

Physiological, Phytochemical And Microbiological Investigations, And Evaluation Of Resources Of *Glycyrrhiza Glabra* L. In Azerbaijan

Sevinj Ismayilova-Abduyeva
Biyon Products LLC, Baku State
University
Baku, Azerbaijan
sevinc.abduyeva@gmail.com

Sayyara Ibadullayeva
Institute of Botany of ANAS,
Biyon Products LLC
Baku, Azerbaijan
Corresponding author email:
ibadullayeva.sayyara@mail.ru

Panah Muradov, Konul Bakhshaliyeva
Institute of Microbiology of ANAS
Baku, Azerbaijan
mpanah678@rambler.ru

Abstract—The article deals with the microbiological effect specifications of *Glycyrrhiza glabra* L. species, collected from the populations in Aghdash, Mingachevir, and Aghdam regions of Azerbaijan. The effect of the abnormalities in the roots of licorice in occupied borderline areas on the chemical component of plant bodies was investigated. The microbiological investigations revealed that the roots of *G. glabra* species in the regions and in occupied territories of Azerbaijan are clean, which shows the academic innovation of this study. It became obvious that the antifungal effect of the plant on fungi is different: in the populations collected from Aghdam region *Fusarium oxysporium* and *Alternaria cucumerinadid* not develop in the liquid extract of the plant, that is the extract showed a strong antifungal effect. The fungicide effect was low in other fungi. The liquid extract decreased the formation of biocompound in *Aspergillus niger* for about 30.1 percent, in *Aspergillus awamorii* about 10 percent, and in *Apergillus repens* for about 28.6 percent. This can be evaluated as fungostatic action. The raw material, collected from the other two regions, effected fungi differently. The heavy metal accumulation in plant roots is normal. The collected plants can be used for medical purposes.

Keywords—*Glycyrrhiza glabra* L., polluted soils, antifungal effect

Introduction

First of all, in order to preserve the ecological balance of the environment it is urgent to define the pollution sources of air, water, and soil, and their contamination degree. If the study object is a medicinal raw material, it is very necessary to apply quality control and ecological investigation methods, and define heavy metals and toxic elements in the samples [Ibadullayeva et al., 2018]. Investigations are of great importance in the study of natural raw material resources of wild plants and their proper use.

Some wild species are more than the demanded amount, such as *Glycyrrhiza glabra* L. in Azerbaijan, tons of which are being exported every year. This plant can bring millions of income to Azerbaijan. Dietary supplements and medicinal products are made of this plant. We can contribute to the development of non-oil sector in Azerbaijan, by exporting such products.

Glycyrrhiza glabra L. is a perennial plant, which can grow up to 50 to 200 cm. Its trunk is bare, straight, and simple, with branches. Its leaves are alike a complex feather with 5 to 20 cm length, bright, strong, longish, and sticky. Its flowers are 12 mm long, and ovary is white pink. Its fruit is longish straight or a bit bent. The root system consists of primary and lateral roots, and go deeper to 8 m; the plant is kept in soil firmly by its root system. The aboveground trunk develops from the primary root. The plant blossoms in May-June, fruit is mature in September. The root and trunk are of cylindrical shape and of different length; thickness can range between 0.5 cm to 5 cm, and even more. The well-developed roots are smooth and 15 cm-thick. The colour of the cleaned raw material changes from light yellow to brownish yellow. It has no smell, has a strong sweet flavour, and a bit irritant.

Glycyrrhiza glabra L. is widespread in Middle Asia, Kazakhstan, Caucasus, Northern Caucasus, and southern Europe. It is spread in everywhere in the Azerbaijani flora. It belongs to the Mediterranean Sea, Iran-Turan, and xerophiles areal, and covers ancient Mediterranean Sea, Iran-Turan, Central Anatolia, Northern Sinai, Gobi Desert, Western Himalayas, and Eastern Mediterranean territories [Flora of Azerbaijan, 1954].

This plant is widespread in riverbanks and near ditches, in shallow ravines, and cultivated areas in Azerbaijan. It is also cultivated in the plantations with modern technologies by Biyon Products LLC in Aghdash region. The roots and trunk of perennial wild *Glycyrrhiza glabra* are collected during different seasons of the year and are used as a valuable raw material for medicinal products. If the raw materials are collected properly every year it will not decrease

the number of the plant, vice versa promote the development of new branches. Raw material consists of 70 to 75 percent of roots, vegetative organs, and branches. Roots are collected by digging the soil. Raw material can be recollected after 4 or 5 years, however some references say it is possible to collect the raw material every year.

