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Morphologic and chemical characterizations of some salep orchids

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Abstract

Salep orchids have been used as a food for centuries without cultivating but, mainly harvesting from nature. After powdering, their tubers are utilized as hot beverage and in ice-cream industry as a stabilizer. Due to their importance in food industry, it is important to characterize the morphology and chemistry of salep orchids. In this study, it was aimed to determine some morphological (fresh weight, dry weight and dry matter ratio of tubers and the number of tubers required for 1 kg salep powder) and chemical (glucomannan, starch, protein, moisture, ash, nitrogen, phosphorus, calcium, magnesium, potassium, iron, copper, manganese and zinc contents and glucomannan/starch ratio of tubers) properties of 10 salep orchid species from 6 genus namely, *Anacamptis pyramidalis*, *Dactylorhiza romana*, *Himantoglossum caprinum*, *Himantoglossum comperianum*, *Ophrys apifera*, *Ophrys mammosa*, *Orchis coriophora*, *Orchis morio*, *Orchis tridendata*, *Serapias vomeracea* grown in nature of Black Sea Region of Türkiye. Such a kinds of data is the first and can be beneficial in utilizing the researched species in food industry.

Keywords: Mineral contents, Orchidacea, sahlep, terrestrial orchids, tuber.

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Introduction

The Orchidacea family is the second one that has the highest number of species, naturally grown in many diversified habitats of the world. This family consists of terrestrial, tropical, epiphytic and lithophilic species and is one of the most biologically complex taxon of the plant kingdom (Swarts and Dixon, 2009). Terrestrial orchids are naturally distributed in temperate climatic regions and many species of this group form a tuber under the ground. Tubers of those orchids have been used as food, healing agents and aphrodisiac for centuries. Tuberous orchids, which are widely used in India, Nepal, China, Turkey, the Middle East, Europe and other temperate regions of the world, are called salep orchids (Farazi et al., 2013; Bozdoğan and Yaşar, 2016; Tığlı and Fakir, 2017). Some species are widespread throughout the world, while others adapted to the climate and soil structure of each region and altitude differences and therefore spread in certain areas. In other words, the areas where salep orchids grow are different due to their ecological demands on a species basis.

Today, Salep is largely collected in Asia Minor, Germany, Greece, Iran, Afghanistan and India (Turgay and Cinar, 2017). It is not possible to mention the total collected amount of tubers as they are not domesticated and are mainly obtained by wild-crafters. Many species from 10 different genera are used in the production of salep in Turkey. The number of salep orchids in Turkish flora is indicated as 38 to 60 (Sezik, 2002; Tamer et al., 2006; Kreutz, 2009).

Salep, which has been consumed fondly for centuries as a hot beverage due to its medicinal properties, is also an indispensable raw material in the production of Maraş type ice cream (Kasparek and Grim, 1999; Kurt and Kahyaoglu, 2017). The history of salep goes back thousands of years. In Historia Plantarum, Theophrastus (372-286 BC) attributed medicinal properties to orchid species, and in De Materia Medica Dioscorides (50-70

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AD) ascribed healing properties were ascribed to two terrestrial orchids based on the resemblance of their tubers to testicles (Kreziou et al., 2015). In traditional medicine, salep has been prescribed for dressing and treating glottal inflammations and intestine disorders, tuberculosis, diarrhea, parkinson, cancer, fever, and is especially used to strengthen the sexual activity, erectile dysfunction therapy, physical strength enhancement and increase vigorousness (Thakur et al. 2009; Tekinşen and Güner, 2010; Farazi et al., 2013; Pourahmad et al., 2015; Atashpour et al., 2017; Tatiya et al., 2018).

