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The Study of Ajara and Ajara-Lazica Endemics on the Content of Biologically Active Compound Flavonoids

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ABSTRACT

The article deals with the gain of biologically active substance flavonoid in endemic plant species in Ajara and Ajara-Lazica applying tandem chromate mass spectrometry (GC-MS/MS) method. In the object under analysis, for the purpose of identification of the target substance, mass spectrums of the peaks existing on chromatographs were compared with the mass spectrums of the substances existing in the database (NIST 2011). Out of the examined 21 species, flavonoids appeared in the following 3 species, *Scutellaria pontica* C. Koch. - Labiatae L.; *Astragalus adzharicus* M. Pop. - Fabaceae Lindl; *Linaria adzharica* Kem.-Nath. (=L.syspirensis C. Koch.) - Scrophulariaceae Juss. Material under current analysis was taken in 2014-2015. The following has been identified in the examined endemic plant species, *Scutellaria pontica* C. Koch. - Labiatae L. Luteolin, apigenin; *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; quercetin, kaempferol; Linaria adzharica Kem.-Nath. (=*L.syspirensis* C. Koch.) - Scrophulariaceae Juss; catechin.

1. INTRODUCTION

For the last period, the interest towards biologically active substances of plant origin, one of the groups of flavonoids of phenol nature compounds has considerably increased. For modern medicine flavonoids represent important medicinal means and are distinguished by effectiveness and wide specter of action, do not reveal any side effects and contraindications [1]. They possess anti-diabetic action and can regulate glucose level in blood [3].

Presumably, flavonoids exist in the plant kingdom over a billion years. Practically they are met in almost every food product such as fruits and vegetable. Therefore, they are consumed in significant quantities. It is regarded that human demand for flavonoids comprises several thousand milligrams a day. Besides, flavonoids are found in medicinal plants. They have been widely used in popular medicine worldwide, especially in China [2].

Scientists have proved that as a group, flavonoids possess quite high antiviral activity, slowing reproduction and activation of viruses. Their content in plants changes annually according to environmental conditions. The primary producers are plants but they are synthesized in some insect species as well [3].

Flavonoids activate ferments; they are characterized by actions against allergy, inflammation and tumor; majority of them inhibit the replication of human immunodeficiency virus [5].

As it has been mentioned, flavonoids reveal a wide spectrum of pharmacological actions. It means anti-inflammatory, antibacterial, antiviral and anti-cytotoxic tumor actions. Apart from this, flavonoids are famous as inhibitors of peroxide oxidation of lipids and platelet aggregation [6].

Therefore, in the conducted research we set as our aim to study the endemic plant species in Ajara and Ajara-Lazica on the content of biologically active substance flavonoids. Consequently, our objective was to obtain methanol extracts from plant grass and roots and study them with tandem chromate mass spectrometry (GC-MS/MS) method.

2. MATERIALS AND METHODS

For the purpose of the study of the biologically active substances, herbaceous as well as woody endemic plant species (total 21) have been selected, spread in Georgia, namely Ajara and Ajara-Lazica.

Endemic plant species of Ajara are:

Allium adzharicum M.Pop. – Liliaceae Juss; *Angelica adzharica* M.Pop. Umbelliferae Juss., Apiaceae Lindl; *Astragalus doluchanovii* Manden. – Fabaceae Lindl; *Centaurea adzharica* Sosn. Asteraceae Dumort. (Compositae Giseke); *Erysimum contractum* Somm. et Levier. - Cruciferae Juss.(=Brassicaceae Burnett.);Psoralea acaulisvar.adzharica - Fabaceae Lindl; *Ranunculus ampellophylus* var. adzharica - Ranunculaceae Juss; *Rubusa dzharicus* Sanadze - Rosaceae Jus

Endemic plant species of Ajara are:

