Vol.66: e23210265, 2023
https://doi.org/10.1590/1678-4324-2023210265
ISSN 1678-4324 Online Edition

*Article - Biological and Applied Sciences*

# Gas Chromatography-Mass Spectrometry Analysis and Antimicrobial and Antioxidant Activities of Some Orchid (Orchidaceae) Species Growing in Turkey

**Ömer Ertürk**[1]
https://orcid.org/0000-0001-5837-6893

**Melek Çol Ayvaz**[2]*
https://orcid.org/0000-0001-5155-5784

**Elif Çil**[3]
https://orcid.org/0000-0003-1420-8729

**Emine Bağdatlı**[2]
https://orcid.org/0000-0001-5946-2988

[1]Ordu University, Faculty of Art and Sciences, Department of Molecular Biology and Genetics, Ordu, Turkey; [2]Ordu University, Faculty of Art and Sciences, Department of Chemistry, Ordu, Turkey; [3]Ordu University, Faculty of Education, Department of Primary School Education, Ordu, Turkey.

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Najeh Maissar Khalil

Received: 24-Apr-2021; Accepted: 23-Aug-2022

*Correspondence: melekcol@hotmail.com; Tel.: +00-90-4522265200 (M.Ç.A.)

**HIGHLIGHTS**

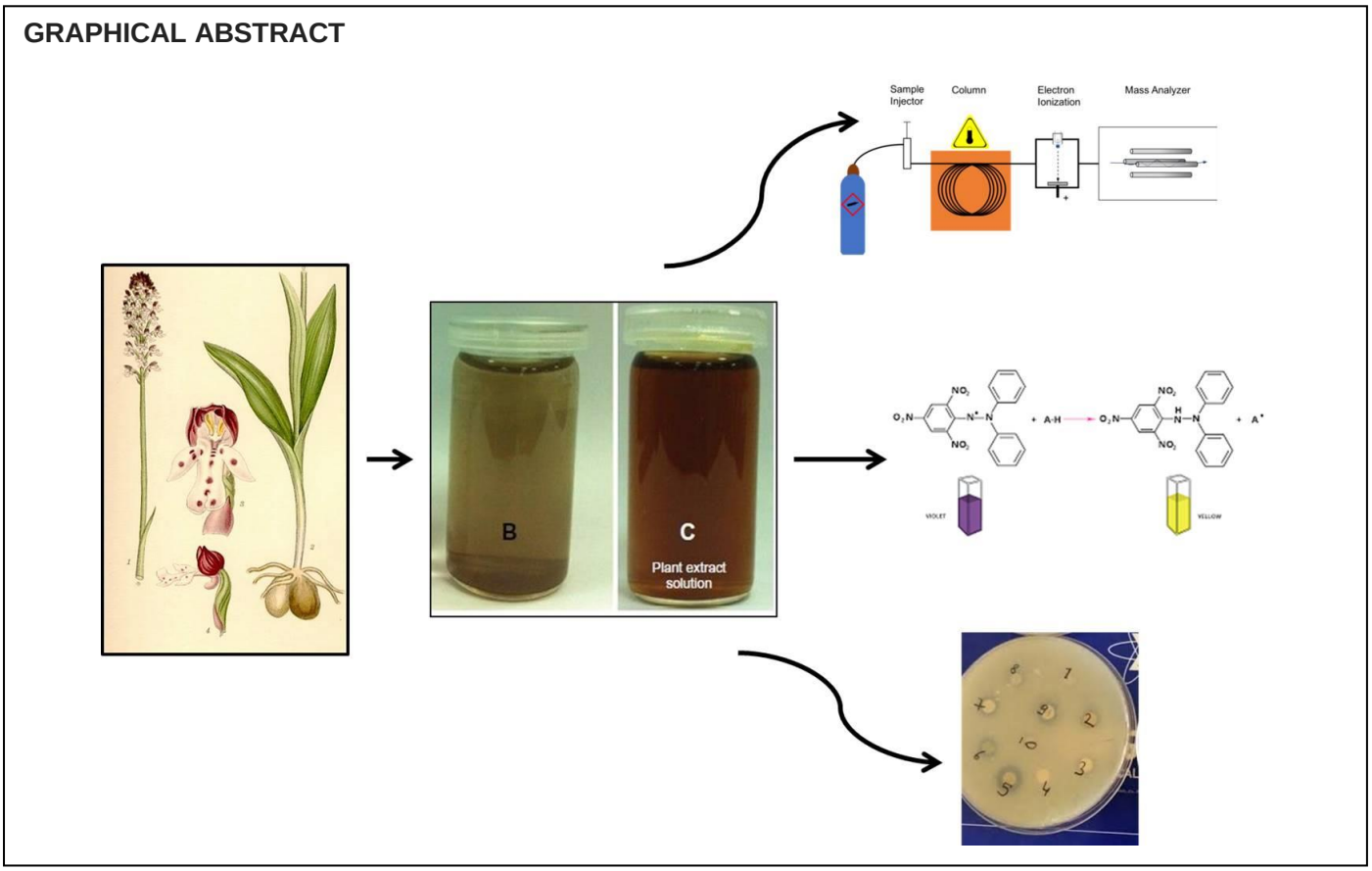
- Phytocomponent content of each sample is quite high and different from each other.
- Tuber extract of *Orchis purpurea* is the most effective againts to pathogens.
- Most of the extracts are better antioxidants than ascorbic acid according to DPPH test results.

**Abstract:** Plants are well known throughout the world for their medical activities. The history of orchids used in many different countries around the world probably started with their use for medicinal purposes thanks to their therapeutic properties. The aim of this study, to investigate antioxidant and antimicrobial activity of ethanol extracts of plant and tuber parts of eight different epiphytic orchids from Turkey as well as their chemical composition. The antimicrobial screening of extracts was performed against 8 bacterial and 2 fungal species. To determine general chemical profile of them gas chromatography-mass spectrometry analysis was done. Antioxidative potentials of the species were proved based on 2,2-Diphenyl-1-picrylhydrazyl radical scavenging and ferric reducing antioxidant power assays. All tested extracts prepared from orchid species especially showed intermediate inhibition activity against *Proteus vulgaris* and *Yersina enterocolitica*. The Gas Chromatography and Mass Spectrometry results showed that the phytoconstituent contents of the samples were high. In the case of all extracts, more than 100 compounds were identified. But, distinctive differences in composition between each orchid species were not observed. A good correlation degree between the results of the two antioxidant tests was calculated. These well - determined antioxidant activity

values can be attributed to the total phenolic content in terms of gallic acid equivalent, determined in a relatively high range (491.41-14082.94 mg GAE/g sample). These data show that different parts of the orchid samples obtained from Turkey with a past history have remarkable antimicrobial and antioxidant activity.

