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BIOACTIVE PROFILE OF *OROBANCHE CARYOPHYLLACEA* EXTRACTS DEPENDING ON THE HOST PLANT AND ENVIRONMENTAL CONDITIONS

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The impact on parasitic plants is complex and influenced by a combination of biotic and abiotic factors operating at various spatial and temporal scales. In parasite-host relationships as biotic factors, we consider the various host plants impact parasitic plants in varied ways. Our study examined also the correlation between the bioactive profile of the parasite plant and different environmental conditions.

Our results demonstrated a correlation between the total polyphenols and antioxidant activity of water-alcohol solutions extracted from the flowers and stems of *Orobanche caryophyllacea* (bedstraw broomrape) under varying environmental conditions and host plants: *Galium verum* and *Galium humifusum*.

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Introduction. Exploring natural reservoirs exhibiting different biological properties remains relevant for contemporary biology, agriculture, and pharmaceuticals [1].

The pharmacological characteristics of plants are influenced by abiotic and biotic factors. This interest in natural components is particularly pronounced in the context of parasitic plants, which have not been thoroughly investigated [2, 3].

The various host plants, influenced by different abiotic pressures, impact parasitic plants in varied ways. Our study examined the correlation between the bioactive profile in the parasite-host relationship and environmental conditions.

The genus Orobanche L. belongs to the family Orobanchaceae Vent., the order Lamiales Bromhead, and the phylum Angiosperms. The 204 species falling into the genus Orobanche have a global distribution [4]. Based on the most recent information, Armenia is home to 18 Orobanche species, among which 6 (*O. arpica* Piwow., Ó. Sánchez & Moreno Mor., *O. coerulescens* Stephan, *O. grenieri* F.W. Schultz, *O. javakhetica* Piwow., Ó. Sánchez & Moreno Mor., *O. laxissima* Uhlich & Rätzel, *O. zajaciorum* Piwow.) are newly discovered species for the country [5, 6].

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Orobanche caryophyllacea Sm. (bedstraw broomrape, clove-scented broomrape) is a Western European Asiatic species [7]. The native distribution of this species is Europe to Anatolia, the Caucasus, Iran, Pakistan, Xinjiang, and NW Africa [8] (Fig. 1).

Fig. 1. The native distribution of *Orobanche caryophyllacea* (© Copyright 2023 World Checklist of Vascular Plants).

O. caryophyllacea is a holoparasite that grows primarily in the temperate biome. Its host plants include species of the genera Galium, Sherardia and Asperula, all from the family Rubiaceae. In Central Europe, it mostly occurs on *G. mollugo* L., *G. verum* L., *G. boreale* L. and *G. odoratum* (L.) Scop. In Armenia it has been reported on *G. verum*, rarely *G. humifusum* M. Bieb., and *G. aparine* L. (Fig. 2). Species inhabit xerothermic rocky grasslands, steppe habitats, subalpine meadows, and the peripheries of forests and shrubs, typically found at elevations ranging from 800 to 2000 *m*, with occasional occurrences up to 2400 *m* above the sea level [5, 7, 9].

Fig. 2. *Orobanche caryophyllacea* with host plant *Galium humifusum.*

The exploration of *O. caryophyllacea*'s phytochemical composition is not comprehensive, and there is limited literature available on the bioactivity of this species. Polish researchers were the pioneers in detailing the presence of phenylethanoid glycosides and iridoids, specifically asperuloside and asperulosidic acid in *O. caryophyllacea*. These particular compounds have only been verified in *O. caryophyllacea* thus far and can function as distinctive chemical markers for identifying this species in chemotaxonomy [10]. Phenylpropanoid glycosides also extracted from this species demonstrated radical scavenging activity that exceeds other Orobanche species by more than 20% [11].

In our study, we focused on assessing the total polyphenols and antioxidant activity of water-alcohol solutions extracted from the flowers and stems of *Orobanche caryophyllacea* Sm. which grew under different environmental conditions and in the presence of various host plants.

Materials and Methods.

Plant Material. The species *O. caryophyllacea* with their host plants were collected from two locations: Zaritap Village, Vayots Dzor Province, host plant *Galium humifusum* – located in south Armenia, and Meghradzor Village, Kotayk Province, host plant *Galium verum* – located in central Armenia.

The parasitic species were identified by monograph "Holoparasitic Orobanchaceae (*Cistanche*, *Diphelypaea*, *Orobanche*, *Phelipanche*) in Armenia: distribution, habitats, host range and taxonomic problems" (2019). The voucher specimens have been stored in the herbarium of the Yerevan State University (ERCB).