Amaracus rotundifolius (Boiss.) Briq. (=Origanum rotundifolium) - family Lamiaceae Juss(=Labiaceae); *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; *Astragalus sommieri* Freyn. - Fabaceae Lindl;*Cyclamen adzharicum* Pobed.(=C.adjaricum var. ibericum) – Primulaceae Vent; Galanthus krasnovii Khokhr. – Liliaceae Juss; *Galanthus rizechensis* Stern.(= G.cilicicus Baker., G. glaucescens Khokhr.); *Hypericum nordmanni* Khokhr.- Hypericaceae Juss; *Hypericum ptarmicifolium* var.adzharicum - Hypericaceae Juss;*Linaria adzharica* Kem.-Nath.(=L.syspirensis C. Koch.)- Scrophulariaceae Juss; *Osmanthus decorus* (Boiss. et Bal.)- Oleaceae Hoffm. et Link; *Primula megasaefolia* boiss. Et Bal. Primulaceae Vent; Quercus petra var. dshorochensisc. Koch.- Fabaceae Lindl; *Rhododendron smirnovii* Trautv.- Ericaceae DC; *Rhododendron ungernii* Trautv. –Ericaceae DC; *Rhynchospora caucasica* Vahl. - Cyperaceae Juss; *Scrophularia chloranta* Ky et Boiss. - Scrophulariaceae Juss; *Scrophularia sosnovskyi* Kem.-Nath.- Scrophulariaceae Juss; *Scutellaria pontica C.* Koch. - Labiatae L; *Seseli foliosum* (Somm. et Lev.) Mand. - Umbelliferae Juss., Apiaceae Lindl.

It should be noted that the great part of the given plants is under a strong impact of anthropogenic factors. Some of them are under threat of extinction. Almost majority of them represent the species included into the Red List (9) and vulnerable species under threat (10). Therefore, their timely study is important for conservation and preservation purposes as well.

Materials for analysis were taken in 2014-2015, early spring (566) and late summer periods when plants are in the active growing and flowering phases. This is the period when they are especially rich in biologically active substances. For the purpose of the study of the stated substances, the experiment was conducted applying gas chromatography (GC/MS/MS method at the Toxicology and Chemical Expertise laboratory of Levan Samkharauli Court

Expertise National Bureau, Georgia) in the following conditions for analysis we took grass and roots, dried them and crushed the obtained sample in accordance with the requirements of tenth edition of state pharmacopeia. Afterward, 25 ml methanol was added to 5 grams of crushed raw material. After evaporation of the organic solvent, 55-50 mkl *BSTFA/ EtAc* (40:10) were separately added to the dry remains and heated to 70^0C temperature for 20 minutes. After cooling, 1-1 mkl was studied with tandem chromate-mass spectrometry – device: *Agilent Technologies 7000 GC/MS/MS Triple Quad;* column - *Elite 5-MS; 30MX250 µm X 0, 25 µm*; furnace temperature: 60^0C-310^0C (program regime); injector temperature - 250^0C; transfer line temperature - 310^0C; 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).

3. RESULTS AND DISCUSSIONS

As a result of the experiment out of 21 endemic species flavonoids were obtained in 3 endemic species: *Scutellaria pontica* C. Koch. - Labiatae L. Luteolin, Apigenin. *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; quercetin, cemperol; *Linaria adzharica* Kem.-Nath. (=L.syspirensis C. Koch.)- Scrophulariaceae Juss; catechin.