**Keywords:** antimicrobial; antioxidant; epiphytic orchids; GC-MS; phenolic content.

**GRAPHICAL ABSTRACT**




## INTRODUCTION

Since the Vedas era, various medicinal aspects of plants have been utilized. The ancient medication practice, which comes under Ayurveda, governs plant extract usage for curing various diseases [1]. The chemical bases of the plant's medicinal efficacy are by-product and end-product of varied metabolic processes. As known, antibiotic resistance is a growing problem. Some of this is because of the overuse of antibiotics in humans, but some of it is presumably because of antibiotics as growth promoters in animals' food [2]. Turkey has a long medicinal tradition and traditional learning of plant remedies. The use of wild plants in medicine by the Anatolian people goes back to ancient times. Records of plant names in recipes in Hittite medicine tablets are presented as proof of this concept. Additionally, it was known that several drugs prepared in Anatolia were exported to other countries during the Hittite and Byzantian periods [3]. Between 50000 and 70000 plant species are known to be used in traditional and modern medicinal systems [4] and more than 500 plant species are used to treat several kinds of diseases in Turkey [3].

Dendrobium species of the Orchidaceae family has been credited as a traditional medicine over the centuries in Asia, Europe, and Australia with more than 1100 species. Orchidaceae, the largest and most evolved family of the flowering plants, comprises 25000 to 35000 species under 750 to 850 genera [5]. Orchids are both ornamental and have medicinal value but are neglected among the plants used in medicine. In addition to these uses, orchids are also used as ecological indicators [6]. The history of orchids probably started with their use for medicinal purposes. Thanks to their therapeutic properties, orchid species are used in many different countries globally.

The origin of orchids on the earth and scientific research of them dates back 120 million years. It was first cultivated and identified by the Chinese [7]. However, available written records are as early as 4th millennium B.C. only. Orchids have been used as a source of herbal remedies in China since 2800 B.C. [8]. Since the Vedic period (2000 B.C.–600 B.C.), some orchids have been used by Indians for their curative and aphrodisiac properties [9]. In terms of terrestrial orchid, Turkey is one of the wealthiest countries in Europe,

and the Middle East. The Orchidaceae family is represented by 22 genera in our country [10]. On the other hand, salep comes from several species of diminishing orchids is well known as flavor enhancers in the food industry and has been widely used for a long time. Besides being used as a flavoring agent in ice cream or beverage, salep is also used as a perfume additive [11]. Salep is produced from different wild Orchidaceae species in different regions of Turkey, it does not have a standard chemical composition The chemical composition of salep and especially its glucomannan and starch content affect the quality of products originating from salep. Salep orchids are used in our country for ulcers and upper respiratory tract diseases, as an anti-diarrheal, tonic and food [12].

It is vital to search for new compounds not based on existing synthetic antimicrobial agents to overcome antibiotic resistance of pathogenic species [13]. The previous studies on orchids have shown that they have a wide range of chemical and biochemical compounds including carbohydrates, flavonoids, alkaloids, glycosides, and other phytochemical contents all of which have great importance in the medicinal field. Some of these chemical compounds have been recently isolated from the orchid species as alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes terpenoids [7,14].

In the present study, extracts from plant and tuber parts of eight different epiphytic orchids harvested from the north of Turkey were checked for their antimicrobial properties against some pathogenic Gram-positive, Gram-negative bacteria and fungi species. Furthermore, total phenolic contents and antioxidative activities of these samples were researched. In addition to these, chemical constituents of the samples were screened by the Gas chromatography and mass spectrometry **(**GC-MS) method.

## MATERIAL AND METHODS

### Plant materials

Plant samples were collected from a large area is located in A4 square according to the grid system extending from Bartin to Ordu during 2012-2014. Orchid species were identified according to Davis's book titled "Flora of Turkey'' [15], and Adil Güner's book [10]. The new record samples diagnosed are placed at the Herbarium of Ondokuz Mayıs University, Faculty of Science and Art (OMUB) [16]. Scientific and local names of the species are listed in Table 1.

**Table 1.** Scientific and local names of studied species

| Sample No | Scientific Name | Local Name |
|---|---|---|
| 1 | *Ophrys sphegodes* Mill. subsp. *caucasica* (Woronow ex Grossheim) Soo | Kafablamutu |
| 2 | *Orchis coriophora* L. subsp. *coriophora* | Pirinç çiçeği |
| 3 | *Orchis laxiflora* subsp. *laxiflora* Lam. | Salep sümbülü |
| 4 | *Serapias vomeracea* (Burm.fil.) Briq. subsp. *orientalis* | Sağır kulağı |
| 5 | *Orchis purpurea* Huds. subsp. *purpurea* | Hasancık |
| 6 | *Ophrys oestifera* M. Bieb. subsp. *oestifera* | Sinek salebi |
| 7 | *Orchis tridentate* Scop. | Katran alacası |
| 8 | *Orchis provincialis* Balb. ex Lam.& DC. | Katrancık |

### Preparation of extracts

The whole plant and tubers of plants were cleared of soil residues and dried under pressure at 23-35 °C for 3 to 4 weeks. The extracts from these parts were prepared according to the methods described by Ertürk [17] with a slight modification. The powdered plant materials were extracted using 95% ethanol in the ratio of 1:5 (w/v) at room temperature. The extracts were kept at 4 °C for 5 days (for a thorough extraction of the plant with ethanol) and were then filtered through a 0.45 µm membrane filter. The solvent was evaporated. The crude extracts were stored at –20 °C until used.

### Antimicrobial analysis

#### *Microorganisms and culture media*

Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection) and NRRL (Agricultural Research Service, United States of America). The antimicrobial activity of orchid samples was studied using eight bacterial (gram-positive and gram-negative; *Staphylococcus aureus* ATCC®25923, *Bacillus subtilis* NRRL B-209, *Micrococcus luteus* NRRL B-1018, *Proteus vulgaris* NRRL B-123, *Escherichia*

*coli* ATCC®25922, *Klebsiella pneumoniae* ATCC®13883, *Pseudomonas aeruginosa* ATCC®27853, *Yersina enterocolitica* ATCC®27729), and two fungal (*Candida albicans* ATCC®10231, *Saccharomyces cerevisiae* ATCC®9763) species. Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and fungal cells, respectively.