The main components of salep tubers are glucomannan and starch both of which are carbohydrate derivative (Farhoosh and Riazi, 2007). Besides, salep contains different compounds such as nitrogenous substances, protein, particularly calcium, potassium, iron, chlorides and phosphates, and some trace levels of volatile oils, ferulic acid, quercetin, daucosterol, cirsilineol and steroids. The most important factor that affects chemical composition and quality is the species of orchids from which salep powder is produced. Ecological conditions of plant habitat also affect the chemistry and quality of salep (Hossain, 2011; Lalika et al., 2013). Glucomannan is a polysaccharide formed by the binding of β-D-glucose and α-D-mannose molecules with β-1,4 bonds (Yaşar et al., 2009). One gram of glucomannan is capable of absorbing 200 ml of water. Doi (1995) reported glucomannan can absorb up to 50 times its weight in water, making it one of the most viscous dietary fibers known. With this feature, glucomannan gives consistency to salep drink, which is a hot beverage. It gives hardness and late melting properties to Maraş type ice cream and also delays the melting time of ice cream.

Salep orchids are taken under protection around the world due to the fact that they are collected from nature without cultivation (Dixon et al., 2003). The international trade of naturally grown plants in the world is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which was put into practice for the first time in 1976. This convention has been signed by 183 countries. The European Union (EU), on the other hand, put into practice the conditions required by the CITES contract with the Council Regulation No. 3626/82, which entered into force in 1984 (Jenkings and Oldfield, 1992). Annex I of this regulation defines the endangered orchid species whose trade is prohibited. All salep species, except those in Annex-I, are included in the CITES Annex-II list (Yaman, 2013).

Despite all the prohibitions, the collection of salep tubers from the natural flora continues today. It is not known exactly how much salep is collected, as it is the product that is collected from nature without cultivating. However, it is estimated that around 500 tons of salep tubers are collected annually throughout Turkey, 80-100 tons of salep flour is obtained by drying and grinding them, used in ice cream production and consumed as a beverage (Caliskan et al., 2019, 2020).

In the present study, it was aimed to determine the morphological features and chemical properties of the tubers of some salep orchids (*Anacamptis pyramidalis* (L.) Rich., *Dactylorhiza romana* (Sebast.) Soo, *Himantoglossum caprinum* (M.Bieb.) Spreng., *Himantoglossum comperianum* (Steven) P.Delforge, *Ophrys apifera* (Huds.), *Ophrys mammosa* (Desf.), *Orchis coriophora* (L.), *Orchis morio* (L.), *Orchis tridendata* (Scop.) and *Serapias vomeracea* (Burm.f.) Briq.) grown wildly in the nature of Central Black Sea Region, Türkiye. This study could be the first report on tuber morphology, mineral element composition and chemical content of the tested species.

Material and Methods

The research was carried out in the Central Black Sea region, within the borders of Samsun province. The total area of studied region is 1055 km^2 and the altitude of sampling sites varies from 0-1500 m causing high diversity in salep flora. The districts, altitudes and coordinates for plant-growing sites are shown in Table 1. In this region, which has a rich flora in terms of the diversity of salep orchids, field trips were made in March-June and samplings were done.

Table 1. Geographical data for sampling sites where the tested salep orchids were collected

Species	District *	Altitude (m)	Latitude (N)	Longitude (E)
Anacamptis pyramidalis (L.) Rich.	Yakakent	132	41° 62′ N	35°51′ E
Dactylorhiza romana (Sebast.) Soo.	Bafra	192	41° 47′ N	35° 91′ E
Himantoglossum caprinum (M.Bieb.) Spreng.	Bafra	1011	41° 37′ N	35° 54′ E
Himantoglossum comperianum (Steven) P.Delforge	Bafra	1005	41° 39′ N	35° 58′ E
Ophrys apifera (Huds.)	Çarşamba	248	41° 09′ N	36° 64′ E
Ophrys mammosa (Desf.)	Bafra	8	41° 64′ N	35° 92′ E
Orchis coriophora (L.)	Ondokuzmayıs	1	41° 66′ N	36° 05′ E
Orchis morio (L.)	Ladik	740	41° 55′ N	36° 07′ E
Orchis tridendata (Scop.)	Ondokuzmayıs	208	41° 45′ N	36° 03′ E
Serapias vomeracea (Burm.f.) Briq	Çarşamba	49	41° 23′ N	36° 05′ E

*Samsun province in Turkey.

