-2-enone 2.Crypton 3.Cryptone	$C_9H_{14}O$	138	138.1044655	500-02-7	384023	69389

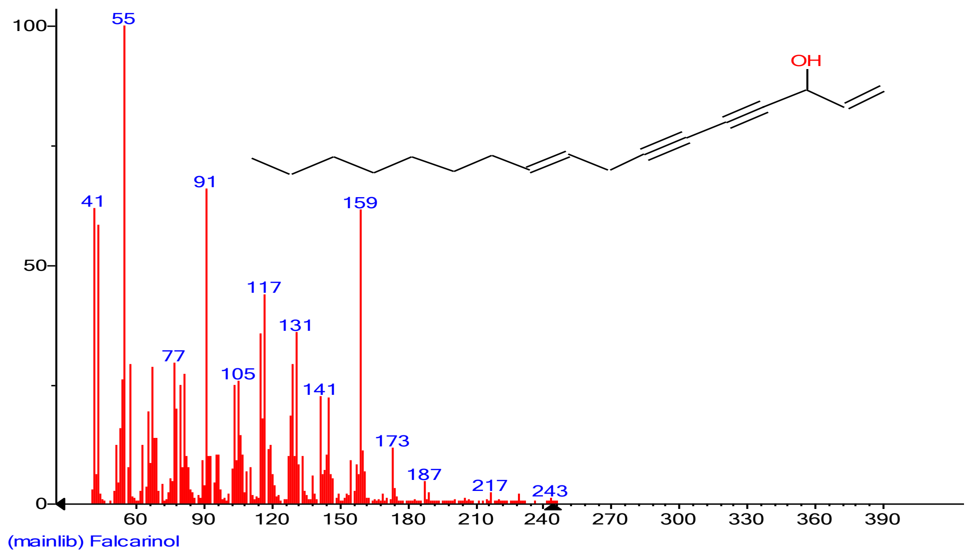


Pic.4. *Seseli foliosum* junenol chromatograph

Table 6

Seseli foliosum GC /MS/MS characteristics of *junenol*

Name	Formula	MW	Exact Mass	CAS	NIST	ID
Junenol	$C_{15}H_{26}O$	222	222.198365	472-07-1	413704	85568

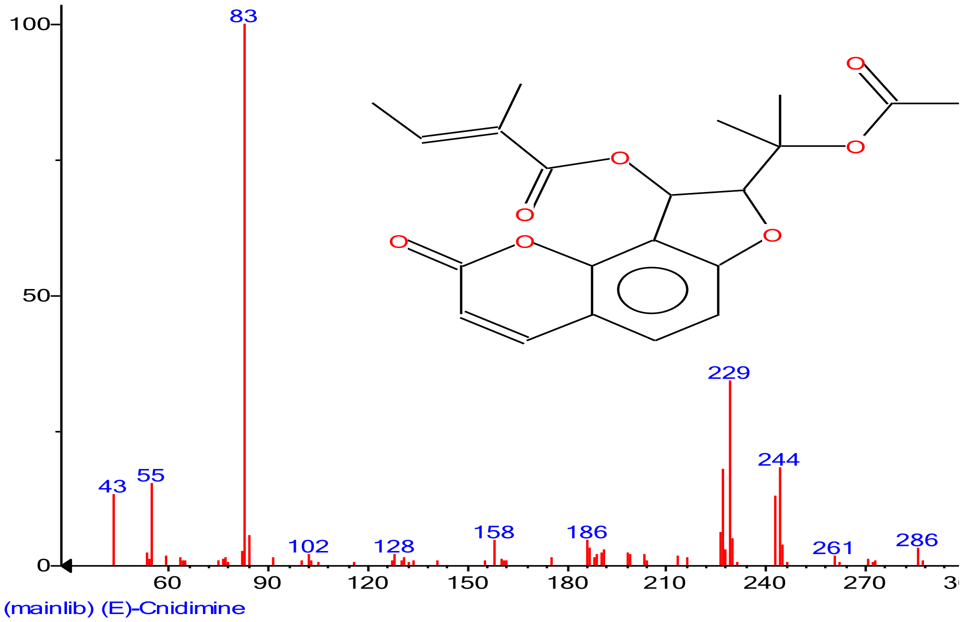


Pic.5. *Seseli foliosum falcarinol* chromatograph

Table 7

Seseli foliosum GC /MS/MS characteristics of falcarinol

Name	MW	Exact Mass	CAS	NIST	ID
Falcarinol	244	244.182715	21852-80-2	112661	20979

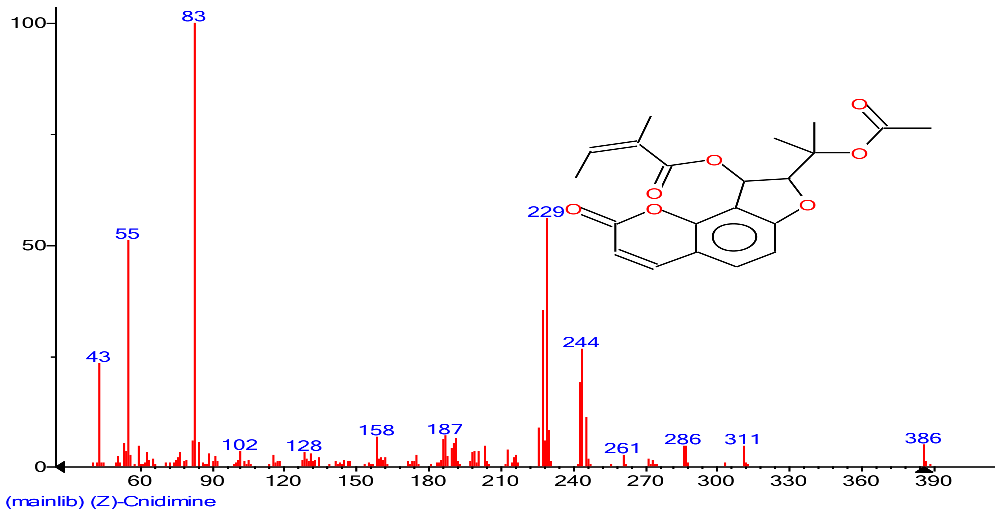


Pic. 6. *Seseli foliosum ,E - crimidine chromatograph*

Table 8

Seseli foliosum, E- GC /MS/MS characteristics of crimidine

Name	Formula	MW	Exact Mass	NIST	ID
(E)Cnidimine	$C_{21}H_{22}O_7$	386	386.136553	412441	53378