*Antibacterial and antifungal assay*

Antibacterial and antifungal activities were firstly measured using disk diffusion methods on agar plates [18]. Then, the extracts of orchid samples dissolved in ethanol were investigated by broth microdilution methods according to the Clinical and Laboratory Standards Institute standard procedures [19-21]. All bacterial strains were grown in MHB for 24 h, at 37 °C and fungal strains were grown in SDB at 30 °C for 48 h. The turbidity of bacterial and fungal suspensions was adjusted as 0.5 and 1.0 McFarland, respectively. Thus, the concentration of bacterial and fungal suspensions was adjusted to $10^8$ cells/mL and $3x10^8$ cells/mL, respectively. Then, sterile paper discs (6 mm in diameter) were placed on the agar to load 50 µL of each extract (10 mg/mL). One hundred units of nystatin (NY100) for fungus and Ampicillin (AM10) and Cephazolin (KZ30) for bacteria, all obtained from a local pharmacy, were used as positive controls, and alcohol was used as a negative control. After appropriate time incubation at growth temperature, inhibition zones of different organisms by different samples were measured with the digital caliper's help to estimate antibacterial and antifungal substances' potency and tabulated. All tests were made in triplicate.

*Minimum inhibition concentration (MIC)*

The broth dilution method, described by Vanden Berghe and Vlietinck [22] was used for the antibacterial screening with slight modifications using 96 well plates (Corning). 100 µL of each extract solution prepared at 1, 0.75, and 0.5 mg/mL concentration was transferred into each plate's well. After solidification, each well was inoculated with 10 µL of freshly prepared bacterial suspension of $10^8$ bacteria/mL and incubated at 37 °C for 24 h. and fungal suspension of $10^7$ fungi/mL and incubated at 27 °C for 48 h. The densities of microorganisms were prepared according to 0.5 Mc Farland. The uninoculated test medium was used as a blank. Positive control (bacteria/fungus and growth media) were used for each test. All assays were performed in triplicate.

*Minimum bactericidal and fungicidal concentrations (MBC and MFC)*

To determine the MBC and MFC, each well exhibiting no visible growth (viability) after 18 h was tested for viable organisms by subculturing 50 µL samples of each well onto nutrient agar/sabouraud dextrose agar media. The bacterial plates were incubated at 37 °C to observe any colony's growth after 24 h, and fungal plates were incubated at 30 °C to observe any colony's growth after 48 h. Plates that yielded zero or less than 10 single colonies were accounted for MBC and MFC value determination.

## GC-MS analysis

GC-MS analysis of the *Orchidaceae* plants and their ethanolic tuber extracts were performed using GC-MS (Hewlett Packard 5890 Series II GC Plus-Hewlett Packard 5971 Series MS) equipped with a column (Innowax 19091N-136, 60 m×0.250 mm i.d.; film thickness 0.25 µm). GC-MS conditions were adjusted as follows: The oven temperature was 70 °C at first and finally increased to 240 °C by raising 5 °C/min. The carrier gas was helium with a flow rate of 0.77 mL/min. The electron ionization detector's voltage was 70 eV, and the detector temperature was adjusted as 280 °C. The compounds absorbed by ethanol were injected into GC-MS in the splitless mode. The compounds were identified by comparing their molecular weights and fragmentations with spectra from the libraries of Wiley and Aromsa.

## Determination of total phenolic contents

The total phenolic content (TPC) of each extract was determined as gallic acid equivalent (mg GAE/g extract) according to the modified method based on Folin-Ciocalteu reagent developed by Singleton and Rossi [23].

## Antioxidative assays

*Measurement of DPPH free radical scavenging activity*

DPPH free radical scavenging activities of the extracts were tested by following the bleaching of the purple-colored methanol solution of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) at 517 nm after the addition of extract at different concentrations as an antioxidant substance to DPPH solution. The inhibition ratio value obtained for each concentration was calculated using the following equation.

$$\text{Inhibition ratio (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

where $A_{blank}$ is the absorbance of the blank tube containing DPPH solution and extract solvent and $A_{sample}$ is the absorbance of the mixture of extract and DPPH solution.

$SC_{50}$ values (extract concentrations providing 50% scavenging) were also calculated using the graph drawn between activity and concentration values [24].

*Measurement of ferric-reducing/antioxidant power (FRAP)*

The FRAP assay was performed following the method based on the principle of reducing the Fe (III)-TPTZ complex in the presence of antioxidants to form blue Fe (II)-TPTZ complex and measurement of maximum absorbance at 595 nm [25]. For this purpose; the appropriate amount of the extracts were combined with the FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution prepared in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio just before use and heated to 37°C). The mixtures were incubated at 37°C for 30 min, and after this, the resulting absorbances were measured at 593 nm. FRAP values for samples were calculated as $FeSO_4$ equivalents (µmol $FeSO_4$/mg sample) from graph drawn using $FeSO_4$.

Both antioxidant tests were performed under the same conditions for the standard antioxidant ascorbic acid, and the degrees of the effects of the samples compared to the standard antioxidant were compared.

## Statistical analysis

The results were presented as mean and standard deviations. The data were analyzed on PASW version 18 (Chicago, IL, USA, 2009) was used for statistical evaluation. The analysis implemented has been mainly descriptive, correlation analysis by subsets, and parametric and non-parametric analysis. It was found out that the numerical variables of TPC and antioxidant activity data can be considered as normal. However, not homogeneous distributed, so we used the robust test of Welch in the absence of homogeneity for comparing means. All test results were represented as the Average±SE. The statistical differences represented by letters were obtained through a one-way analysis of variance (ANOVA). For each statistically significant factor, means were compared using Tamhane's T2 test (all the distributions were heteroscedastic) with $p < 0.05$. The Spearman rank correlations are also presented. Data from the antimicrobial activity test can be regarded as not normally distributed, so we used a non-parametric test (Kruskal-Wallis) for statistical evaluation. Antimicrobial activity correlation analysis was performed using the Crosstabs test.

# RESULTS

## Antimicrobial activity evaluations

As shown in Table 2, the whole plant and tuber extracts of eight orchid species at 10 mg/mL concentration showed intermediate inhibition against all bacteria and fungi strains tested. The normality of the inhibition zone diameter results was tested and it was found that the data were not parametric. Then inhibition zone diameter data were compared by the Kruskal-Wallis test, with a significance level at $p<0.05$. The antimicrobial activity data showed that at least two microorganisms statistically different from each other ($X^2$ (9; 570)=53.861; $p<0.05$). The synthetic antibiotics (AM10, KZ30, and NY100) used as a positive control exhibited the highest antimicrobial activity. Results can be classified into three levels of qualitatively sensitive (inhibition zone diameter ≥ 20 mm), moderate antimicrobial activity (15 mm ≤ inhibition zone diameter ≥ 19 mm), and resistant (inhibition zone diameter ≤ 14 mm) based on the region size interpretative table of the Clinical and Laboratory Standards Institute [21]. The degree of inhibition on all microorganisms varied from species to species and from the extract origin. Generally, in most extracts, pathogens exhibited intermediate antimicrobial activity. The results of all microorganisms' pairwise comparisons of inhibition zone diameters were modeled in the SPSS program (Figure 1). Interpretation of Kruskal-Wallis post-hoc pairwise

comparisons is given in Figure 1. The blue line represents a statistically significant result, but the red line is not ($p < 0.05$).