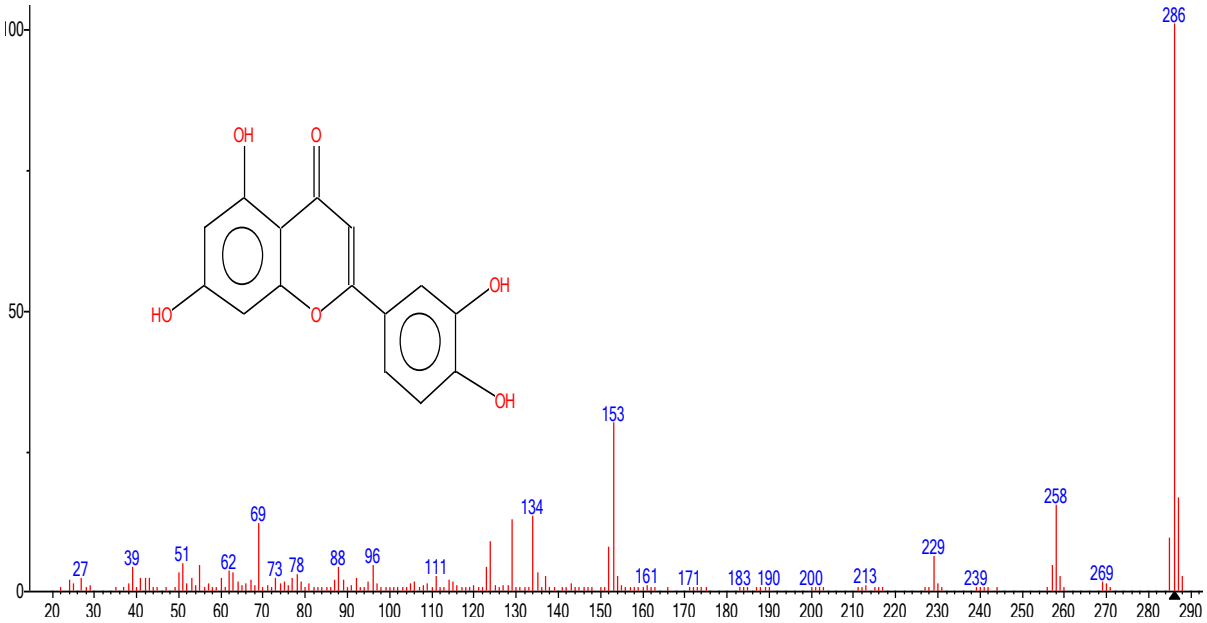

Fig. -3.1 *Scutellaria pontica* C. Koch. - Labiateae L. Luteolin chromatogram

Table -3.1 *Scutellaria pontica* C. Koch. - Labiateae L. Chromatographic characteristics of Luteolin

Name: Luteolin Formula: $C_{15}H_{10}O_6$ MW: 286 Exact Mass: 286.047737 CAS#: 491-70-3 NIST#: 153070 ID#: 221528 DB: mainlib 10 largest peaks: 286 999 | 153 296 | 287 163 | 258 149 | 134 132 | 129 124 | 69 119 | 285 91 | 124 87 | 152 75 |

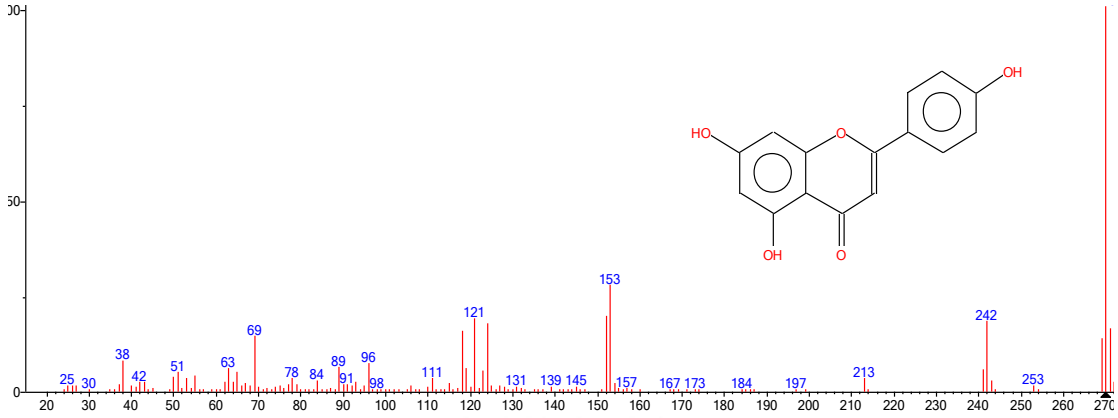

Fig. 3.2 *Scutellaria pontica* C. Koch. - Labiateae L. Apigenine chromatogram.