Required legal tuber collection permission was obtained before the study. The number of species in the region is more than twenty, but rare species were ignored. With this purpose the common wild-growing species namely, *Anacamptis pyramidalis* (L.) Rich., *Dactylorhiza romana* (Sebast.) Soo, *Himantoglossum caprinum* (M.Bieb.) Spreng., *Himantoglossum comperianum* (Steven) P.Delforge, *Ophrys apifera* (Huds.), *Ophrys mammosa* (Desf.), *Orchis coriophora* (L.), *Orchis morio* (L.), *Orchis tridendata* (Scop.) and *Serapias vomeracea* (Burm.f.) Briq were sampled to obtain 10 gram of dry sample for each species.

The fresh weights of tubers collected from natural flora were determined after washing and cleaning. The tubers were boiled in hot water at 110-120°C for 6-8 minutes and left to dry. By dividing the fresh and dry weights by the number of tubers, the average single tuber weight and the number of tubers required to obtain one kilogram of dry salep were calculated. Thus, data for tuber size and morphology were obtained. The dried and powdered samples were objected to chemical analyses to determine their macro and micro nutrients contents as detailed below.

Total moisture, protein and ash contents

The gravimetric method was used to determine moisture the content at 105°C. Dried plant samples were ground to powder size before analysis. Ash contents of plant samples were determined in a muffle furnace at 550°C for 24 h. Protein content was determined according to the Kjeldahl method based on the nitrogen conversion factor of 5.7. For P contents by spectrophotometric method, for K, Ca, Mg, Fe, Mn, Zn, and Cu contents using atomic absorption spectrophotometer (AOAC, 2000; Kacar and Inal, 2008).

Glucomannan and starch contents

Glucomannan (1) (K-GLUM 12/19) and starch (2) (K-TSTA 11/20) assay kit purchased from Megazyme International Ireland Ltd. was used to determine glucomannan and starch content of salep samples. Absorbance values of prepared blank and sample solutions were measured to determine the glucomannan and starch contents of the samples using a UV-Vis Spectrophotometer at 340 nm for glucomannan and 510 nm for starch. The following formulas were used to calculate the contents (Megazyme, 2004a,b):

$$\Delta \text{Aglucomannan} = (\text{A3} - \text{A1})\text{sample} - (\text{A3} - \text{A1})\text{blank x } 36.8\ [\text{g}/100\text{g}] \tag{1}$$

where A1 and A3 were the measured absorbance values of prepared blank and sample solutions for glucomannan at the end of reaction approximately after 3 min and 20 min, respectively.

$$\text{Starch} = \Delta\text{A x F/W x } 9.18\ [\text{g}/100\text{g}] \tag{2}$$

where

ΔA = absorbance value of sample solution against reagent blank after 50°C for 20 min incubation

W = the weight of the sample

F = (100 (μg of glucose control)) / (Absorbance value of glucose control (1.111))

Data analysis

Data for morphological and chemical tuber characters for each species tested were objected to one-way analysis of variance (ANOVA) and significant differences among mean values were tested with the Duncan Multiple Range Test (P<0.01) by using the statistical software package XLSTAT2010 Trial Version.

Results and Discussion

Morphological characters

The tubers of salep orchids are divided into two groups in terms of morphological appearance. The *Dactylorhiza* genus, which has palmate tuber shape and is called footed or fingered, is in one group, while other oval-shaped genera are in the other group. As shown in Figure 1, *D. romana* which is one of the tested species is always recognized with two bulges, formed on lower part of its tuber. Tuber shape is oval, close to round or narrow oval for the other tested genera. Variations in tuber shape have been attributed to genetic factors, varied with species (Aybeke, 2012; Kurt, 2020). Fresh and dry tuber weights of the tested species are shown in Table 2. The number of tubers, required to obtain 1 kg dry salep was calculated based on tuber dry weights.