When Table 2 is examined in detail, the extract prepared from the whole plant of *O. sphegodes* subsp. *caucasica* showed intermediate inhibition activity against *Y. enterocolitica*, and the tuber extract of this species showed intermediate inhibition activity against *B. subtilis* and *Y. enterocolitica*. Still, both extracts had weak activity against all other bacteria. The whole plant extract of *O. coriophora* showed intermediate inhibition activity against *M. luteus* > *Y. enterocolitica* > *E. coli* > *P. vulgaris*. At the same time, the tuber extract of *O. coriophora* has intermediate inhibition activity against *Y. enterocolitica* and *S. cerevisiae,* but *P. vulgaris* was sensitive (20.20 mm/50 µL inhibition zone). The whole plant extract of *O. laxiflora* only showed intermediate inhibition activity against *Y. enterocolitica*, but the tuber extract of *O. laxiflora* showed intermediate inhibition activity against *P. vulgaris* > *E. coli* > *B. subtilis* > *M. luteus* respectively. At the same time, it showed intermediate antifungal activity against *C. albicans* > *S. cerevisiae*. The antibacterial effect of *S. vomeracea* subsp. *orientalis* extracts of the whole plant were intermediate to *P. aeruginosa* > *E. coli* > *S. aureus*. It also had intermediate antifungal activity to *S. cerevisiae* > *C. albicans*. The tuber extract of this species showed intermediate antibacterial activity against *P. vulgaris* > *Y. enterocolitica* > *P. auroginosa* and intermediate antifungal activity against *S. cerevisiae*. Both extracts of *O. purpurea* subsp. *purpurea* showed intermediate antimicrobial activity against *M. luteus*, *Y. enterocolitica*, *C. albicans*, and *S. cerevisiae*. It can be said that *B. subtilis* was sensitive to tuber extract of it. The whole extract of *O. oestifera* subsp. *oestifera* showed intermediate inhibition activity against *P. vulgaris* > *B. subtilis*> *Y. enterocolitica* > *M. luteus* > *C. albicans* > *S. cerevisiae*. Almost similar results are valid for the tuber extract, but *S. aureus* and *E. coli* instead of *B. subtilis* and *M. luteus* showed moderate antimicrobial activity. *O. tridentate*'s whole plant extract showed intermediate antimicrobial activity against all strains but *K. pneumoniae* and *P. vulgaris*. *K. pneumoniae* was resistant to the extract, while *P. vulgaris* was sensitive. The whole extract gave better results than the tuber extract. Only two strains (*B. subtilis* and *Y. enterocolitica*) showed intermediate antibacterial activity in the extract. The whole extract of *O. provincialis* showed moderate antimicrobial activity except *S. aureus*, *B. subtilis*, and *C. albicans*. In contrast, tuber extract of *O. provincialis* showed only intermediate antimicrobial activity against *B. subtilis, M. luteus*, and *C. albicans*.

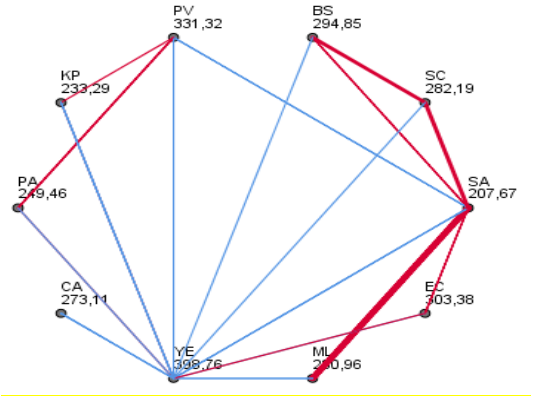



**Figure 1.** Pairwise comparisons of inhibition zone diameters of whole microorganisms in the study. Each node shows the sample average rank of the microorganisms. Asymptotic significances (2-sided tests) are displayed *p* value < 0.05 Significance values have been adjusted by the Bonferroni correction for multiple tests.
SA*: Staphylococcus aureus;* BS*: Bacillus subtilis;* ML*: Micrococcus luteus;* EC*: Escherichia coli;* PA*: Pseudomonas aeruginosa;* PV*: Proteus vulgaris;* YE*: Yersina enterocolitica;* KP*: Klebsiella pneumoniae;* CA*: Candida albicans;* SC*: Saccharomyces cerevisiae*

Normality of the MIC and MBC assessments was tested and the data were found to be nonparametric in the same way as calculated for disk diffusion test results. Additionally, MIC and MBC data were compared by the Kruskal-Wallis test and it was determined that the MIC and MBC data obtained for all extracts differed statistically at the 0.05 significance level according to the orchid variety category. In fact at least two microorganism groups statistically different from each other (MIC $X^2$ (5; 288)=29.027; MBC $X^2$ (5; 288)=34.485 $p$<0.05). There was a weak correlation between MIC values and microorganism types (Cramer $V$=0,314 $p$<0.05). There were moderately significant correlations between MIC values and the samples (Cramer $V$=0,596 $p$<0.05) and also between MIC values and extract origin (whole plant/tuber) φ =0,610 $p$<0.05). MIC and MBC results were showed that the effective minimum extract concentration was 0.02 mg/mL, and the most sensitive microorganism is *S. cerevisiae* (Table 3 and Table 4).