Pic.7 . *Seseli foliosum, Z* - crimidine *chromatograph*

Table 9

Seseli foliosum Z - GC /MS/MS characteristics of crimidine

Name	Formula	MW	Exact Mass	CAS	NIST	ID
(Z)Cnidimine	$C_{21}H_{22}O_7$	386.136553	386.136553	15591-75	412440	53377

Table 10

Seseli foliosum – research outcomes with GC-MS-MS

Name	Formula	Score	RT	m/z	Base	Mass
.alpha.-Phellandrene	$C_{10}H_{16}$	82.92	5.08	93	93	136.1
Cyclopetane,1-methyl-3-(1-methylethyl)-	C_9H_{18}	62.2	5.878	55.1	55.1	126.1
Octen-1-ol,acetate	$C_{10}H_{18}O_2$	74.31	6.106	43.1	43.1	170.1
3-Octanol,acetate	$C_{10}H_{20}O_2$	67.57	6.206	43.1	43.1	172.1
3-Oxatricyclo[4.1.1.0(2,4)]octane,2,7,7-trymethyl-	$C_{10}H_{16}O$	73.11	6.277	109	109	152.1
1,7,7-Trimethylbicyclo[2.2.	$C_{10}H_{16}O$	86.99	6.385	108	108	152.1

1]hept-5-en-2-ol						
Bicyclo[3.1,1]hept-3-en-2-ol,4,6,6-trimethyl-,[1S-(1.a..	$C_{10}H_{16}O$	86.71	6.546	109	109	152.1
6-Bytl-1,4-cycloheptadiene	$C_{11}H_{18}$	57.51	6.611	69	69	150.1
2-Cyclohexen-1-one,4-(1-methylethyl)-	$C_{9}H_{14}O$	83.78	6.888	96	96	183.1
Cyclohexene,4-ethyl-3-ethylidene-	$C_{10}H_{16}$	63.63	6.954	79	79	136.1
(.+/-.)-Lavandulol, acetate	$C_{12}H_{20}O_2$	76.09	5.151	93	93	196.1
2-Ethyl-4-Methylanisole	$C_{10}H_{14}O$	65.72	17.048	107	107	150.1
2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-	$C_{10}H_{16}O$	64.62	7.084	109	109	152.1
Benzene,1-ethyl-4-nitro-	$C_{8}H_{9}NO_{2}$	66.83	7.464	151	151	151.1
1,3-Cyclopentadiene, 1,3bis(1-methylethyl)-	$C_{11}H_{18}$	57.14	7.645	91	91	150.1
2-Adamantanol, 2-(bromomethyl)-	$C_{11}H_{17}BrO$	69.43	7.787	151	151	244
1,3-Cyclohexadiene, 1methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	52.89	7.885	121	121	136.1
2,3-Dimethylphenoxyacetic acid	$C_{10}H_{12}O_{3}$	77.85	7.979	105	105	180.1
Copaene	$C_{15}H_{24}$	91.84	8.264	161.1	161.1	204.2
Benzene,1,2,4-trmethyl-	$C_{9}H_{12}$	65.78	5.225	105	105	120.1
Cyclobuta[1,2;3,4]diclopentene, decahydro-3a-met…	$C_{16}H_{24}$	65.55	8.337	81	81	204.2
Bicyclo[3.1.1]hept-2-ene-2,6-dimethyl-6-(4-methyl-3-…	$C_{15}H_{24}$	91.01	8.6	119	119	204.2
Isocaryophillene	$C_{15}H_{24}$	68.32	8.653	69	69	204.2

Spiro[5.5]undec-2-ene,3,7,7-trimethyl-11-metylene-…	$C_{15}H_{24}$	81.55	8.809	119	119	204.2
Silane,trimethyl[(1-methylexyl)oxy]-	$C_{10}H_{24}OSi$	63.13	8.9	73	73	188.2
1H-Cyclopenta[1,3cyclopropa[1,2]benzene,octahydr…	$C_{15}H_{24}$	91.1	8.985	161.1	161.1	204.2
Cyclohexane,1-ethenyl-1methyl-2-(1-methylethenyl	$C_{15}H_{24}$	64.93	9.075	121	121	204.2
n-Propylcyclopropanemethylamine	$C_{7}H_{15}N$	58.41	5.295	55.1	55.1	113.1
Isocaryophillene	$C_{15}H_{24}$	80.8	9.176	69	69	204.2
Bicyclo[2.2.1]heptan-2ol,1-methyl-,acetate	$C_{10}H_{16}O_{2}$	67.65	9.316	126	126	168.1
3,6,10,13-Tetraoxa –2,14-disilapentadecane,2,2,14,1.	$C_{13}H_{32}O_{4}Si_{2}$	57.16	9.609	73	73	308.2
1,2-Ethanediol,1,2-di-2-pyridimyl-	$C_{12}H_{12}N_{2}O_{2}$	50.94	9.647	109	109	216.1
2,2-Dimethyl-5-[2-(2-trymethylsilyletoxymetoxy)-pro…	$C_{15}H_{30}O_{5}Si$	70.83	9.718	73	73	312.2
3,6-Dioxa-2,7-disilaoctane,2,2,7,7-tertamethyl-4,5-di…	$C_{20}H_{30}O_{2}Si_{2}$	57.46	9.813	73	73	358.2
3-Methyl-2-butenoic acid,2,6-dimethylnon-1-en-3-yn-…	$C_{16}H_{24}O_{4}$	58.88	9.92	83	83	248.2
Ethane,1-vinylthio-2[(trimethyloxy)ethylthio]-	$C_{9}H_{20}OS_{2}Si$	54.45	10.058	87	87	236.1
.alpha.-Phellandrene	$C_{10}H_{16}$	82.65	5.355	93	93	136.1
Pentanoc acid,trymethyl ester	$C_{8}H_{18}O_{2}Si$	57.85	10.144	73	73	174.1
Propanetriol,2-methyl-,tris-o-(trimethylsilyl)-	$C_{13}H_{34}O_{3}Si_{3}$	56.38	10.262	73	73	322.2