Table -3.2 *Scutellaria pontica* C. Koch. - Labiateae L. Chromatographic characteristics of Apigenine.

Name: Apigenin Formula: $C_{15}H_{10}O_5$ MW: 270 Exact Mass: 270.052824 CAS#: 520-36-5 NIST#: 153076 ID#: 216769 DB: mainlib 10 largest peaks: 270 999 | 153 276 | 152 195 | 121 189 | 242 182 | 124 175 | 271 164 | 118 155 | 69 143 | 269 137 |

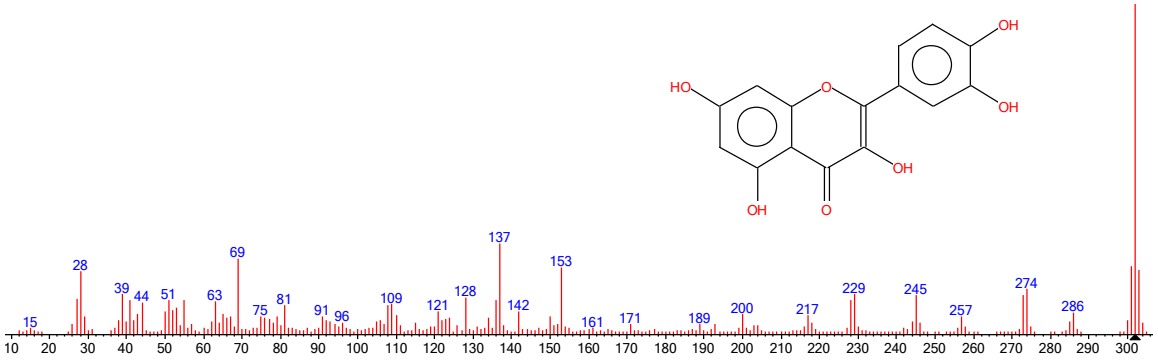

Fig. 3.3 *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; Quercetin chromatogram

Table 3.3 *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; Chromatographic characteristics

Name: Quercetin
Formula: $C_{15}H_{10}O_7$
MW: 302 Exact Mass: 302.042652 CAS#: 117-39-5 NIST#: 229372 ID#: 225416 DB: mainlib
10 largest peaks:
302 999 | 137 230 | 69 192 | 301 174 | 153 170 |
303 164 | 28 159 | 274 114 | 229 103 | 39 101 |

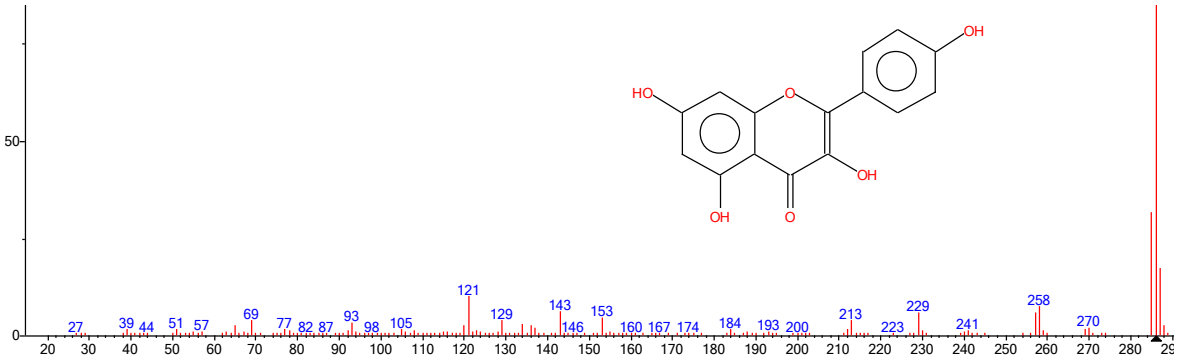

Fig. 3.4 *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; Cemperol chromatogram