**Table 2.** Inhibition zone diameter (IZD; mm) of whole and tuber extracts of orchid samples, and drugs as positive controls against bacterial and fungal strains

| | Part of the plant | IZD (mm)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Gram Positive Bacteria | | | Gram Negative Bacteria | | | | | Fungi | |
| | | SA | BS | ML | EC | PA | PV | YE | KP | CA | SC |
| **1** | Whole | 12.98±0.011 | 8.84±0.02 | 10.60±0.015 | 12.28±0.005 | 14.19±0.01 | 6±0.00 | 15.26±0.025 | 12.40±0.01 | 12.72±0.01 | 12.20±0.05 |
| **1** | Tuber | 9.16±0.01 | 15.10±0.011 | 6±0.00 | 12.19±0.2 | 12.72±0.1 | 14.96±0.15 | 16.58±0.15 | 9.90±0.1 | 11.25±0.05 | 11.43±0.1 |
| **2** | Whole | 9.25±0.02 | 6±0.00 | 18.23±0.05 | 15.19±0.05 | 14.87±0.05 | 15.13±0.05 | 17.08±0.1 | 13.00±0.01 | 14.93±0.02 | 14.57±0.01 |
| **2** | Tuber | 13.90±0.01 | 13.60±0.005 | 13.55±0.005 | 14.71±0.005 | 9.56±0.005 | 20.20±0.01 | 17.84±0.02 | 10.57±0.02 | 13.61±0.01 | 16.05±0.01 |
| **3** | Whole | 12.57±0.015 | 9.63±0.01 | 12.04±0.005 | 13.31±0.01 | 14.04±0.005 | 10.28±0.01 | 16.07±0.015 | 13.15±0.015 | 14.77±0.03 | 14.83±0.02 |
| **3** | Tuber | 14.12±0.015 | 17.93±0.015 | 15.22±0.02 | 18.37±0.011 | 9.36±0.01 | 18.47±0.11 | 13.67±0.12 | 14.09±0.015 | 19.63±0.05 | 15.63±0.05 |
| ***4*** | Whole | 15.30±0.02 | 11.48±0.011 | 13.89±0.20 | 15.54±0.01 | 15.59±0.02 | 14.76±0.01 | 14.87±0.01 | 13.62±0.15 | 15.62±0.10 | 17.30±0.36 |
| **4** | Tuber | 14.38±0.02 | 13.55±0.02 | 14.47±0.01 | 13.37±0.15 | 15.09±0.2 | 16.58±0.5 | 15.57±0.02 | 14.55±0.01 | 12.72±0.01 | 15.24±0.01 |
| **5** | Whole | 13.37±0.015 | 11.82±0.01 | 15.97±0.015 | 15.53±0.015 | 14.19±0.015 | 16.51±0.01 | 15.57±0.01 | 13.77±0.02 | 16.96±0.015 | 15.82±0.01 |
| **5** | Tuber | 16.71±0.035 | 22.07±0.01 | 19.53±0.005 | 14.24±0.005 | 12.74±0.015 | 14.07±0.002 | 17.90±0.036 | 10.31±0.023 | 16.21±0.01 | 15.18±0.01 |
| **6** | Whole | 13.49±0.01 | 18.49±0.03 | 16.39±0.023 | 14.61±0.005 | 9.40±0.02 | 18.87±0.015 | 16.47±0.015 | 13.81±0.005 | 16.20±0.005 | 15.260.005 |
| **6** | Tuber | 17.51±0.025 | 12.24±0.01 | 13.51±0.02 | 15.61±0.005 | 14.76±0.011 | 19.39±0.005 | 18.54±0.01 | 16.82±0.01 | 17.74±0.01 | 17.48±0.01 |
| **7** | Whole | 16.52±0.015 | 16.25±0.011 | 16.94±0.005 | 16.24±0.015 | 15.19±0.011 | 20.76±0.005 | 15.71±0.12 | 14.92±0.005 | 15.10±0.026 | 15.21±0.005 |
| **7** | Tuber | 10.58±0.01 | 16.70±0.011 | 12.53±0.025 | 12.91±0.026 | 11.58±0.017 | 13.94±0.02 | 15.06±0.015 | 9.30±0.011 | 12.35±0.015 | 13.15±0.01 |
| **8** | Whole | 12.59±0.005 | 14.48±0.01 | 17.13±0.01 | 16.39±0.005 | 17.16±0.005 | 17.51±0.005 | 17.56±0.011 | 15.69±0.005 | 6±0.00 | 16.40±0.005 |
| **8** | Tuber | 14.28±0.01 | 19.65±0.005 | 15.19±0.00 | 12.65±0.005 | 10.63±0.005 | 13.57±0.015 | 16.75±0.02 | 11.23±0.01 | 15.53±0.01 | 11.83±0.005 |
| Alcohol | | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 | 6±0.00 |
| Ampicillin | | 11±0.01 | 36±0.06 | 6±0.23 | 40.12±0.03 | 30.27±0.26 | 28±0.57 | 26.44±0.011 | 40.41±0.017 | NT | NT |
| Cefazolin | | 6±0.1 | 38±0.29 | 35±0.02 | 47.16±0.23 | 29.24±0.56 | 6±0.04 | 34.25±0.12 | 44.11±0.14 | NT | NT |
| Nystain | | NT | NT | NT | NT | NT | NT | NT | NT | 15.02±0.15 | 14.01±0.03 |

[a]Average values are expressed as mean of triplicates ± SD (Standart Deviation). NT, not tested

*SA: Staphylococcus aureus;* BS: *Bacillus subtilis;* ML: *Micrococcus luteus;* EC: *Escherichia coli;* PA: *Pseudomonas aeruginosa;* PV: *Proteus vulgaris;* YE: *Yersina enterocolitica;* KP: *Klebsiella pneumoniae;* CA: *Candida albicans;* SC: *Saccharomyces cerevisiae*

**Table 3.** Minimum inhibitory concentrations (MICs) (mg/mL) of orchid extracts towards selected strains of Gram (+), Gram (-) bacteria and fungi

| Sample No | Part of the plant | Gram Positive Bacteria | | Gram Negative Bacteria | | Fungi | |
|---|---|---|---|---|---|---|---|
| | | SA | BS | EC | YE | CA | SC |
| 1 | Whole | 0.2≥ | 0.1≥ | 0.05≥ | 0.2≥ | 0.1≥ | 0.05≥ |
| | Tuber | 0.03≥ | 0.03≥ | 0.03≥ | 0.03≥ | 0.06≥ | 0.24≥ |
| 2 | Whole | 0.06≥ | - | 0.24≥ | 0.24≥ | 0.24≥ | 0.06≥ |
| | Tuber | 0.08≥ | 0.48≥ | 0.17≥ | 0.48≥ | 0.08≥ | 0.08≥ |
| 3 | Whole | 0.03≥ | 0.03≥ | 0.06≥ | 0.24≥ | 0.24≥ | 0.03≥ |
| | Tuber | 0.05≥ | 0.03≥ | 0.03≥ | 0.2≥ | 0.05≥ | 0.03≥ |
| 4 | Whole | 0.02≥ | 0.02≥ | 0.02≥ | 0.16≥ | 0.02≥ | 0.02≥ |
| | Tuber | 0.03≥ | 0.03≥ | 0.03≥ | 0.14≥ | 0.03≥ | 0.03≥ |
| 5 | Whole | 0.24≥ | 0.03≥ | 0.03≥ | 0.03≥ | 0.03≥ | 0.03≥ |
| | Tuber | 0.36≥ | 0.36≥ | 0.36≥ | 0.36≥ | 0.36≥ | 0.09≥ |
| 6 | Whole | 0.16≥ | 0.02≥ | 0.02≥ | 0.02≥ | 0.02≥ | 0.02≥ |
| | Tuber | 0.07≥ | 0.24≥ | 0.04≥ | 0.24≥ | 0.14≥ | 0.04≥ |
| 7 | Whole | 0.03≥ | 0.2≥ | 0.2≥ | 0.2≥ | 0.2≥ | 0.2≥ |
| | Tuber | 0.16≥ | 0.16≥ | 0.16≥ | 0.16≥ | 0.16≥ | 0.03≥ |
| 8 | Whole | 0.02≥ | 0.16≥ | 0.02≥ | 0.16≥ | - | 0.02≥ |
| | Tuber | 0.02≥ | 0.16≥ | 0.02≥ | 0.16≥ | 0.16≥ | 0.02≥ |