Pentanoic acid, trimethylsilyl ester	$C_8H_{18}O_2Si$	50.75	10.424	73	73	174.1
2,5-O-Methylene- D-mannitol	$C_7H_{14}O_6$	51.44	10.665	73	73	194.1
3,6,10,13-Tetraoxa-2,14-disilapentadecane,22,14,1..	$C_{13}H_{32}O_4Si_2$	58.26	10.813	117	117	308.2
6-Octen-1-ol,3,7-dimethyl-,propanoate	$C_{13}H_{24}O_2$	59.05	10.863	117	117	212.2
Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	92	11.9	117	117	328.3
3,3-Dimethoxy-6,6-dimethyl-cyclohexa -1,4-diene	$C_{10}H_{16}O_2$	68.29	5.47	137	137	168.1
8,8-Dimethyl-2-oxo-2,8,9,10 – tetrahydropyrano(2,3-)c…	$C_{24}H_{26}O_7$	61.08	15.395	83	83	426.2
8,8-Dimethyl-2-oxo-2,8,9,10 – tetrahydropyrano(2,3-)c…	$C_{24}H_{26}O_7$	74.43	17.362	83	83	426.2
Cholest-5-en-3-ol,(3 alpha)	$C_{27}H_{46}O$	72.83	18.943	95	95	386.4
Benzene,1,2,3,5-tetramethyl	$C_{10}H_{14}$	70.8	5.514	119	119	134.1
.gamma.-Sitosterol	$C_{29}H_{50}O$	79.43	20.901	414.2	414.2	414.4
4-Methyl-2,4-bis(4-trimethylsilyloxyphenyl)pentene-1	$C_{24}H_{36}O_2Si_2$	64.44	22.1	207	207	412.2
Bicyclo[3,1,0]hex-2-ene,4-methyl-1-(methylethyl)	$C_{10}H_{16}$	86.33	5.583	93	93	136.1
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	78.45	5.789	93	93	136.1

21 compounds were identified while studying Ajara-Lazeti endemics ***Astragalus sommieri.*** Trans-2-Hexenyl… [R/T 5] is characterized with the shortest period of retaining. Beta-Amyrin [R/T 21.549] was the last detected. Dominant substances such as halfordin and crimidine were also identified together with carbonic, organic, fatty phenol acids: tartaric, hydroxypyridine tartaric, malic,

benzoic, wine, ferulic, petroseline, a-linolenic acids. Sugars are also identified: xylose, ribitol, glactopyranoside, D-tagatofuranose, D-psicofuranose, D-lyxofuranose, D-pinitol, L-fucopyranose, dulcitol; polyphenolic compounds: (chromen products), coumarines – crimidine and archangelicine; flavonoids: quercetin, kempferol; triterpenoid compounds: campesterol, stigmasterol, B-sitosterol, etc.

24 compounds were identified while studying Ajara-Lazeti endemics ***Astragalus adzharicus*** by GC-MS-MS research. *Beta pineni (R/T5.133)* is characterized with the shortest period of retaining, while *cholesterol trimetily (R/T 19.045)* has the longest period of retaining. Identified compounds are distinguished by various biological activities. Coumarines: furocoumarine, psoralene, isopsoralene; flavonoids: quercetin, kempferol; organic acids: malic acids; fatty acids: palmitin acids; phenolic acids: benzoic and gallic acids; terpenoids: a-pinene, carene, a-murolene, cadinene, etc. Dominant substances are isopsoralen, quercetin, and kempferol.

20 compounds were identified while studying Ajara endemics ***Psoralea acaulis.*** *Cyclopentane, 1 (R/T 7.606)* is characterized with the shortest period of retaining and Silane, dimethyl (R/T 24.409) has the longest period of retaining. The following compounds were detected: organic compounds: malic acids, fumaric acids; fatty acids: caproic and butyric acids; phenolic acids: benzoic acids, hydroxybenzoic acids, vanillic and protocatechuic acids; amino acids: L-valine, L-alanine; ethers: methylethylmalonate; sugar: L- threose, erythropentulose, xylitole, anhydrogalactopyranose, deoxyribose; polyphelonic compounds: furocoumarines – psoralene and angelicine, coumarines – umbelliferone, etc. Dominant substances are psoralene and hydroxycoumarine.

40 compounds were identified while studying Ajara-Lazica endemics ***Scutellaria pontica***. (-/+)Lavandulo.. [R/T 5.149] is characterized with the shortest period of retaining and 6-Fluro -2-trifluo… [R/T 21,749] has the longest period of retaining. Dominant substances are polyphelonic compounds: flavonoids: luteolin, apigenine; organic acids: malic acids, gallic acids; fatty acids: lactic acids; phenolic acids: protocatechuic, gallic acids; sugar: ribofuranose, psichofuranose, indole and isoindole products; aminoacids: L-leucine; terpenoids: a-terpinyl acetate, etc.

55 compounds were identified while studying Ajara-Lazeti endemics ***Quercus petra var. Dshorochensis***. Nonane, 2-meth [R/T 5.229] is characterized with the shortest period of retaining, while Benzamide, 4-flu [R/T 15.706] has the longest period of retaining. The following bioactive compounds are identified: carbon, fatty and phenolic acids: threone, citric, malic, shikimic, traumatic, canine, gallic and palmitin acids; sugar: anhydrydoglucitol, ribitol, arabinofuranose, allofuranose, fructose, galactopyranose, mannopyranose, tagatofuranose, ramnose; benzylquinoline products; cumaroylquinic acids products; pentacyclic triterpenoidic compound – friedeline; polyphenolic compound – catechine, triterpenoidic compounds: friedelan-3-1, 5-0-cumarole-D-chinine acids and traumatic acids.

