

ASSESSMENT OF ANTIOXIDANT ACTIVITY OF SOME
OROBANCHACEAE SPECIES FROM ARMENIA

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Holoparasitic plants of the family Orobanchaceae are represented by 36 species belonging to 4 genera in Armenia. Despite the fact that the secondary metabolites and their pharmacological properties are not well studied, the limited data on this topic is already a promising source for different bioactive compounds. In this paper, we are representing the antioxidant properties and phenolic content of four species belonging to Orobanchaceae family. The highest antioxidant activities were recorded for *Orobanche kurdica* alcoholic extract. Water soluble phenols may explain the great antioxidant capacity of the aqueous extract of *Diphelypaea tournefortii*.

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Keywords: Orobanchaceae, parasitic plants, antioxidant activity, phenols.

Introduction. The parasitic plants of Armenia hold approximately 80 species and represent only 2% of the Armenian flora [1]. The family Orobanchaceae is the largest parasitic family and includes 36 species root parasites from 4 genera: *Cistanche* Hoffmanns & Link, *Diphelypaea* Nicolson, *Orobanche* L. and *Phelipanche* Pomel.

Armenia as a part of the Caucasus is one of the most important biodiversity hotspots of the family Orobanchaceae in the world. In recent years 10 species of Orobanchaceae family were found in Armenia first time, including 3 endemic new species *Phelipanche hajastanica* Piwow., Ó. Sánchez & Moreno Mor., *Phelipanche zangezurica* Piwow., Ó. Sánchez & Moreno Mor., *Phelipanche sevanensis* Piwow., Ó. Sánchez & Moreno Mor. [2].

In root parasites, the parasitic lifestyle is characterized by developing a specific organ called a haustorium, which penetrates host plant tissue and connects the two plants. Unlike hemiparasites, holoparasites lost all their photosynthetic properties, have lower transpiration rates and absorb water and nutrients from host-plant vascular tissue [3–5]. Haustorium cells generate host-metabolites, creating a parasite-specific metabolic profile, which may affect hosts' organic and inorganic nutrients [6–10].

Some ethnobotanical and ethnomedicinal usage of plants from this family has already been reported worldwide. One can use different species of plants as toys, medicine or food [11–14].

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From 2100 species of the family Orobanchaceae [15] only a few dozen species have been investigated for their secondary metabolites. Revealed that they have many pharmacological activities such as antiaging, anticancer, anti-inflammatory, antimicrobial, antioxidant, antiviral, cytotoxic, immunomodulating, neuroprotective, and enzyme inhibitory factors [16–21]. Most of these studies indicate antitumor and skin-protecting activities and mentioned that traditionally plants are used also against impotence and female infertility.

There is very limitedly studied antifungal activity. From Armenian species only for *Orobanche crenata* mentioned antifungal activity. Phenolic extracts of the plant showed significant activity against *Aspergillus carbonarius*, *Botrytis cinerea*, *Candida spp.*, *Monilinia laxa*, *Penicillium digitatum*, *P. expansum*, *P. italicum*, and showed less activity against *Monilinia fructicola* [22, 23].

The Polish team studied about 10 species belonging to the genera *Cistanche*, *Orobanche*, *Phelipanche* and *Phelypaea* and reported that they are rich in PhGs and their number is greater than in *Herba Cistanches*, which has a high antioxidant potential and widely used in Chinese medicine as a part of nutraceutical and medicinal preparations [8–10, 12, 17, 19, 24].

An intensive analysis of the published literature revealed that the chemical content only of a few of Armenian species of this family is thoroughly studied [8–10, 19, 21, 25].

Despite all these possible applications Orobanchaceae family species being holoparasites are threatening different plant communities. Being evolutionary close to the species they parasite on it is extremely difficult to get rid of them without damaging the host organism. That is why understanding their unique metabolic pathways of synthesis of secondary metabolites also will be crucial in terms of developing highly specific and ecologically friendly herbicides to protect the plant communities from their destructive effect.

The aim of our research was to assess the phytochemical composition and biological activity of the aqueous and hydroalcoholic extracts of *Orobanche caryophyllacea*, *O. kurdica*, *Phelipanche libanotica* and *Diphelypaea tournefortii*. To date, the *O. kurdica* and *P. libanotica* have not been studied for phytochemical composition. A study of the secondary metabolites of parasitic species can be used to discover a new source for biologically active food additives and disease treatment products.

Materials and Methods.