- no inhibition

SA: *Staphylococcus aureus;* BS: *Bacillus subtilis;* EC: *Escherichia coli;* YE: *Yersina enterocolitica;* CA: *Candida albicans;* SC: *Saccharomyces cerevisiae*

**Table 4.** Minimum bactericidal and fungicidal concentrations (MBCs and MFCs) (mg/mL) of orchid whole/tuber extracts towards selected strains of Gram (+), Gram (-) bacteria and fungi.

| Sample No | Part of the plant | Gram Positive Bacteria | | Gram Negative Bacteria | | Fungi | |
|---|---|---|---|---|---|---|---|
| | | SA | BS | EC | YE | CA | SC |
| 1 | Whole | 0.1≥ | 0.05≥ | 0.05≥ | 0.1≥ | 0.05≥ | 0.05≥ |
| | Tuber | 0.03≥ | 0.03≥ | 0.03≥ | 0.03≥ | 0.06≥ | 0.12≥ |
| 2 | Whole | 0.06≥ | - | 0.12≥ | 0.12≥ | 0.12≥ | 0.06≥ |
| | Tuber | 0.08≥ | 0.24≥ | 0.17≥ | 0.24≥ | 0.08≥ | 0.08≥ |
| 3 | Whole | 0.03≥ | 0.03≥ | 0.06≥ | 0.12≥ | 0.12≥ | 0.03≥ |
| | Tuber | 0.05≥ | 0.03≥ | 0.03≥ | 0.1≥ | 0.05≥ | 0.03≥ |
| 4 | Whole | 0.02≥ | 0.02≥ | 0.02≥ | 0.08≥ | 0.02≥ | 0.02≥ |
| | Tuber | 0.03≥ | 0.03≥ | 0.03≥ | 0.07≥ | 0.03≥ | 0.03≥ |
| 5 | Whole | 0.12≥ | 0.03≥ | 0.03≥ | 0.03≥ | 0.03≥ | 0.03≥ |
| | Tuber | 0.18≥ | 0.18≥ | 0.18≥ | 0.18≥ | 0.18≥ | 0.09≥ |
| 6 | Whole | 0.08≥ | 0.02≥ | 0.02≥ | 0.02≥ | 0.02≥ | 0.02≥ |
| | Tuber | 0.07≥ | 0.14≥ | 0.04≥ | 0.14≥ | 0.07≥ | 0.04≥ |
| 7 | Whole | 0.03≥ | 0.1≥ | 0.1≥ | 0.1≥ | 0.1≥ | 0.1≥ |
| | Tuber | 0.08≥ | 0.08≥ | 0.08≥ | 0.08≥ | 0.08≥ | 0.03≥ |
| 8 | Whole | 0.02≥ | 0.08≥ | 0.02≥ | 0.08≥ | - | 0.02≥ |
| | Tuber | 0.02≥ | 0.08≥ | 0.02≥ | 0.08≥ | 0.08≥ | 0.02≥ |

-no inhibition

SA: *Staphylococcus aureus;* BS: *Bacillus subtilis;* EC: *Escherichia coli;* YE: *Yersina enterocolitica;* CA: *Candida albicans;* SC: *Saccharomyces cerevisiae*

*Serapias vomeracea* subsp. *orientalis* whole plant extract showed moderate antimicrobial activity to all tested microorganisms. Disk diffusion test results of this extract were supported by MIC and MBC data. *Orchis provincialis*, *Serapias vomeracea* subsp. *orientalis, O. purpurea* subsp. *purpurea and O. oestifera* subsp. *oestifera* tuber extracts exhibied antimicrobial effect on all microorganisms too. *Orchis laxiflora* subsp. *laxiflora* tuber extract also displayed moderate antimicrobial activity against all tested microorganisms but *P. aeroginosa*.

**Findings of GC-MS analysis**

The compounds were determined by comparison of their molecular weights and molecular fragmentations according to their molecular structures with spectra from the libraries of Wiley and Aromsa. In this work, ethanolic extracts of the plant and its tuber parts of six different species which belong to *Orchidaceae* family were investigated for determination of their chemical constituents. The relative amount (%) of each component expresses a comparison of its average peak area to the total areas. The peaks amounting to at least 1.6% of the total compounds were taken into account. Total ion chromatograms (TIC is a chromatogram formed by summing up intensities of all mass peaks at the same scan) of *O. provincialis* plant and its ethanolic tuber extracts can be seen below as representative examples (Figure 2). The chromatograms' peaks were integrated and compared with the GC-MS library database containing the spectra of known components. After the analysis, the library search identification results and the MS fragmentation of the TIC peaks of the samples were also examined and the compounds were determined.

The results were set into their functionalities with their retention times ($t_R$) and area % values (shows the relative abundances of the components) for the whole plant and its tuber extracts separately in the Table 5.