Details regarding the collected plant materials, including the host plant, location, GPS coordinates, geographical features, and etc. are outlined in Tab. 1.

Table 1

Details regarding the collected plant materials

Parasitic species	*O. caryophyllacea* Hum	*O. caryophyllacea* Ver
Host species	*Galium humifusum*	*Galium verum*
Location	Zaritap Village, Vayots Dzor Province	Meghradzor Village, Kotayk Province
GPS coordinates	39°38'06"N 45°30'39"E	40°36'32"N 44°37'16"E
Geographical features	South Armenia	Central Armenia
Annual precipitation	500 *mm*	823 *mm*
Duration of sunshine	2600 *h*	2200 *h*
Harvest period	May	June

The climate in Zaritap Village is dry continental with moderately cold winters and hot summers. The average air temperature is –16°C in January and +26°C in August. In general, the annual duration of sunshine in the region is 2600 *h*. The annual amount of precipitation is about 500 *mm*, and it falls mainly in the form of rain, according to which the maximum is observed in spring. The climate in Meghradzor Village is mild, moderately hot in summer (average temperature in July–August: 18°C), moderately cold in winter (average temperature in January – 7°C) and the duration of sunshine is about 2200 *h* per year. The annual amount of precipitation is 823 *mm* [12].

The collected plant material was dried, finely (<1 *mm* size of particles) ground using an electric grinder, and stored at 4°C before the extraction procedure.

Obtaining Extracts. Fresh plant samples were air dried under room conditions for 14 days. Dried and grindered samples were placed for 24 *h* on a magnetic stirrer with 70% ethanol in a ratio of 1:10 for extraction. Once extraction is complete extracts were filtered using a filter with pore sizes of 0.45 μm. All the extracts were used within 3 *h* after preparation.

Determination of Antioxidant Activity. The antioxidant activity (AOA) was determined by potentiometric measurements of change of oxidation-reduction potential of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediatory system caused by pro- and anti-oxidants in extracts. Briefly, an aliquot of the sample extract (1.0 *mL*) was transferred to tubes containing $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediatory system (3.0 *mL*). Then the mixture was allowed to stand at 45°C for 30 *min*. The platinum electrode and reference electrode were used for measurements. The AOA of samples was derived from the standard curve of ascorbic acid ranging from 10^{-4} *g*/*L* to 10^{-2} *g*/*L* [13, 14].

Determination of Total Phenolic Content. The content of total phenols was determined by spectrophotometry using gallic acid as standard [15, 16]. An aliquot of the sample extract (1.0 *mL*) was transferred to tubes containing a Folin-Ciocalteu's reagent (0.5 *mL*, 2.0 *N*) and distilled water (7.0 *mL*). After 3 min a sodium carbonate solution (0.5 *mL*, 7.5% w/v) was added. The tubes were then allowed to stand at 45°C for 60 *min* before absorbance at 750 *nm* was measured. The concentration of polyphenols in samples was derived from the standard curve of gallic acid ranging from 10 to 50 $\mu g/mL$.

Results and Discussion. Our study demonstrated the correlation between the total polyphenols and antioxidant activity of water-alcohol solutions extracted from the flowers and stems of *O. caryophyllacea* under varying environmental conditions and in the presence of different host plants: *G. verum* and *G. humifusum.*

According to our results, the highest amount of phenolic compounds was recorded for ethanolic extracts of *O. caryophyllacea* with the host plant *G. humifusum* (Tab. 2).

Table 2

Total phenolic content and antioxidant activity of ethanolic extracts of Orobanche caryophyllacea with different host plants

Ethanolic extracts	Phenolic compounds *mg* gallic acid per *g* of dried plant sample ± SD	Antioxidant activity $\times 10^{-4}$ *g* vit C eq. per *L* ± SD (negative values show prooxidant activity)
O. caryophyllacea Hum flowers	3.76 ± 0.003	–18 ± 0.004
O. caryophyllacea Hum stems	3.73 ± 0.009	–17 ± 0.012
O. caryophyllacea Ver flowers	1.98 ± 0.002	48 ± 0.003
O. caryophyllacea Ver stems	1.47 ± 0.005	45 ± 0.009

In contrast, the total phenolic content of ethanolic extract of *O. caryophyllacea* with host plant *G. verum* was approximately two times less. This can be explained by the fact that the host species *G. humifusum* also contains a high amount of phenolic compounds what parasite absorbs during infestation.

Phenolic compounds are known for their antioxidant properties. Generally, higher concentrations of phenolic compounds correlate with higher antioxidant activity. However, in this dataset, there seems to be some inconsistency. The ethanolic extracts of *O. caryophyllacea* with the host plant *G. verum* have higher antioxidant activity despite having lower concentrations of phenolic compounds than samples of *O. caryophyllacea* with the host plant *G. humifusum*. This suggests that chemicals other than phenolic compounds contribute to the antioxidant activity observed in these extracts.