As shown in Table 2, *Himantoglossum comperianum* produced the highest values for fresh and dry tuber weights (9.09 and 0.32 g, respectively), while *Orchis morio* tubers had the lowest dry and fresh weights (2.35 and 0.32 g, respectively). Interestingly, mean values of tuber fresh weight were lower than 3 g for *Dactylorhiza romana* and *Ophrys mammosa* together with *Orchis morio*, thus these species can be characterized as small tuberous ones. As for dry matter ratio of tubers, mean values varied from 13.6-22.3% depending on species. As in the cases of fresh and dry tuber weights, *Orchis morio* produced the lowest tuber dry matter ratio, but this character reached its highest levels in *D. romana* (22.1%), *O. coriophora* (22.3%) and *S. vomeracea* (22.2%). It is noteworthy to note that *H. caprinum* and *H. comperianum* which have large tubers also produced lower dry matter ratio when compared to the other tested species. Based on the data, presented in Table 2, it can be concluded that about 7 kg of the fresh tuber is required approximately to obtain 1 kg of dry salep.

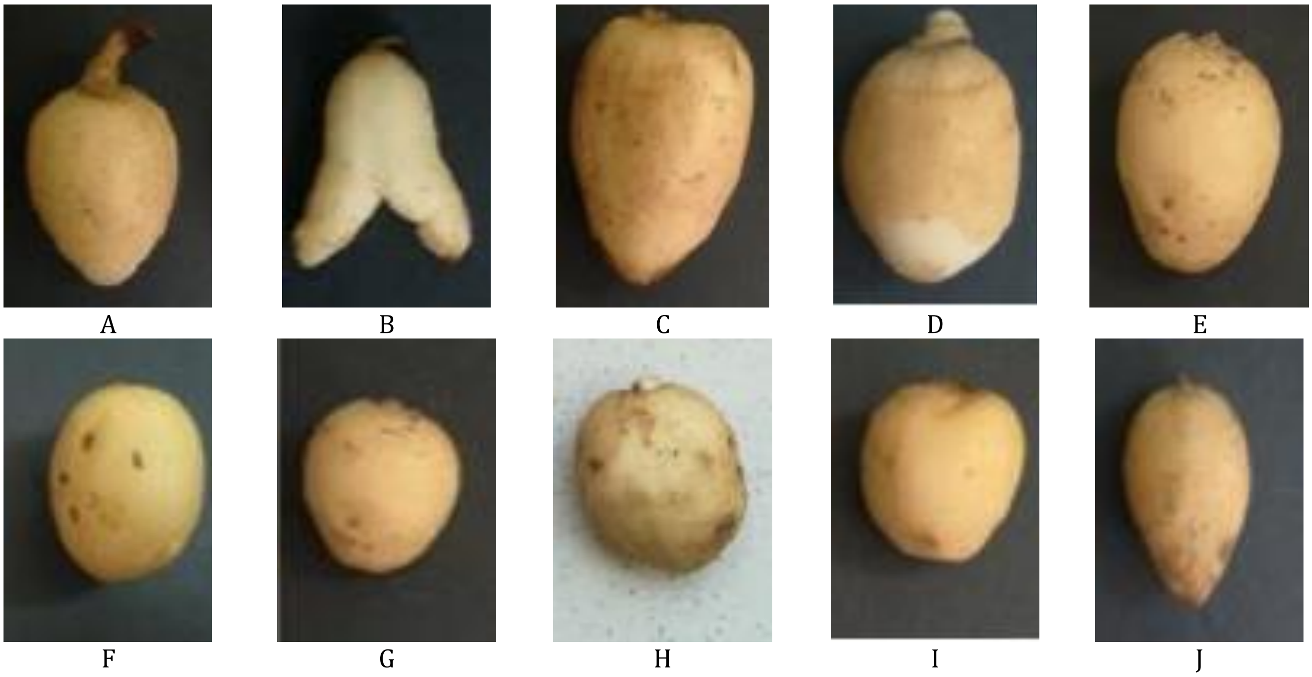

Figure 1. Views from tubers of the studied orchid species. A; *Anacamptis pyramidalis* (L.) Rich., B; *Dactylorhiza romana* (Sebast.) Soo, C; *Himantoglossum caprinum* (M.Bieb.) Spreng., D; *Himantoglossum comperianum* (Steven) P.Delforge, E; *Ophrys apifera* (Huds.), F; *Ophrys mammosa* (Desf.), G; *Orchis coriophora* (L.), H; *Orchis morio* (L.), I; *Orchis tridendata* (Scop.) and J; *Serapias vomeracea* (Burm.f.) Briq.

Table 2. Some morphologic characters of the tubers of salep orchids tested

Species	Fresh tuber weight (g /tuber)	Dry tuber weight (g /tuber)	Dry matter ratio (%)	The number of tuber required to obtain one kg salep powder
Anacamptis pyramidalis (L.) Rich.	5.88	1.16	19.8	859
Dactylorhiza romana (Sebast.) Soo.	2.99	0.66	22.1	1516
Himantoglossum caprinum (M.Bieb.) Spreng.	6.00	0.88	14.7	1134
Himantoglossum comperianum (Steven) P.Delforge	9.09	1.32	14.5	757
Ophrys apifera (Huds.)	3.20	0.64	20.0	1565
Ophrys mammosa (Desf.)	2.55	0.40	15.8	2488
Orchis coriophora (L.)	3.11	0.69	22.3	1445
Orchis morio (L.)	2.35	0.32	13.6	3139
Orchis tridendata (Scop.)	4.18	0.83	19.9	1201