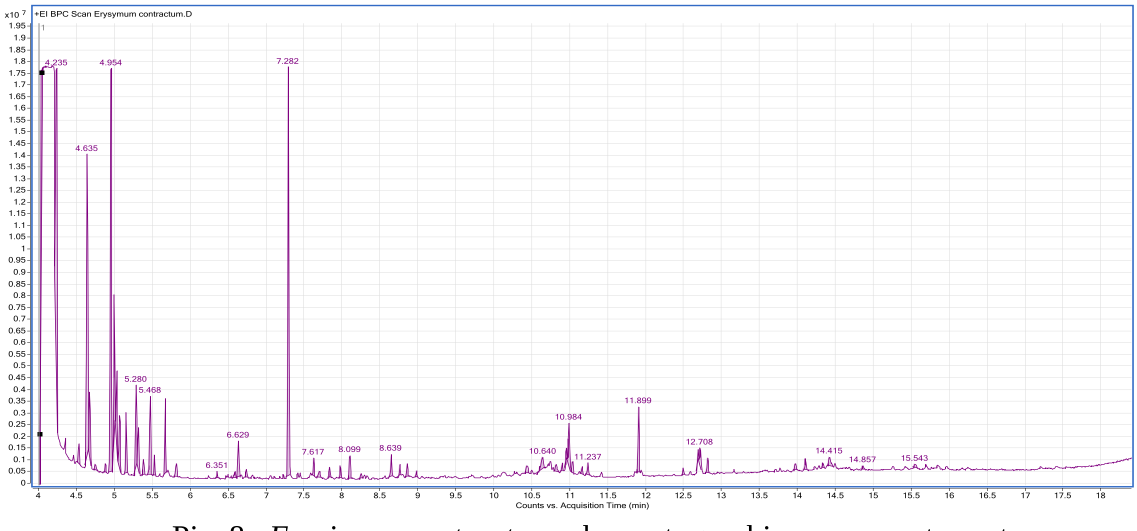
41 compounds were identified while studying Ajara-Lazeti endemics ***Rhododendron smirnovii***. *Hexane,2,4-dim -ƅ [R/T 5.231]* is characterized with the shortest period of retaining and 4-*Methyl-2,4-bis(…. [R/T 25.246]* has the longest period of retaining. It was identified that the plant contains biologically active substance: carbonic, organic, fatty and phenolic acids – lactic, malic, succinic, valeric, citric, chinine, palmitin, liloneline, oxalic acids; aminoacids – L-leucine, sugar: galactopyranose, ribofuranose; sesquiterpenic compound – ledol; benzodihydropyrone products; steroid compounds - a-sitosterol. The dominant substance is a sesquiterpenic compound – ledol.

26 compounds were identified while studying Ajara-Lazeti endemics ***Rhododendron ungernii***. *Undecane, 4,4-d [R/T 5.232]* is characterized with the shortest period of retaining and *4-Methyl-2,4-bis(…[R/T 24.507*] has the longest period of retaining. The following bioactive compounds are revealed: carbonic, organic, fatty and phenolic acids – lactic, malic, citric, chinine, protocatechuic,

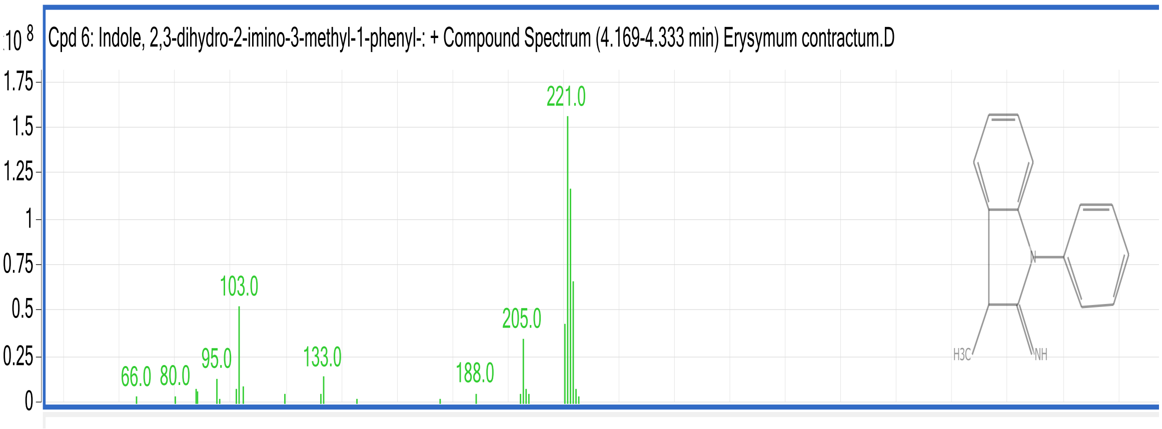
lignoceric, gallic, palmitin, liloneline, oxalic acids; sugar: arbitol, fructofuranose, drohechitole; sesquiterpenic compound – ledol; polyphenolic compounds – flavonoids, catechine; triterpenoidic compounds: lupeol, a-amyrine products, friedelanol, friedeline, ursolic acids; steroid compounds, etc.

32 compounds were identified while studying Ajara-Lazeti endemics ***Linaria adzharica***. *Octane, 1,1-oxy… [R/T 5.919]* is characterized with the shortest period of retaining, while *Cholesterol trim [R/T 19.041*] has the longest period of retaining. The following organic acids are identified: malic acids, hydroxypyridine tartaric acids, succinic; phenolic acids: benzoic acids; sugar: cylose, galactopyranose, methylglucopyranoside, deoxyribose; polyphnolic compounds, catechine. Dominant substances are catechine, hydroxypyridine tartaric acids, etc.

32 compounds were identified while studying Ajara endemics ***Erysimum contractum*** (pic. 8) (table 9)**.** Octane, 1,1-oxy… [R/T 5.919] is characterized with the shortest period of retaining and Cholesterol trim [R/T 19,041] has the longest period of retaining. The following bioactive compounds were detected:carbonic acids: trans-cinnamic acids, malic acids; fatty acids: caproic acids, ketobutyric acids, palmitin acids; phenolic acids – benzoic acids; sugar: D-turanose, methylhexsopyranoside, xylitole, ribofuranose, mannopyranose, arbitol, methylglucose; sulphuric compounds: dimethylsulphone; steroid compounds – a-sitosterol, etc. (pic. 8; 9) (Table 9; 10)



Pic. 8. *Erysimum contractum*, chromatographic-mass spectrometry



Pic. 9. *Erysimum contractum,* indole 2,3 dihydro-2-amino 2-3-methyl-1-fenil chromatograph

Table 11

GC /MS/MS characteristics of *Erysimum contractum* , indole 2,3 dihydro-2-amino 2-3-methyl-1-fenil