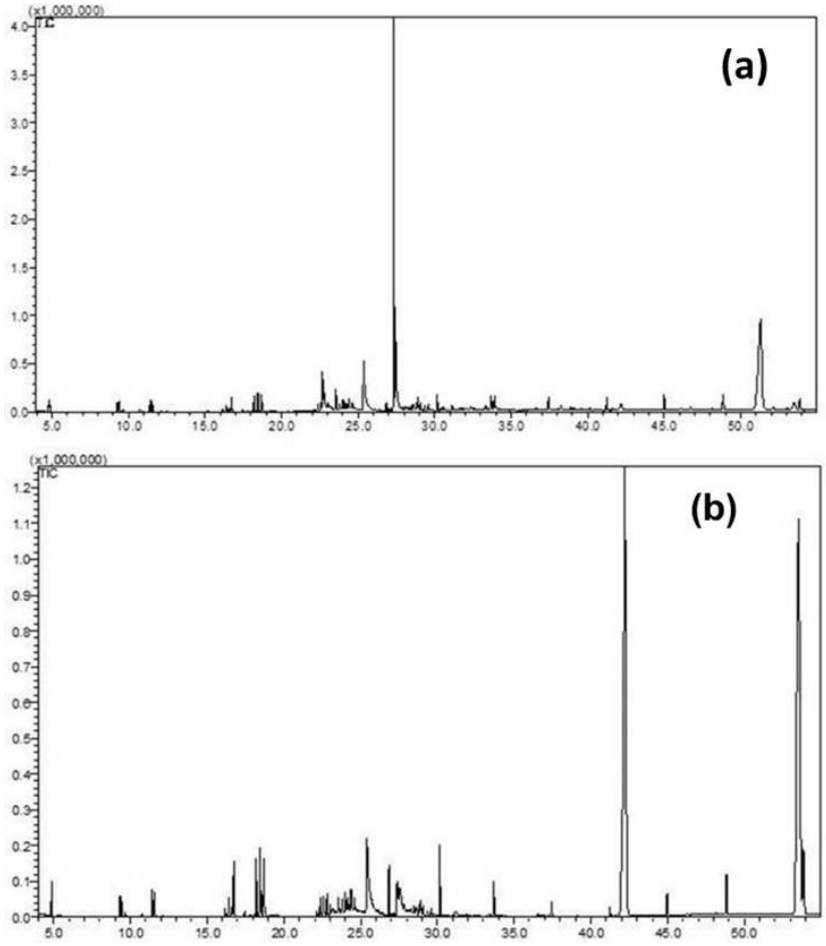



**Figure 2.** TIC chromatograms of ethanolic extracts of *Orchis provincialis* Balb. ex Lam.& DC plant **(a)** and its tuber **(b).**

The results indicated the existence of ketones, esters (saturated or unsaturated aromatic and aliphatic esters), alkenes, alkanes, alcohols (saturated or unsaturated aromatic and aliphatic alcohols), amines (aromatic and aliphatic amines), and other aromatic compounds. The primary compound class is an aromatic substituted amine that was detected in *O. purpurea* tuber, *O. provincialis* plant, and its tuber, *O. laxiflora* subsp. *laxiflora* tuber, *O. sphegodes* subsp. *caucasia* plant and its tuber and *S. vomeracea* tuber extracts. The cyclic ketone was the major chemical component for *O. purpurea* subsp. *purpurea* whole plant extract while *O. laxifllora* subsp. *laxiflora* whole plant and *O. oestifera* subsp. *oestifera* tuber extracts had the unsaturated ester as the major compound class. *O. oestifera* subsp. *oestifera* and *S. vomeracea* subsp. orientalis whole plant extracts showed the presence of aromatic ester as the major organic functionality.

**Table 5.** GC-MS results of the extracts with general structural functionalities

| Extracts/ Peaks[1] | $t_R$[2] | Area (%) | Compound class | Extracts/ Peaks | $t_R$ | Area (%) | Compound class |
|---|---|---|---|---|---|---|---|
| **Plant Material** | | | | **Plant Tuber** | | | |
| ***Or. purpurea*** | | | | ***Or. purpurea*** | | | |
| 1 | 27.35 | 45.81 | Cyclic ketone | 1 | 48.80 | 11.70 | Aromatic |
| 2 | 27.49 | 16.0 | Ester | 2 | 53.80 | 9.19 | substituted amine |
| 3 | 22.64 | 12.91 | Alkene | 3 | 44.95 | 7.90 | ″ |
| 4 | 23.54 | 1.85 | Aromatic alcohol | 4 | 23.52 | 2.98 | Aromatic alcohol |
| 5 | 30.53 | 1.72 | Ketone | 5 | 23.01 | 2.83 | ″ |
| | | | | 6 | 24.07 | 2.70 | Alkene |
| | | | | 7 | 23.97 | 2.69 | Tricyclic alkane |
| | | | | 8 | 23.71 | 1.79 | Ester |
| | | | | 9 | 22.8 | 1.73 | ″ |
| ***Or. provincialis*** | | | | ***Or. provincialis*** | | | |
| 1 | 51.30 | 23.19 | Aromatic substituted amine | 1 | 53.51 | 33.42 | Aromatic substituted amine |
| 2 | 27.33 | 18.27 | Unsaturated ester | 2 | 42.22 | 31.47 | ″ |
| 3 | 27.49 | 7.16 | ″ | 3 | 25.41 | 4.91 | Aromatic ester |
| 4 | 25.37 | 6.46 | ″ | 4 | 53.85 | 2.53 | Aromatic amine |
| 5 | 22.66 | 3.38 | Alcohol | 5 | 2.21 | 1.62 | Alkane |
| 6 | 53.50 | 1.62 | Aromatic amine | 6 | 18.45 | 4.04 | Alkene |
| ***Or. laxiflora*** | | | | ***Or. laxiflora*** | | | |
| 1 | 27.35 | 10.24 | Unsaturated ester | 1 | 48.84 | 9.33 | Aromatic amine |
| 2 | 27.51 | 8.25 | Ester | 2 | 53.86 | 8.37 | ″ |
| 3 | 25.43 | 8.23 | Aromatic ester | 3 | 27.34 | 7.84 | Unsaturated ester |
| 4 | 18.45 | 4.98 | Alkene | 4 | 27.51 | 6.94 | Ester |
| 5 | 18.70 | 4.33 | Alcohol | 5 | 44.98 | 5.80 | Aromatic amine |
| 6 | 4.87 | 3.83 | Alkene | 6 | 18.45 | 1.79 | Mercaptan |
| 7 | 18.21 | 3.48 | ″ | | | | |
| 8 | 9.41 | 3.13 | Alkane | | | | |
| 9 | 11.55 | 2.95 | Alkene | | | | |
| 10 | 9.29 | 2.76 | Alkane | | | | |
| 11 | 11.43 | 2.74 | Alcohol | | | | |
| 12 | 16.73 | 2.67 | Aromatic ring | | | | |
| 13 | 23.55 | 1.84 | Aromatic alcohol | | | | |
| 14 | 16.41 | 1.75 | Alkane | | | | |
| ***Op. sphegodes*** | | | | ***Op. sphegodes*** | | | |
| 1 | 53.87 | 13.48 | Aromatic substituted amine | 1 | 53.80 | 23.07 | Aromatic substituted amine |
| 3 | 25.35 | 7.03 | Aromatic ester | 2 | 48.80 | 17.27 | ″ |
| 4 | 27.32 | 6.62 | Ester | 3 | 44.96 | 10.79 | ″ |
| 5 | 44.99 | 5.83 | Aromatic substituted amine | 4 | 25.36 | 5.78 | Aromatic ester |
| 6 | 25.31 | 3.84 | Aromatic ester | 5 | 27.32 | 3.44 | Unsaturated ester |
| 7 | 27.49 | 3.37 | Ester | | | | |
| 8 | 23.79 | 3.27 | Aromatic ester | | | | |
| 9 | 23.54 | 3.17 | Aromatic ring | | | | |
| 10 | 23.99 | 2.15 | Alcohol | | | | |
| **11** | **23.64** | **2.05** | **Bicyclic ylidene** | | | | |