Serapias vomeracea (Burm.f.) Briq	2.40	0.53	22.2	1877

Ghorbani et al. (2014) reported that 1 kg of salep is produced from 4-8 kg of fresh tubers, depending on plant age, species and harvest time. They also reported that the number salep tubers, required to obtain one kg dry salep is 605±219 for *Dactylorhiza* genus and 1117±236 for other species. Here we observed that one kg dry salep could be produced from 1516 salep tubers for *D. romana*. The fresh weight of single tuber in *Anacamptis sancta* was 4.9 g (Parlak, 2016). In a previous study, conducted out on Bucak district of Burdur province, fresh tuber weight was reported to be 1.17 g for *Orchis mammosa*, 1.04 g for *Orchis anatolica,* 16.8 g for *H. comperianum*, 1.7 g for *Ophrys rein* subsp*. leucotaenia*, 1.57 g for *Ophrys amanensis* subsp*. antalyensis* and 1.65 g for *Orchis palustris* (Tığlı and Fakir, 2017). As in our case, *O. mammossa* was characterized as small tuberous, but *H. comperianum* as large tuberous one in this previous report. Quantitative differences, however, between the present and previous results could be ascribed the evidently different ecological conditions of plant habitats as the two sampling areas are separated by a distance of 850 km and Bucak district of Burdur province has higher temperature and precipitation values.

The number of tubers to be used to obtain ready-to-eat salep flour is a reflection of the wet tuber weight and dry matter ratios. We observed a great variation for the number of tuber required to obtain 1 kg salep powder varying with 757-3139 depending of the species tested. While producing salep powder by using dry tubers, a loss of 3% occurs. Considering this loss, it appears that the number of tuber required to obtain 1 kg salep powder, in fact, is higher than that of our detection for many of species. This number was reported to be 1000-4000 (Sezik, 2002) and 1000-4350 (Lande, 1998) without indicating the orchid species used. Here, it is the first time we have reported the number of tuber required to obtain 1 kg salep powder for each species which has practical meaning in evaluating the tested species in food industry and protecting the wild plant populations.