Name	Formula	Score	RT	m/z	Base-	MASS
Indole, 2,3-Dihidro-2-imino-3-methyl-1-phenyl	$C_{15} H_{14}N_2$	62,39	4,235	221	221	222

Table 12

GC-MS-MS research outcomes of Erysimum contractum

Name	Formula	Score	Mass	Base peak	m/z	RT
Trifluormethyltri…	$C_4H_9F_3Si$	59,97	142	73	73	4,136
Indole 2,3-dihyd..	$C_{15}H_{14}N_2$	62,39	222.1	221	221	4,235
2-3-2 H-4-Methyl…	$C_4H_6N_2O$	66,17	98	98	4.506	0.212
Thiazole tetrahy	C_3H_7NS	50,7	89	89	89	4,526
Disiloxane hexa…	$C_6H_{18}OSi_2$	78,33	126,1	147	147	4,636
Ethanedioic acid	$C_8H_{18}O_4Si_2$	69,08	234,1	147	147	4,949
Benzene 1- ethyl…	C_9H_{12}	90,53	120,1	105	105	4,995
Benzene1,2,4-t	C_9H_{12}	84,55	120,1	105	105	5,065

Benzene 1,2,3,t..	C_9H_{12}	82,64	120,1	105	105	5,15
Cycloheptane, m…	C_8H_{16}	79,94	112,1	55,1	55,1	5,188
Cis-2-Hexen-1-ol	$C_9H_{20}Si$	66,58	172,1	75	75	5,311
Butane, 2-Pheny…	$C_{13}H_{22}OSi$	54,94	222,1	117	117	5,665
Trimethylsilyl eth..	$C_{12}H_{32}O_3Si_3$	87,1	308,2	147	147	7,288
Butane, 1,2,3-tri…	$C_{13}H_{34}O_3Si_3$	68,99	322,2	116	116	3,244
2-Ethyl (dimeyl…	$C_9H_{22}OSi$	55,87	174,1	75	75	10,143
Benzoic acid,3…	$C_{16}H_{30}O_4Si$ 3	77,21	370,1	193,1	193,1	10,737
Butane,1,2,3,tri.	$C_{13}H_{34}O_3Si_3$	54,41	322,2	73	73	10,812
Beta-DL-Arabinofu…	$C_{15}H_{36}O_5Si_3$	67.08	380,2	73	73	11,236
Hexadecanoic acid	$C_{19}H_4O_2Si$	91,63	328,3	117	117	11,9
Alpha – linolenic	$C_{21}H_{38}O_2Si$	67.28	350,3	73	73	12,714
Octadecanoic acid	$C_{21}H_{44}O_2Si$	87,71	356,3	117	117	12,81
Glucofuranoside	$C_{19}H_{46}O_6Si$	59,58	482,2	73	73	14,099
D-Turanose, hepta	$C_{19}H_{46}O_6Si_4$	59,58	484,2	73	73	14,33
Glucofuranoside	$C_{19}H_{46}O_6Si_4$	52,14	482,2	73	73	15,543
Tetracosamethyl…	$C_{24}H_{72}O_{12}Si_{12}$	73,4	888,2	73	73	16,238
L-Valin,N(2,5…	$C_{21}H_{27}F_6NO_3$	57,82	455,2	241,1	241,1	18,749
Cholesterol trim	$C_{30}H_{54}OSi$	92,68	458,4	129	129	19,045
4-Methyl -2-4 –bis(	$C_{24}H_{36}OSi_2$	58,63	412,2	207	207	23,81

17 compounds were identified while studying Ajara endemics ***Centaurea adzharica*** . *Benzene,1-ethyl… [RT 7.462]* is characterized with the shortest period of retaining and *gamma.-Sitoste [R/T 20.903]* has the longest period of retaining. The plant contains secondary metabolites- organic, fatty and phenolic acids: malic, shikimic, citric, chinine, behenic, myristic, linolenic, monopalmitine acids; sugar- arabitol, deoxyribopyranose, galactopyranose, D-(-)- tagatofuranose, D-(+)- talofuranose,

fructofuranose, ribofuranose; triterpenoidic compounds – b-amyrine products; polyphenoloc compounds – isopsoralene; steroid compounds –stigmasterol, etc.

13 compounds were identified while studying Ajara-Lazeti endemics ***Osmanthus decorus***. *Hexane.3,3,4,4-… RT 5.3* is characterized with the shortest period of retaining, while 4-Methyl-2,4-bis(… [R/T 21.114] has the longest period of retaining. The following biologically active compounds were detected: carbonic, fatty and phenolic acids – linolenic, citric, malic, tartaric, benzoic acids; sugar – xylitol, methyl-lixyfuranoside, methyl-D-glucofuranoside, mannopyraonse; anthracen products; acetoxypropilene; benzylalcohol, indole products; diethyldodecane; diglycerol; phytole, etc.

41 compounds were identified while studying Ajara-Lazeti endemics ***Scropularia chloranta***. *Cyclopentane, 1…. [RT 5.92]* is characterized with the shortest period of retaining, while *Cholesterol trim [R/T19.035]* has the longest period of retaining. The following biologically active compounds were detected: carbonic, organic, fatty and phenolic acids – a-linolene, butyric, lactic, gallic, chinine, malic, succinic, protocatechuic acids; sugar – furanose, arabinofuranose, deoxypentofuranose, turanose, naphtofuranose; pyrole products, etc.

39 compounds were identified while studying Ajara-Lazeti endemics ***Hypericum ptarmicifolium***. 2-Oxabicyclo *[2.2…RT 5.108]* is characterized with the shortest period of retaining, while *4-Methyl-2,4-bis [R/T20.058]* has the longest period of retaining. The following biologically active compounds were detected: carbonic, organic, fatty and phenolic acids: sorrelic, vanillic, protocatechuic, coumaric, palmitin, linolenic acids; tetracyclic sesquiterpenic compounds – grandisine; heteropentacyclic compound – versicolorin; diterpenoid compound – isopimanirol, manole oxide, isopimaral; triteterpenoid compounds - kauren products; polyphenolic compounds – flavonoids: hyperoside, quercetin, rutin; steroid compounds – campesterol, epicampesterol, stigmasterol, etc.