The collected raw materials are dried at the Biyan Industrial Park of Biyan Products LLC by applying modern technologies. Later, the dried products are stored in modern well-ventilated warehouses. This plant can be stored for about 10 years. In some enterprises of Azerbaijan, the sliced raw material is stored in veneer boxes, and powder is stored in glass jars.

Glycyrrhiza glabra L. is included in the State Pharmacopeia (DF X, p. 573 and DS 22839-77; 42-0296-2339-02, 42-0273-1781-01 and 0296-2339-02).

Licorice is widely used in the Azerbaijani folk medicine against colds accompanied by cough. Licorice root of half teaspoon is mixed with a teaspoon of honey and taken thrice a day. For stomach and gallbladder diseases the root part is chewed 5 to 7 times a day.

Considering the importance of this medicinal herb, we started to collect this plant, which is widespread in the Azerbaijani flora. Nevertheless, the abnormalities, like unsmooth and destroyed roots of collected plants from Aghdam population, emerged the necessity to study the plant thoroughly and in comparison with the other populations. Therefore, we started to investigate the plant roots from different point-of-view and collected plant roots from Aghdash and Mingachevir population, decided to carry out microbiological investigations of the roots.

MATERIAL AND METHOD

In 2017 and 2018, the Ethnobotany Laboratory of the Botany Institute of the Azerbaijan National Academy of Sciences (ANAS) and employees of the Biyan Industrial Park of Biyan Products LLC collected *G. glabra* from three populations – Aghdam (SP1), Mingachevir (SP2), and Aghdash (SP3) – in order to define the ecological purity of the plant and the effect of pollutants on the microbiological activity of the plant. The method of collecting medicinal herbs was applied while collecting the raw material [Krylova et al., 1971].

Soil samples were also collected from each populations. The collected plant roots [State Pharma., 1990] and soil samples were dried, grinded, and filtered for further analyses. The heavy metal amount in soil and root samples were analysed at the Laboratory in Biyan Industrial Park of Biyan Products LLC. The technical quality document for licorice raw material doesn't list the requirements for allowed concentration, so while defining the amount of iron, manganese, and nickel we based on the references

[Tamahina et al., 2015; Guidelines ., 1992]. The natural compounds and active components, as well as pharmacological activity of materials have a great role in defining the main therapeutic properties of medicinal herbs, which are considered biologically active substances.

The microbiological analyses of the plant roots from each population were carried out by the employees of the Microbiology Laboratory of ANAS based upon general methodology.

EXPERIMENTS

In August of 2018, academic investigations were carried out in one of the occupied territories of Azerbaijan – Aghdam region, Mingachevir, and in 2017, in Aghdash region. It became clear that 3 500 tons of raw material can be achieved from these areas. However, the collection of plants revealed some abnormalities in the plant roots (Fig. 1). One can immediately observe the abnormalities when looking at the image. It may have different reasons: plants have not been collected for a long period of time because of the borderline and consequently underwent some destructions, or they are too old and affected by the sewage water of the enemies in that area. Therefore, several investigations were carried on these roots (accumulation of heavy metals on plant roots, quality content and microbiological analyses of glycyrrhizic acid and other biologically active substances of the chemical component of the plant).

First, distribution of iron, manganese, and nickel in organs of *Glycyrrhiza glabra* L. were studied. The academic investigations revealed the amount of several heavy metals in underground parts and root system of *G. glabra* in areas where these elements were abundant or deficit. It became clear that the amount of biogenic metals – iron and manganese – are lower in underground parts of the plant, which are used for medicinal purposes (Fig. 2).



Figure 1. 1. Licorice root, collected from Aghdam region (on top)
2. Licorice root, collected from Aghdash region (below)

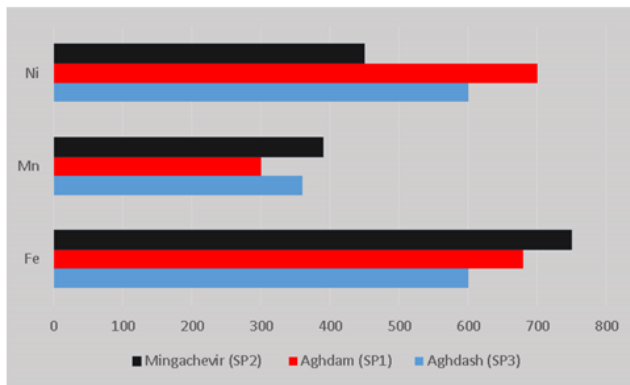


Fig. 2. Distribution of heavy metals in licorice roots from different populations