Table 3.4 *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; Chromatographic characteristics of Cemperol

Name: Kaempferol
Formula: $C_{15}H_{10}O_6$
MW: 286 Exact Mass: 286.047737 CAS#: 520-18-3 NIST#: 234055 ID#: 221662 DB: mainlib
10 largest peaks:
286 999 | 285 310 | 287 170 | 121 97 | 258 72 |
143 60 | 257 57 | 229 55 | 153 44 | 129 37 |

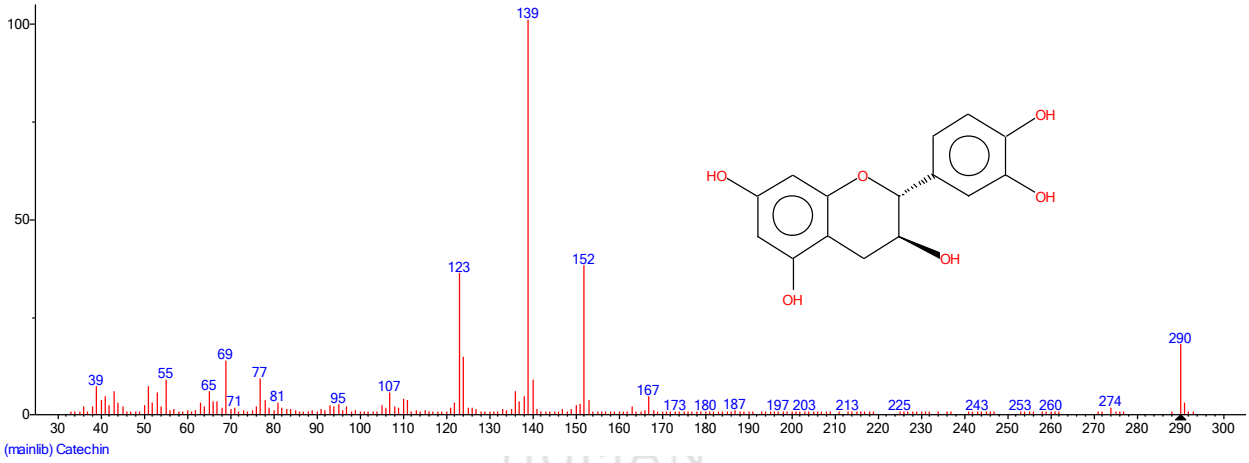

Fig. 3.5. *Linaria adzharica* Kem.-Nath.(=L.syspirensis C. Koch.)- Scrophulariaceae Juss; Catechin chromatogram

Table -3.5. *Linaria adzharica* Kem.-Nath.(=L.syspirensis C. Koch.)- Scrophulariaceae Juss; Chromatographic characteristics of Catechin

Name: Catechin
Formula: $C_{15}H_{14}O_6$
MW: 290 Exact Mass: 290.079039 CAS#: 154-23-4 NIST#: 272941 ID#: 126700 DB: mainlib
139 999 | 152 375 | 123 356 | 290 177 | 124 145 |
69 135 | 77 90 | 55 84 | 140 84 | 39 70 |

CONCLUSION

Thus, as a result of the analysis of 21 plant species endemics to Ajara and Ajara-Lazica with the tandem chromate mass spectrometry (GC-MS/MS) method, the following 3 species have been identified containing biologically active flavonoids substances: *Scutellaria pontica* C. Koch. - Labiatae L.; *Astragalus adzharicus* M.Pop. - Fabaceae Lindl; *Linaria adzharica* Kem.-Nath.(=L.syspirensis C. Koch.) - *Scrophulariaceae Jus*s. The research is a novelty and will continue in future cytotoxic effect as well.

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