It has been established that the pressure of abiotic factors initiates adaptation processes affecting the growth and development of plants, as well as the physiological and biochemical restructuring of metabolic processes. Thus, the following pattern is observed: the greater the amount of precipitation, the higher the content of flavor-noids. On the other hand, considering the influence of the light factor on the content of flavonoids, an undoubted dependence on the accumulation of flavonoids was revealed, one of the physiological functions of which is protection from ultraviolet radiation. It is obvious that in plants in more illuminated habitats, the total content of flavonoids is higher than in plants growing in more shaded conditions [17].

Comparing habitat conditions, we can conclude that insolation and annual precipitation differ for both regions and as a result, in central Armenia, plants receive more humidity and synthesize more antioxidant compounds. In the southern region, a high amount of sunshine is a reason for polyphenols' active synthesis. Apparently, this explains the difference in the bioactivity of samples of the same species growing in different regions of Armenia.

Overall, further analysis would be required to understand what influences the relationship between phenolic compounds and antioxidant activity in these plant samples. The host plants and environmental factors prevailing in different regions: the humid zone of central Armenia and the drier zone of southern Armenia, play a role in the quantity of chemical compounds and determining antioxidant activity of the investigated species *O. caryophyllacea*.

Conclusion. In summary, our study reveals the complex interplay between environmental conditions, host plant species, and the biochemical composition of *O. caryophyllacea* extracts.

We found that host plants influence variations in phenolic compounds and antioxidant activity, with *G. verum* extracts exhibiting higher antioxidant activity despite lower phenolic content compared to *G. humifusum*.

Furthermore, our investigation into environmental factors revealed distinct patterns in the synthesis of bioactive compounds. Specifically, plants in central Armenia, characterized by higher humidity, synthesized more antioxidant compounds, whereas those in southern Armenia, exposed to greater sunlight, exhibited active synthesis of polyphenols.

These findings underscore the importance of considering both biotic and abiotic factors in understanding the bioactivity of plant extracts. Further research is warran-ted to delve deeper into the underlying mechanisms driving the observed variations.

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REFERENCES

1. Lourenço S., Moldão-Martins M., Alves V. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules* **24** (2019), 4132. https://doi.org/10.3390/molecules24224132
2. Piwowarczyk R., Ochmian I., et al. Phytochemical Parasite-host Relations and Interactions: a *Cistanche armena* Case Study. *Sci. Total Environ.* **716** (2020), 137071. https://doi.org/10.1016/j.scitotenv.2020.137071
3. Piwowarczyk R., Ochmian I., et al. Correlational Nutritional Relationships and Interactions Between Expansive Holoparasite *Orobanche laxissima* and Woody Hosts on Metal-rich Soils. *Phytochemistry* **190** (2021), 112844. https://doi.org/10.1016/j.phytochem.2021.112844
4. *The WFO Plant List* (2024). https://www.worldfloraonline.org/taxon/wfo-4000027206
5. Piwowarczyk R., Pedraja Ó., et al. Holoparasitic Orobanchaceae (*Cistanche, Diphelypaea, Orobanche, Phelipanche*) in Armenia: Distribution, Habitats, Host Range and Taxonomic Problems. *Phytotaxa* **386** (2019), 001–106. https://doi.org/10.11646/phytotaxa.386.1.1
6. *Flora of Armenia* (ed. A.L. Takhtajian). Yerevan **8** (1987), 419 (in Russian).
7. Piwowarczyk R. *Orobanche caryophyllacea* Sm. (Orobanchaceae) in Poland: Current Distribution, Taxonomy, Plant Communities and Hosts. *Acta Agrobotanica* **67** (2014), 97–118. https://doi.org/10.5586/aa.2014.035
8. *Plants of the World Online* (2024). https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:662213-1
9. Tsaturyan T.G., Gevorgyan M.L. *Parasitic and Hemiparasitic Flowering Plants of Armenia*. Yerevan, YSU Press (2009), 137 (in Russian).
10. Jedrejek D., Pawelec S., et al. Identification and Occurrence of Phenylethanoid and Iridoid Glycosides in Six Polish Broomrapes (Orobanche spp. and Phelipanche spp., Orobanchaceae). *Phytochemistry* **170** (2020), 112189. https://doi.org/10.1016/j.phytochem.2019.112189
11. Skalski B., Pawelec S., et al. Antioxidant and Anticoagulant Effects of Phenylpropanoid Glycosides Isolated from Broomrapes (*Orobanche caryophyllacea, Phelipanche arenaria*, and *P. ramosa*). *Biomedicine & Pharmacotherapy* **139** (2021), 111618. https://doi.org/10.1016/j.biopha.2021.111618
12. *Hydrometeorology and Monitoring Center. Climate* (2024) (in Armenian). http://www.armmonitoring.am/page/366
13. Braynina H.Z., Ivanova A.V., Sharafutdinova E.N. Assessment of Antioxidant Activity of Food Products via Potentiometric Method. News of Institutes of Higher Education. *Food Technology* **4** (2004), 73–75 (in Russian).
14. Gevorgyan V.S., Chantikyan A.A., Seferyan T.Ye. Potentiometric Method for Measuring Antioxidant Activities of Plant and Fungal Aquatic and Alcoholic Extracts. *High Technol., Basic and Applied Research. in Physiol. and Med.* (2016), 28–31 (in Russian).
15. Vamanu E., Nita S. Antioxidant Capacity and the Correlation with Major Phenolic Compounds, Anthocyanin, and Tocopherol Content in Various Extracts from the Wild Edible *Boletus edulis* Mushroom. *BioMed. Res. Internat.* (2013). ID 313905. https://doi.org/10.1155/2013/313905
16. Gevorgyan V.S., Nanagulyan S.G., et al. Assessment of Antioxidant Activities of Some Medicinal Fungal Extracts. *Proc. of the YSU. Chem. and Biol. Sci*. **51** (2017), 163–165. https://doi.org/10.46991/PYSU:B/2017.51.3.163
17. Lomboeva S.S., Tankhaeva L.M., Olennikov D.N. Dynamics of Flavonoid Accumulation in the Aerial Part of *Orthilia secunda* (L.) House. *Chemistry of Plant Raw Material* **3** (2008), 83–88.