Plant Material. The species were collected from Vayots Dzor Province of Armenia in June 2022. Specimens were dried under natural conditions. Voucher specimens were deposited in the Herbarium of Yerevan State University (ERCB). The plant names were updated according to [2, 26].

Chemicals. $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6] \cdot 3H_2O$, $NaCH_3COO \cdot 3H_2O$, acetic acid, ethyl alcohol, ascorbic acid, Folin-Ciocalteu's reagent, gallic acid, sodium carbonate were purchased from "Sigma-Aldrich" GmbH (Sternheim, Germany).

Obtaining of Extracts. Air dried samples (5.0 g) were placed for 24 h on magnetic stirrer with distilled water (50 mL) for the aqueous and water-ethanol mixture (3:7 (v/v), 50 mL) for alcoholic extract preparation. After the incubation, extracts were filtered and used.

Determination of Antioxidant Activity. The antioxidant activity (AOA) was determined by potentiometric measurements of change of ORP of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ mediatory system caused by antioxidants in extracts [27, 28].

Determination of Total Phenolic Content. The content of total phenols was determined by spectrophotometry, with some changes, using gallic acid as standard [29, 30].

Results and Discussion. Species from the family Orobanchaceae in our country are known generally as root holoparasitic weeds affecting the majority of crops, especially potatoes, tomatoes and eggplants. Population didn't distinguish species from genera *Orobanche*, *Phelipanche*, *Cistanche*. All species from these genera they called *tchragakhot* (torch plant) or *zarazikha* (from Russian name of genus *Orobanche*). The species of genus of *Orobanche* traditional uses insignificant percentage of the population as a medicinal plant for blood diseases, as well as tea and dye for threads and eggs.

Armenians are well acquainted only with representatives of genus *Diphelypaea*. They called them different in various regions: *chibukh* (chibouk), *vayri tup* (wild tulip) in Tavush Marz; *lala* (wild tulip) or *sari lala* (mountain tulip) in Aragatsotn and Syunik Marzes, *arji mushtuk* (cigarette holder of bear) in Vayots Dzor. In Central Armenia species from genus *Diphelypaea* population called *yot eghbor aryun* (blood of seven brothers), *arabi aryun* (Arabian blood) or *turqi lala* (Turkish tulip) and have superstition that plant brings unhappiness and death. These species are traditional uses generally as decorative plants, rarely as medicinal plants for arthrosis and as a dye for threads and eggs.

Diphelypaea tournefortii (Desf.) Nicolson occurs as a parasite on the roots of *Tanacetum polycephalum* subsp. *argyrophyllum* (K. Koch) Podlech [2]. It grows on the rocky steppe slopes of Armenia, 1200–2500 m above sea level (Fig. 1).



Fig. 1. *Diphelypaea tournefortii* parasitic on the roots of *Tanacetum polycephalum* subsp. *Argyrophyllum*.



Fig. 2. *Orobanche caryophyllacea* parasites on *Galium verum*

Orobanche caryophyllacea Smith parasites exclusively on the Rubiaceae family species, in our case on *Geranium verum* L. (Fig. 2). Plant grows on steppe communities, subalpine meadows, edges of forests and shrubs, 800–2400 m above sea level [2].

Orobanche kurdica Boiss. & Hausskn. grows on rocky slopes of extinct volcanos, from lower to upper mountain belts, subalpine meadows, and forests with *Quercus macranthera* Fisch. & C.A. Mey. ex Hohen. Hosts plant is *Prangos ferulacea* (L.) Lindl. from Apiaceae family.

Phelipanche libanotica (Schweinf. ex Boiss.) Soják is typical in southern and southeastern regions of Armenia, especially on dry rocky slopes 670–1900 m above sea level, solely on *Prunus fenziiana* R. M. Fritsch and *P. nairica* (Fed. & Takht.) (Rosaceae) [2].

According to our results, the highest amount of phenolic compounds was recorded for alcoholic extracts of *O. kurdica* (Tab. 1). This can be explained due to the fact that the host species *Prangos ferulacea* also contains a high amount of phenolic compounds [31].