55 compounds were identified while studying Ajara-Lazeti endemics ***Hypericum nordmanni***. 1-Methyltriclyclo] is characterized with the shortest period of retaining, while DI-.alpha.-Tocop… [R/T20.058] has the longest period of retaining. The following biologically active compounds were detected: carbonic, fatty and phenolic acids – vanillic, protocatechuic, benzoic acids, salicil, palmitin, linolene; sugar – aribitol, deyoxypentofuranose, ribofuranose, glucopuranoside; polyphenolic compounds – flavonoids: hyperoside, quercetin, rutin.

55 compounds were identified while studying Ajara-Lazeti endemics ***Rhynchospora caucasica***. *Cyclohexane,et... RT 5.277* is characterized with the shortest period of retaining, while *Benzimidazo[2,1.. [R/T 22.168]* has the longest period of retaining. The following compounds were identified: carbonic, organic, fatty and phenolic acids – malic, hydroxybenzoic, shikimic, vanillic, protocatechuic, syringic, cumaric, ferulic, linolenic, pentanoic products; sugar: deoxyribose, ribofuranose, galactofuranose, erythrofuranose; benzidazol products; lignane-styrenisol; triterpenoid compounds: lupeol, a-amyrine and b-amyrine products; steroid compounds: stigmasterol, a-sitosterol, etc.

33 compounds were identified while studying Ajara endemics ***Angelica adzharica***. *Silane,(2,2-dim… [RT 5.14*] is characterized with the shortest period of retaining, *while 3-Hydroxy-2-but... [RT 20.652]* has the longest period of retaining. The following compounds were identified: carbonic, organic, fatty and phenolic acids: lactic, malic, protocatechuic, chinine, citric, cumaric, palmitin, linolene; sugar – arabitol, fructofuranose, arabifuranose, and other bioactive substances.

55 compounds were identified while studying Ajara endemics ***Rubus adzharicus***. Cyclohexane,et... RT 5.277 is characterized with the shortest period of retaining, while Benzimidazo[2,1.. [R/T 22.168] has the longest period of retaining. The following compounds were identified: carbonic, organic, fatty and phenolic acids – malic, hydroxybenzoic, shikimic, vanillic, protocatechuic, syringic, cumaric, ferulic, linolenic, pentanoic products; sugar: deoxyribose,

ribofuranose, galactofuranose, erythrofuranose; benzidazol products; lignane-styrenisol; triterpenoid compounds: lupeol, a-amyrine and b-amyrine products; steroid compounds: stigmasterol, a-sitosterol, etc.

55 compounds were identified while studying Ajara-Lazica endemics ***Amaracus rotundifolius***. Cyclohexane,et... RT 5.277 is characterized with the shortest period of retaining, while Benzimidazo[2,1.. [R/T 22.168] has the longest period of retaining. The following compounds were identified: carbonic, organic, fatty and phenolic acids – malic, hydroxybenzoic, shikimic, vanillic, protocatechuic, syringic, cumaric, ferulic, linolenic, pentanoic products; sugar: deoxyribose, ribofuranose, galactofuranose, erythrofuranose; benzidazol products; lignane-styrenisol; triterpenoid compounds: lupeol, a-amyrine and b-amyrine products; steroid compounds: stigmasterol, a-sitosterol, etc.

5. The study of Ajara and Ajara-Lazica endemics on cytotoxic activities

The aim of this stage of research was studying methanol extracts of Ajara and Ajara-Lazica endemics rich in bioactive substances (Ajara endemics: Allium adzharicum M.Pop., Angelica adzharica M.Pop., Centaurea adzharica Sosn., Erysimum contractum Somm. Et Levier., Psoralea acaulis var.adzharica, Ranunculus ampellophylus var.adzharica, Rubus adzharicus Sanadze and Ajara-Lazica endemics: Astragalus adzharicus M.Pop.) on cytotoxic activities for the identification of perspective species.

Human skin fibroblasts WS1 (ATCC CRL-1502), lung carcinoma A-549 (ATCC CCL-185), colon carcinoma DLD-1 (ATCC CCL-221) and RAW 264, 7 (ATCC TIB-71) are gained from American type of culture (Manassas, VA, USA). Description of cultures and experiment process are described in ordered sequence in the dissertation. Outcomes are presented below.

A study of cytotoxic action of methanol extracts of Adjara and Adjara-Lazeti endemic plants has been established thatmethanol extract of *Erysimum contractum* has inhibitory activities on lung carcinoma cells (A-549) IC-11,4 ± 0,4 µg/ml, less inhibitory activities on colon carcinoma DLD-1 IC-59 ± 10 µg/ml and it has no toxicity to skin normal fibroblasts (WS-1) unlike IC-121 ± - 32 µg/ml etoposids. Other research samples in some cases have revealed no inhibitory activities or high toxicity. For the identification of compounds responsible for cytotoxic activities, the investigation was continued on new galenic preparation received from Erysimum contractum Somm. Et Levier grass and its liposomal nanoparticles. Compounds with anti-cancer activities received from plant raw materials and its nanoparticles have a significant importance as they have selective activities on cancer cells of target organs.

New galenic preparation, which was received from Erysimum contractum grass and studied in accordance with Rasazorin method, revealed inhibitory activities on lung carcinoma cells (A-549) IC-12 ± 2 µg/ml and colon carcinoma IC - 8.8 ± 0.5 µg/ml and low toxicity to skin normal fibroblasts (WS-1) - IC - 56 ± 13 µg/ml. Almost the same outcomes were received with Hoechst method, the only difference was that the research object showed no toxicity to skin normal fibroblasts (WS-1) - IC ->200 µg/ml.

The liposomal nanoparticles of new galenic preparation revealed selective activities while studying them with Resazorin and Hoechst methods. They have high cytotoxic activity on cancer cells and at the same time have no toxicity to skin normal fibroblasts.

According to our research strategy – identification of perspective medicinal plant species for the purpose of its medicinal usage and its pharmacological evaluation, the next direction of our research was studying the activity of fraction rich in water, methanol and dry extracts and cardiac glycosides of Erysimum contractum through in vitro testing to cancer cells, in particular, mammary glands cancer cells MCF -7 (human breast adenocarcinoma) and skin keratinocytes HaCaT. As a result of research, the most effective cytotoxic effects have methylene extract and enzyme glycosides. Essentially less effect was revealed in a water extract.