Chemical characters

In the present study conducted out on a total of 10 species from 6 genera, we also observed a great variation in the chemical characters of tubers as in the case of morphologic ones (Table 3). Especially, starch, protein and glucomannan contents of tubers varied significantly with species. We detected a twofold difference between the lowest and highest starch contents (21.09% for *S. vomeracea* and 42.16% for *H. comperianum* % 42.16) and a more than threefold difference between the lowest and highest glucomannan contents (9.68% for *O. mammossa* and 32.54% for *A. piramidalis*) of tubers. Our current data are not in accordance with the previous ones in literature. Starch and glucomannan contents of tubers were reported to be 5.44-38.7% and 17.7-54.6% respectively, depending on species (Tekinşen and Güner, 2010). Sen et al. (2019) reported starch and glucomannan contents of six salep orchids to be 12.24-40.21% and 7.84-43.67%, respectively. Glucomannan content of five salep orchids from south eastern part of Anatolia was reported to vary with 29.47-59.63% namely, 30.13% for *O. lutea*, 59.63% for *S. vomeracea*, 28.60% for *O. mammosa*, 30.17% for *O. umbilicata* and 29.47% for *O. sancta* (Ertaş et al., 2019). The general opinion is that genetic factors are the main factor determining the chemical content of salep species. In addition, environmental factors of plant growing habitat are also indicated to affect the chemical content of orchid tubers (Sen et al,. 2019). Arabacı et al. (2017), for example, reported that starch and glucomannan contents of *Serapias vomeracea* tubers varied significantly with plant developmental stages. In our case, plants were sampled at the end of flowering period.

Table 3. Starch, glucomannan, protein and moisture contents and glucomannan / starch ratio of the tubers of salep orchids tested

Species	Starch (%)	Glucomannan (%)	Glucomannan / starch ratio (%)	Protein (%)	Moisture (%)
Anacamptis pyramidalis (L.) Rich.	21.34 d	32.54 a	1.52	3.25 de	10.93 a
Dactylorhiza romana (Sebast.) Soo.	28.09 c	21.20 c	0.75	4.82 b	8.65 de
Himantoglossum caprinum (M.Bieb.) Spreng.	29.04 c	15.64 d	0.53	3.15 e	8.41 e
Himantoglossum comperianum (Steven) P.Delforge	42.16 a	10.32 e	0.24	7.83 a	9.06 cde
Ophrys apifera (Huds.)	39.66 b	11.74 e	0.30	4.58 bc	9.22 b-e
Ophrys mammosa (Desf.)	41.24 ab	9.68 e	0.23	4.87 b	9.75 a-d
Orchis coriophora (L.)	22.69 d	26.95 ab	1.18	3.99 cd	10.53 ab
Orchis morio (L.)	21.75 d	23.79 bc	1.09	3.97 cd	9.98 abc
Orchis tridendata (Scop.)	40.93 ab	13.48 e	0.32	5.19 b	10.25 abc
Serapias vomeracea (Burm.f.) Briq	21.09 d	30.52 a	1.45	2.43 e	9.71 a-d
CV (%)	2.12	8.54	-	7.37	5.24

Results from the limited number of previous studies revealed a negative relationship between starch and glucomannan contents of salep orchids' tubers as confirmed by our present results. The most important quality character of salep tubers is their glucomannan content which is crucial for hot beverage and ice-cream production and a high ratio is desirable. Thus, glucomannan/starch ratio was supplied for each species tested and presented in Table 3. Such kind of data could be useful for further evaluating the tested species in food industry.