Although the amount of heavy metals are different in each population, the indices are within the norms (Fe-750 mg/kg in Mingachevir, 680 mg/kg in Aghdam, 600 mg/kg in Aghdash; Ni-450 mg/kg in Mingachevir, 700 mg/kg in Aghdam, 450 mg/kg in Aghdash; Mn-390 mg/kg in Mingachevir, 300 mg/kg in Aghdam, 360 mg/kg in Aghdash). The background amount of iron in soil was considered 3800.0 mg/kg [Beslaneev et al., 2012; Perlman, 2000]. If plant absorbed 50 percent of this amount it cannot be used as a medicinal herb. Nevertheless, our investigations proved that the Fe amount in the plant is within the norm. The amount of manganese in dry substance for herbs is between 25.0 to 250 mg/kg. Over 500 mg/kg in dry substance is considered toxic [State Pharmacopeia., 1990]. The allowed concentration amount of manganese in soils is 600 mg/kg [Reutova et al., 2010]. The amount of manganese and cobalt in plant organs is not more than the allowed index in the selected senopopulations.

Although the raw material, collected from Aghdam region, visually seem not sufficient for medicinal usage, its content is more pure.

The quality analyses show that licorice roots contain not only glycyrrhizic acid, but also flavonoids, steroids, essential oils, ascorbic acid, bitter substances, pigments, resin, triterpen saponins, organic acids, and fatty oils. Our investigations coincide with the references [Biologically active substance., 2001].

As the roots will be used, we considered to study the antifungal effect as well. Plant sample in 1:10 ratio was taken and liquid extract was prepared. The extract of 100 ml was poured into flasks and sterilized under 0.5 atm pressure for 30 minutes. Then 5 toxic fungi (*Fusarium oxysporium*, *Aspergillus niger*, *Aspergillus awamorii*, *Apergillus repens* and *Alternaria cucumerina*) were planted in those flasks rich with nutrients. The cultivation was carried out in thermostat for 7 days under 25-27C⁰ temperature. Following the seven days, fungi were filtered, dried and the amount of the dry component was calculated. Figure 3 shows the achieved results.

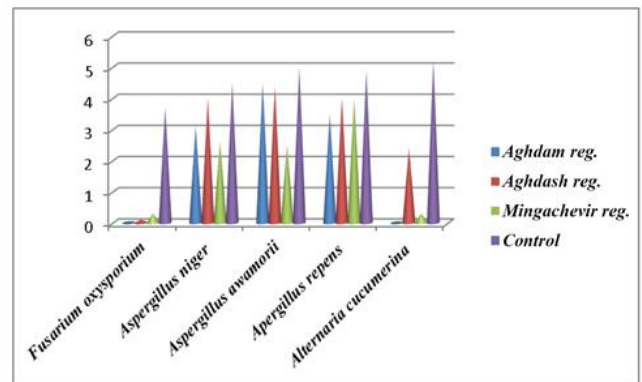


Fig. 3. Antifungal effect of plant extract on some toxicogenic fungi

Note: the information for the chart

Fusarium oxysporium – not developed 3.7 g/l (control)

Aspergillus niger – 3.1 g/l (formed) 4.5 (control)

Aspergillus awamorii – 4.5 g/l 5.0

Apergillus repens – 3.5 g/l 4.9

Alternaria cucumerina – not developed 5.2

Note that, fungi, belonging to *Fusarium*, *Aspergillus*, and *Penicillium* species, diffuses toxins, such as moniliphorme, citrine, patulin, and oxratoxin, which are harmful for human body.

The annual biological resources of *Glycyrrhiza glabra* in the above-stated regions were studied during the investigations (Table 1).

Table1.

Annual biological resources of *Glycyrrhiza glabra* in Aghdam, Mingachevir, and Aghdash populations

Regional populations	Areal of a single population (ha)	Resource density (ha/t)	Biological resources (t)	Exploitation resources (t)
Aghdam	771	8.00 – 17.60	1097.00± 67.84	548.00 ± 35.92
Mingachevir	569		1168.00 ± 71.25	584.00 ± 34.49
Aghdash	900		1670.89± 148.60	835.45 ± 78.60
Total:	4178	8.4± 0.05	3935.89± 228.50	1967. 945±114.45

The table shows that the selected populations are sufficient for the procurement of the plant.

RESULTS

The investigations revealed that the amount of heavy metals in roots of the three populations *Glycyrrhiza glabra* L. is below or within the norm. It is rich in biologically active substances, and its antifungal effect on fungi is quite different. *Fusarium*

oxysporium and *Alternaria cucumerina* did not develop in the liquid plant extract, thus the extract had a strong antifungal effect. In other fungi, fungicide effect was lower. The liquid extract decreased the formation of biocompound in *Aspergillus niger* for about 30.1 percent, in *Aspergillus awamorii* about 10 percent, and in *Apergillus repens* for about 28.6 percent. This can be evaluated as fungostatic action.

These results pave the way for the procurement of licorice and production of licorice products.

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