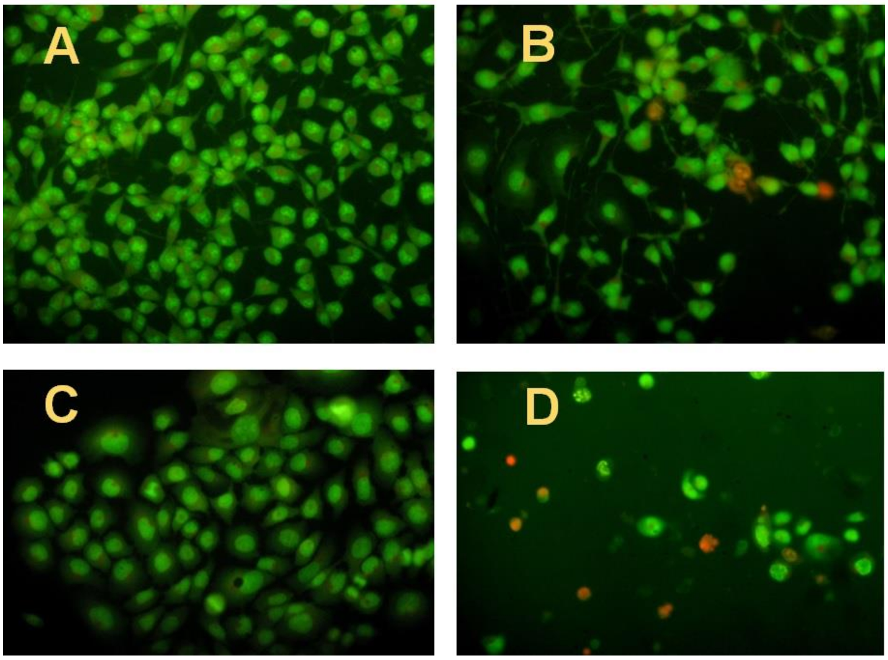
In the subsequent experiments, we examined the fractional cytotoxic action concentrations enriched with watery, methylated extracts and cardiac glycosides, which gave us the possibility of taking dose-effect dependence and the prescribed 50 value. Research processes and results are consistently described in thesis, tables, diagrams and other visualizations.

Based on the results obtained, cytotoxic activity of methanol fraction is mostly apparent and during its determination process, we used reagent *PrestoBlue™ Reagent (Invitrogen).*While using this reagent, it was easier to identify proliferation and survival skills of cultivated cancer cells. This result was approved by standard test using acridine orange and ethidium bromide vital fluorescent dyes on live and dead cells.

Methanol preparation 25 mkg/ml from culture area was added to cultivated cells. Cells painting and evaluation of their quantity and state were carried out after 24 hours using florescence microscope. The picture shows representative fluorescent micro photos of *MCF - 7* and *HaCaT* cells obtained by mixing methanol preparation with DMSO solution (B, D) after 24 hours from the influence of equal volume solutions of DMSO (control). While observing methanol preparation activity on *MCF -7* cells, it is visible, that a general number of cells is less than in control. Moreover, the percentage of dead necrotic cells (red fluorescence) is too little. As appeared, decreasing the number of *MCF-7* cells due to methanol preparation activity is conditioned by suppressing its proliferation and activation of apoptosis. Methanol preparation activity on *HaCaT* cells is more marked. Their number is significantly decreased after 24 hours of incubation. The percentage of dead cells, which might be in the stage of late apoptosis or necrotic is quite high.

Thus, because of investigation, it was identified that the methanol and cardiac glycosides fraction of Ajara endemic plant species (*Erysimum contractum* Somm. et Levier) has efficient cytotoxic activity. Based on dose-response relationship, concentrations of these fractions were detected on cultural area, where the number of viable cancer cells is decreased by 50% (CTD50) in 24 hours. They are presented in the table №13.

According to data given in the table №13, we can conclude, that Erysimum contractum Somm. et Levier preparations of Ajara endemic plant have cytotoxic activity on human breast adenocarcinoma cell line MCF -7, although no equal to anti-cancer activity of curcumin. At the same time, methanol and cardiac glycosides preparations have specific high cytotoxicity on keratinocytes. Therefore, we can count them as potential pharmacological treatments for the external therapy of pathologies caused by high proliferation of keratinocytes.



Pic. 1 : Representative fluorescent micro photos of *MCF*-7 (A,B) და *HaCaT* (C,D) cells: after 24 hours of adding A,C- dimethylsulphoxide (solution); B,D- after 24 hours of activity of methanol preparation (dose – 25 mkg/ml). Dye system: acridine orange + ethidium bromide.

Table №13

Concentrations of water, methanol and enriched cardiac glycosides fractions of *Erysimum contractum*, which reduce viability of cultivated human cells by 50%

Preparation	**TCD_{50}**, (mkg/ml)	
	Cell line *MCF*-7	Cell line *HaCaT*
water	520	410
methanol	16	6
cardiac glycosides	20	4
curcumin	2,2	4,8