Protein contents of the tested species varied from 2.43-7.83% and we observed 2-3-fold differences among mean values. The protein content of tubers was reported to be 3.17-4.95% (Tekinşen and Güner, 2010) and 2.70-11.93% (Sen et al. 2018) for other salep orchids. The protein content of orchid tubers has been affected by several factors such species, soil characteristics and climatic peculiarities. For example, protein content of *Serapias vomeracea* tubers which is one the most prevalent and studied species was reported to be 4.83% (Tekinşen and Güner, 2010), 2.70% (Sen et al., 2019), 12.9% (Arabacı et al., 2017) and 11.2% (Kurt and Kahyaoglu, 2017).

Our present results are within the scope of literature data together with some insignificant differences. For example, protein content of *Orchis tridendata* tubers was reported as 4.94% by (Tekinşen and Güner, 2010) while we detected it as 5.95%. The same authors also reported this value to be 4.95% for *Orchis morio*, but we found it to be 3.97%. Glucomannan contents of the tested orchids exhibited a greater variation when compared to results of the previous studies on the same species. Tekinşen and Güner (2010) reported glucomannan content of *O. mammosa* tubers as 17.7%, but we detected as 9.68%. There is indeed a big difference between these data but it should be noted that the lowest glucomannan content was produced by the same species, *O. mammosa,* among the tested salep orchids in both studies. Based on the present and previous results, it can be concluded that chemical content of salep orchids are affected not only by soil and climatic factors but also genetic ones. Harvesting time, of course, is another factor affecting chemical content of tuber. Likewise, Arabacı et al. (2017) reported that starch content of *Serapias vomeracea* was 14.01% at the beginning of blooming but 21.41% at full blooming in two consecutive years. We detected starch content of the same species as 21.09%.

Ash ratio is regarded as a quality character for salep orchids and this criterion varied significantly with species in the present study. *Himantoglossum caprinum* (2.86%) and *Himantoglossum comperianum* (2.67%) produced the highest ash content while the lowest value for this criteria was supplied by *Orchis tridendata* (0.93%) (Table 4). Türkmen (2019) reported ash ratio of *Anacamptis. pyramidalis, Orchis. mascula, Ophrys apifera, Dactylorhiza majalis, Serapias lingua, Platanthera. grandiflora, Himantoglossum hircinum, Ophrys sphegodes, Habenaria repens, Platanthera chlorantha* tubers to be 1.42-3.37%. As for the nitrogen content of tuber, we detected no significant difference among the tested species and it is noteworthy to note that this criterion does not affect the on quality of salep powder. On the contrary, the moisture and ash contents are important in terms of storage and detecting the adulteration. To store the salep tubers for a long time, the amount of moisture should be less than 10%, and the amount of ash should be less than 5% in order to obtain white salep flour (Sezik, 2002; Karaman et al., 2012).

Table 4. Mineral contents of the tubers of salep orchids tested

Species*	Ash (%)	N (%)	P (%)	Ca (%)	Mg (%)	K (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
A	1.90 c	0.95 b	0.120 de	0.125 d	0.058 bcd	0.495 de	66.55 f	39.13 d	6.99 e	38.06 b
B	1.28 d	1.05 b	0.118 de	0.071 f	0.047 d	0.374 fg	115.89 d	109.89 a	12.40 cd	106.13 a
C	2.86 a	1.03 b	0.134 de	0.097 e	0.065 b	0.526 cd	190.32 a	44.34 d	23.85 b	46.30 b
D	2.69 a	1.56 a	0.210 b	0.152 c	0.100 a	0.662 a	64.81 f	97.82 ab	10.74 de	111.31 a
E	2.37 b	0.99 b	0.170 c	0.193 b	0.106 a	0.584 bc	202.64 a	50.95 cd	12.20 cd	42.38 b
F	1.88 c	1.53 a	0.240 a	0.099 e	0.064 cb	0.619 ab	169.45 b	46.35 d	13.50 cd	49.05 b
G	2.32 b	0.72 c	0.171 c	0.334 a	0.097 a	0.473 de	86.22 e	24.95 e	10.67 de	25.40 b
H	1.79 c	0.98 b	0.109 e	0.099 e	0.045 d	0.440 ef	60.95 f	60.46 c	16.11 c	63.65 ab
I	0.93 e	0.95 b	0.119 de	0.160 c	0.069 b	0.573 bc	138.31 c	92.00 b	11.40 cde	74.35 ab
J	1.25 d	0.88 bc	0.138 d	0.188 b	0.051 cd	0.319 g	112.28 d	48.03 d	61.30 a	39.59 b
CV (%)	6.89	9.63	8.85	5.67	10.79	8.57	9.28	12.44	14.81	5.88