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OROBANCHE CARYOPHYLLACEA ՏԵՍԱԿԻ ԼՈՒԾԱՄԶՎԱԾՔՆԵՐԻ ԿԵՆՍԱԿՏԻՎ ՊՐՈՖԻԼԸ ԿԱԽՎԱԾ ՏԵՐ ԲՈՒՅՍԻՑ ԵՎ ՇՐՋԱԿԱ ՄԻՋԱՎԱՅՐԻ ՊԱՅՄԱՆՆԵՐԻՑ

Մակաբույծ բույսերի վրա ազդեցությունը կրում է բարդ բնույթ և որոշվում է տարբեր տարածական և ժամանակային պայմաններում գործող կենսաբանական և աբիոտիկ գործոնների համադրությամբ: Մակաբույծ-տեր բույս փոխհարաբերություններում մենք որպես բիոտիկ գործոններ դիտարկել ենք տարբեր տեր բույսեր, որոնք տարբեր ձևով են ազդում մակաբույծ բույսի վրա: Հետազոտության ընթացքում ուսումնասիրվել է նաև կենսաակտիվ պրոֆիլի և շրջակա միջավայրի պայմանների միջև կապը:

Մեր հետազոտության արդյունքները ցույց տվեցին արտաքին միջավայրի տարբեր պայմաններում և տարբեր տեր բույսերի՝ *Galium verum* և *Galium humifusum*, ազդեցության տակ աճող *Orobanche caryophyllacea* (ճրագախոտ մեխակային) տեսակի ծաղիկներից և ցողուններից անջատված ջրասպիրտային լուծամզվածքների պոլիֆենոլների գումարի և հակաօքսիդանտային ակտիվության միջև կապը:

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БИОАКТИВНЫЙ ПРОФИЛЬ ЭКСТРАКТОВ *OROBANCHE CARYOPHYLLACEA* В ЗАВИСИМОСТИ ОТ РАСТЕНИЯ-ХОЗЯИНА И УСЛОВИЙ СРЕДЫ ОБИТАНИЯ

Воздействие на растения-паразиты носит комплексный характер и определяется сочетанием биотических и абиотических факторов, действующих в различных пространственных и временных условиях. В отношениях паразит-хозяин в качестве биотических факторов мы рассматриваем различные растения-хозяева, которые по-разному влияют на растение-паразит. В исследовании также изучалась корреляция между биоактивным профилем и условиями окружающей среды.

Наши результаты продемонстрировали корреляцию между суммой полифенолов и антиоксидантной активностью водно-спиртовых экстрактов, выделенных из цветков и стеблей *Orobanche caryophyllacea* (заразиха гвоздичная) в различных условиях внешней среды и под влиянием разных растений-хозяев: *Galium verum* и *Galium humifusum*.