Conclusions

1. Bioecological and pharmacognostic studies of Ajara and Ajara-Lazica endemic plant species spread in Ajara floristic district were conducted.
2. We identified coverage areas and GPS coordinates of Ajara and Ajara-Lazica research endemics. Endemics are spread according to vertical zones from coastal regions to subalpine zone. Their geographical coverage center is Ajaristskali River gorge.
3. There were identified viable forms of research species, among them *Osmanthus decorus, Rhododendron smirnowii, Rhododensron ungernii, Quercus petra* var. dshorochensis is a hardwood plants and the rest of plants are perennial grasses. Morphological characteristics of each research species were described. Global conservation status of Ajara and Ajara-Lazica endemics was analyzed.
4. Screening of 6 species of Ajara endemics and 15 species of Ajara-Lazica endemics on the content of basic classes of bioactive substances was conducted with qualitative analysis and thin-layer chromatography methods. Based on screening, there were revealed 14 alkaloid-containing, 11 flavonoid-containing and 5 coumarine-containing Ajara and Ajara-Lazica endemics.
5. There was studied the content of bioactive substances of 21 Ajara and Ajara-Lazica endemics with the help of tandem chromate mass spectrometry (GC-MS/MS). According to the research:
5.1 bioactive compounds containing nitrogen were identified in 11 species: *Erysimum contractum* Somm. et Levier., Seseli foliosum (Somm. et Lev.) Mand., *Astragalus sommieri* Freyn., *Quercus petra var. dshorochensis* c. Koch., *Rubus adzharicus* Sanadze - Rosaceae Juss., *Rhynchospora caucasica* Vahl., *Amaracus rotundifolius* (Boiss.) Briq., *Rhododendron smirnovii* Trautv., *Rhododendron ungernii* Trautv., *Centaurea adzharica* Sosn., *Astragalus adzharicus* M.Pop.
5.2 Flavonoids were detected in the following two species: *Scutellaria pontica* C. Koch. - luteolin, apigenine . *Astragalus adzharicus* M.Pop. - quercetin, kaempferol.
5.3 Coumarines were detected in the following four species: *Psoralea acaulis* var.adzharica - furocoumarine; Astragalus sommieri Freyn. -crimidine, archangelicine; Seseli foliosum (Somm. et Lev.) Mand. – isophsoralene, edullisin, cridimine, archangelicine; Astragalus adzharicus M.Pop. - furocoumarine, phsoralene.
5.4 Ajara and Ajara-Lazica endemics were investigated in accordance with tandem chromate mass spectrometry GC-MS/MS method and identified other bioactive substances: 8 species of terpenoids and indole products in 4 endemics.
6. There was carried out the screening of methanol extracts of 8 Ajara and Ajara-Lazica endemics on cytotoxic activity. They were studied in vitro testing with the help of Resazorin and Hoechst methods on A-549 (lung carcinoma) and DLD-1 (colon carcinoma). It was also revealed, that they do not damage healthy cells (normal skin fibroblast cell WS1). According to experimental research, *Erysimum contractum* Somm. et Levier is considered to be a prospective species for further investigation.

7. Cytotoxic activity on new galenic forms received form *Erysimum contractum* Somm. et Levier grass on A-549 (lung carcinoma) and DLD-1 (colon carcinoma) were studied. Liposome nanoparticles with new galenic forms received from Erysimum contractum Somm. et Levier grass have selective activity, in particular, high cytotoxic activity and at the same time they don't damage normal skin fibroblast cell culture WS1.

8.Methanol extracts received from *Erysimum contractum* Somm. et Levier grass and cardiac glycoside-enriched fraction have no cytotoxicity unlike curcumin to MCF-7 (human breast adenocarcinoma cell line) and they are also characterized by high specific cytotoxicity toward keratinocytes. Selective, anti-cancer activity of liposome nanoparticles containing new galenic formation received form *Erysimum contractum* Somm. et Levier grass will become a base for new drugs elaboration.

9.Growth and development characteristics in seasonal dynamics of 11 species distinguished by biologically active substances were studied: *Rubus adzharicus; Osmanthus decorus*; *Centaurea adzharica; Erysimum contractum; Psoralea acaulis; Ranunculus ampellophylus; Amaracus rotundifolius; Astragalus adzharicus; Linaria adzharica; Primula megasaefolia; Seseli foliosum* .

10. Based on studies of basic phenophases of research species growth and development (according to months), we identified the following: *Psoralea acaulis, Rubus adzharicus, Amaracus rotundifolius; Seseli foliosum* are distinguished by long period of vegetation, approximately 8 months and *Primula megaseifolia* is distinguished with short period of vegetation, approximately 3 months. The length of vegetation for the rest of species is 5-7 months. Two of research species - *Rubus adzharicus* and Erysimum contractum preserve aboveground organs and they are classified as wintergreen plants.

11. Linaria adzharica, Rubus adzharicus and Seseli foliosum are characterized with the longest blooming period, approximately 6 months and the blooming of the rest of species last 2-3 months.

12. Fruit and seed ripening period is in summer and autumn except *Primula megaseifolia*, which seeds are ripen in spring.

13. Growth and development processes of Ajara and Ajara-Lazica endemics in nature occur normally. There were no problems concerning phenophases during investigation, but it must be mentioned, that their phenodata strongly depends on air temperature. When winter is mild and temperature is higher than normal, they start vegetation and generative development processes one month earlier or more.

14. Ajara and Ajara-Lazica research endemics, especially *Erysimum contractum* needs to be paid special attention in terms of their medicinal usage.

15. Selective, anti-cancer activity of liposome nanoparticles with new galenic forms received from Erysimum contractum Somm. et Levier grass will become a base for the elaboration of new medications.

18. This research should be applied for Ajara and Ajara-Lazica endemics preservation in nature (in situ conservation) and their practical usage.

Publications around the dissertation:

1. „Phytochemical Study and Biological Activities of Selected Adjara and Adjara – Lazica Endemic Plants”
   Intern. Scientif. Conf. „Future Technologies and Quality of Life”, 29 september-1 october 2017, Batumi. Abstracts books, Tbilisi – Batumi, pp. 42-43.
2. „Cytotoxity Screening of Endemic Plants from Adjara“
   Intern. Scientif. Conf. „Future Technologies and Quality of Life”, 29 september-1 october 2017, Batumi. Abstracts books, Tbilisi – Batumi, pp. 95-96.
3. <u>STUDY OF CYTOTOXIC ACTIVITY OF METHANOL EXTRACTS OF THE ENDEMIC PLANT OF THE GENUS ERYSIMUM OF THE ADJARIAN FLORISTIC DISTRICT OF GEORGIA</u>.
   Publication date 2017/5, Journal Georgian medical news, Issue266, Pages 80-85.
4. „Gaz chromatography - mass spectrometry (GC-MS) analysis of bioactive compounds of Ajara and Ajara-Lazica endemic species“.
   International Journal of Current Research, September, 2016, Vol. 8, Issue, 09, pp.38939-38944. www.journalcra.com
5. „The study of Ajara and Ajara-Lazica Endemics on the Content of Biologically Active Compound Flavonoids”.
   International Journal of Science and Research Methodology, November, 2016, Vol.:5, Issue:1, www.ijsrm.humanjournals.com
6. „The study of Ajara and Ajara-Lazica Endemics on the Content of Biologically Active Compound Coumarin”.
   International Journal of Recent Trends in Engineering & Research (IJRTER); Volume 02, Issue 09; September, 2016.
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