*A; *Anacamptis pyramidalis* (L.) Rich., B; *Dactylorhiza romana* (Sebast.) Soo, C; *Himantoglossum caprinum* (M.Bieb.) Spreng., D; *Himantoglossum comperianum* (Steven) P.Delforge, E; *Ophrys apifera* (Huds.), F; *Ophrys mammosa* (Desf.), G; *Orchis coriophora* (L.), H; *Orchis morio* (L.), I; *Orchis tridendata* (Scop.) and J; *Serapias vomeracea* (Burm.f.) Briq.

To the best of our knowledge, no study has been done on mineral content of salep orchids, except for *Dactylorhiza romana* P, Ca, K and Mg contents of which were reported to be 0.170, 0.152, 0.734 and 0.066% respectively. Fe, Mn, Cu and Zn contents of the same species were detected to be 117.62, 12.91, 4.05 and 13.85 ppm, respectively (Palaz et al., 2018). We also determined similar Fe and Mn contents but somewhat different contents of the rest minerals in the present study for *D. romana*. Mean values for mineral contents of the tested salep orchids are shown in Table 4. Mineral contents varied significantly by species and were doubled between the lowest and highest N, P and K contents; we observed a four-fold difference for the Zn content and a nine-fold difference for the Mn content. These evident differences in the mineral contents of tubers could be attributed mainly to soil characters and genetic factors because results from the studies on plant nutrition and soil productivity indicated that mineral contents of plant species depend greatly on the presence of available mineral matter contents in soils. Considering the size of sampling area (1055 m^2), soil characters of plant habitats are greatly different, and the type and amount of minerals, taken from the same soil are significantly different and depend greatly on plant species and genotypes. Mineral contents of the tested species here are reported for the first time.

Conclusion

Salep orchids, which are widely distributed in the temperate zone, have a rich diversity with tens of genera and hundreds of species. Salep orchid species have always been important medicinal plants from historical times to the present. For this reason, they have been illegally collected from the natural flora and offered to the market for hundreds of years. A large number of tissue culture studies are carried out on species around the world and production possibilities are tried to be improved. However, the morphological and chemical characteristics of the species are not known enough. For example, how many salep tubers need to be collected to produce one kg of salep flour is presented here for the first time on a species basis. According to the tuber size and dry matter ratio of the species, this number varied between 757-3139. The mineral content of salep tubers was revealed for the first time in this study. *Himantoglossum caprinum* (M.Bieb.) Spreng. and *Himantoglossum comperianum* (Steven) P.Delforge have the highest ash content. The most important component in salep orchids is glucomannan. It is understood that the glucomannan ratio varies greatly according to the species. *Ophrys mammosa* (Desf.) had the lowest glucomannan ratio (9.68%). The highest glucomannan ratio is in *Anacamptis pyramidalis* and *Serapias vomeracea* (Burm.f.) Briq species (32.54-30.52 %). There is more than a threefold difference in glucomannan content between the species considered. A similar situation is observed when few sources are examined on the chemical content of salep species. A large number of tissue culture studies are carried out on species around the world and production possibilities are tried to be improved. In future studies, tuber chemical content should be taken into account for the food industry, and it will be important to concentrate the research on species with high glucomannan and protein content.

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