Table 1

Total phenolic content of investigated plant extracts

Species	Phenolic compounds mg gallic acid per g of dried plant sample \pm SD	
	aqueous extract	alcoholic extracts
<i>Diphelypaea tournefortii</i>	1.6762 \pm 0.0252	1.2557 \pm 0.0189
<i>Orobanche caryophyllacea</i>	1.0877 \pm 0.0163	1.5774 \pm 0.0237
<i>Orobanche kurdica</i>	0.6663 \pm 0.01	3.5550 \pm 0.0534
<i>Phelipanche libanotica</i>	0.9917 \pm 0.015	1.0192 \pm 0.0153

In addition, it is interesting to note that the aqueous extract of *O. kurdica* showed the smallest content of the total phenols. From aqueous extracts, higher content of phenols was revealed for *D. tournefortii*.

As expected, the highest antioxidant activities were recorded for *O. kurdica* alcoholic extract (Tab. 2). Despite the average content of phenols, significant antioxidant activity was also observed in an alcoholic extract of *O. caryophyllacea*. Water soluble phenols may explain the great antioxidant capacity of the aqueous extract of *D. tournefortii*.

Table 2

Antioxidant activity of investigated plant extracts

Species	Antioxidant activity $\times 10^{-4}$ g vit C eq. per L \pm SD	
	aqueous extract	alcoholic extracts
<i>Diphelypaea tournefortii</i>	56 \pm 2	18 \pm 3
<i>Orobanche caryophyllacea</i>	34 \pm 1	54 \pm 2
<i>Orobanche kurdica</i>	24 \pm 1	74 \pm 2
<i>Phelipanche libanotica</i>	25 \pm 3	24 \pm 3

The results demonstrated that *O. kurdica*, *O. caryophyllacea* and *D. tournefortii* can be considered as a valuable source for obtaining polyphenolic compounds possessing antioxidant and other biological activities.

Conclusion. Species of the Orobanchaceae family in Armenia were very limitedly studied. The biological activities of most of these plants have never been published. Among the investigated species *Orobanche kurdica* shows the highest potential to provide a bunch of secondary metabolites. The plant can serve as a potential source of antioxidant compounds and will continue to study the medical uses of compounds isolated from these parasite plants.

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ՀԱՅԱՍՏԱՆԻ OROBANCHACEAE ԸՆՏԱՆԻՔԻ ՈՐՈՇ ՏԵՍԱԿՆԵՐԻ
ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ԳՆԱՀԱՏՈՒՄ

Հայաստանում *Orobanchaceae* ընտանիքի հոլոպարազիտ բույսերը ներկայացված են 4 ցեղերին պատկանող 36 տեսակներով: Չնայած այն հանգամանքին, որ դրանց երկրորդային մետաբոլիտները և դեղաբանական հատկությունները դեռևս լավ ուսումնասիրված չեն, այս թեմայի վերաբերյալ առկա սահմանափակ տվյալներն արդեն իսկ խոստումնալից են: Աշխատանքում ներկայացված են ՀՀ տարածքից հավաքված *Orobanchaceae* ընտանիքին պատկանող 4 տեսակների հակաօքսիդանտային ակտիվությունը և ֆենոլների պարունակությունը: Ամենաբարձր հակաօքսիդանտային ակտիվությունը գրանցվել է *Orobanche kurdica* տեսակի սպիրտային լուծամզվածքի համար: *Diphelypaea tournefortii* տեսակի ջրային լուծամզվածքի բարձր հակաօքսիդանտային հատկությունը պայմանավորված է ջրալույծ ֆենոլների պարունակությամբ: Ներկայացված ուսումնասիրությունն այս ընտանիքի էթնոբուսաբանական և էթնոբժշկական տվյալների հետ միասին արժեքավոր կլինի հետագա դեղաբանական հետազոտությունների համար:

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ОЦЕНКА АНТИОКСИДАНТНОЙ АКТИВНОСТИ НЕКОТОРЫХ ВИДОВ
СЕМЕЙСТВА OROBANCHACEAE В АРМЕНИИ

В Армении голопаразитные растения семейства *Orobanchaceae* представлены 36 видами, относящимися к 4 родам. Несмотря на то, что во всем мире еще недостаточно изучены их вторичные метаболиты и фармакологические свойства, немногочисленные данные по этой теме уже являются многообещающими. В статье представлены содержание фенолов и антиоксидантная активность 4 видов растений, принадлежащих к семейству *Orobanchaceae*, собранных на территории Республики Армения. Наибольшая антиоксидантная активность зарегистрирована у спиртового экстракта *Orobanche kurdica*. Водорастворимые фенолы объясняют высокую антиоксидантную способность водного экстракта *Diphelypaea tournefortii*. Представленное исследование, наряду с этноботаническими и этномедицинскими данными об этом семействе, будет ценным для дальнейших фармакологических исследований.