**Cont. Table 5**

| ***Op. oestifera*** | | | | ***Op. oestifera*** | | | |
|---|---|---|---|---|---|---|---|
| 1 | 25.33 | 8.52 | Aromatic ester | 1 | 27.34 | 11.79 | Unsaturated ester |
| 2 | 25.26 | 6.08 | ″ | 2 | 27.51 | 11.35 | Ester |
| 3 | 24.97 | 5.48 | ″ | 3 | 25.42 | 10.88 | Aromatic ester |
| 4 | 27.30 | 5.62 | Unsaturated ester | 4 | 4.87 | 2.87 | Alkene |
| 5 | 25.11 | 5.41 | Aromatic ester | 5 | 53.86 | 2.69 | Ester |
| 6 | 25.02 | 4.40 | ″ | 6 | 18.46 | 2.49 | Alkene |
| 7 | 27.46 | 3.06 | Ester | 7 | 18.70 | 2.02 | Cycloalkane |
| 8 | 35.45 | 1.88 | Aromatic substituted amine | 8 | 51.28 | 1.94 | Aromatic substituted amine |
| 9 | 53.77 | 1.71 | ″ | 9 | 9.41 | 1.93 | Alkane |
| | | | | 10 | 42.21 | 1.85 | Aromatic substituted amine |
| | | | | 11 | 48.85 | 1.83 | Ester |
| ***Ser. vomeracea*** | | | | ***Ser. vomeracea*** | | | |
| 1 | 25.40 | 12.46 | Aromatic ester | 1 | 53.85 | 24.01 | Aromatic |
| 2 | 18.43 | 3.46 | Alkene | 2 | 48.83 | 13.96 | substituted amine |
| 3 | 16.70 | 2.92 | Aromatic ring | 3 | 44.99 | 6.35 | ″ |
| 4 | 27.54 | 2.87 | Alkane | 4 | 25.50 | 4.52 | Ester |
| 5 | 53.77 | 2.81 | Ester | 5 | 25.44 | 2.92 | ″ |
| 6 | 18.67 | 2.63 | Alkene | 6 | 18.45 | 2.11 | Alkene |
| 7 | 18.18 | 2.51 | ″ | | | | |
| 8 | 27.60 | 2.09 | Lactone | | | | |
| 9 | 48.98 | 2.05 | Ester | | | | |
| 10 | 27.37 | 2.01 | Unsaturated ester | | | | |
| **11** | 4.84 | 1.60 | Alkene | | | | |

[1]The peaks amounting to at least 1.6 % of the total compounds were taken into account; [2]$t_R$: Retention time in minutes, *Or*.: *Orchis*; *Op*.: *Ophrys*; *Ser*.: *Serapias*

## Total phenolic contents and antioxidative activities of orchid samples

The tuber parts of the plant samples were more affluent than the other part of the plants in half of the samples. This difference is particularly noticeable in the case of *O. sphegodes* subsp. *caucasica.* The phenolic content of the tuber is about 3 times higher than that of the whole plant (Table 6). It was determined that TPC values obtained for orchid extracts differed statistically at the 0.05 significance level according to the orchid variety category ($F$ (7, 16.760)=391.470; $p$<0.05) and also TPC values of *O. sphegodes* subsp. *caucasica, O. purpurea* subsp. *purpurea* and *O. provincialis* extracts differed statistically significantly from the others ($p$<0.05).

The calculated $SC_{50}$ values for the DPPH radical scavenging activities of samples are in the range of 1.35-26.92 µg/mL. The lowest $SC_{50}$ value was calculated for the tuber part of the *O. sphegodes subsp. caucasica* with the highest phenolic content and it can be said that this species' efficacy to sweep the DPPH radical was the highest among the tested samples. In general, there is a high correlation ($R^2$=0.8065) between phenolic content and $SC_{50}$ values obtained by the DPPH test. On the other hand, the highest $SC_{50}$ value was calculated for the tuber part of the *O. laxiflora* subsp. *laxiflora,* and this extract was differed statistically significantly from the others ($F$(7, 15.625)=78.800; $p$<0.05).

FRAP values were calculated in the range of 2.32-70.31 µmol $FeSO_4$/mg sample.

**Table 6.** The results of the total phenolic content (TPC) and antioxidative activity (DPPH and FRAP) measurements of the studied samples.

| Sample No | Part of the plant | TPC (mg GAE/g sample) | DPPH ($SC_{50}$;µg/mL) | FRAP (µmol $FeSO_4$/mg sample) |
|---|---|---|---|---|
| 1 | whole | 4749.19$^{f}$ | 3.74$^{h}$ | 34.26$^{f}$ |
| | tuber | 14082.94$^{a}$ | 1.35$^{l}$ | 70.31$^{b}$ |
| 2 | whole | 1127.82$^{l}$ | 12.81$^{d}$ | 8.85$^{m}$ |
| | tuber | 707.83$^{o}$ | 15.35$^{b}$ | 10.03$^{l}$ |
| 3 | whole | 814.68$^{m}$ | 12.57$^{d}$ | 12.82$^{k}$ |
| | tuber | 491.41$^{p}$ | 26.92$^{a}$ | 2.32$^{n}$ |
| 4 | whole | 777.94$^{n}$ | 13.33$^{c}$ | 8.15$^{m}$ |
| | tuber | 3272.77$^{g}$ | 1.91 | 24.79$^{h}$ |
| 5 | whole | 9969$^{c}$ | 1.75 | 71.15$^{a}$ |
| | tuber | 11084.75$^{b}$ | 2.01$^{j}$ | 59.81$^{c}$ |
| 6 | whole | 2850.64$^{i}$ | 6.06$^{f}$ | 22.32$^{i}$ |
| | tuber | 2499.66$^{j}$ | 8.19$^{e}$ | 16.24$^{j}$ |
| 7 | whole | 1373.26$^{k}$ | 4.97$^{g}$ | 25.36$^{h}$ |
| | tuber | 3016.56$^{h}$ | 3.97$^{h}$ | 26.6$^{g}$ |
| 8 | whole | 8073.84$^{d}$ | 2.41$^{i}$ | 47.94$^{d}$ |
| | tuber | 7449.8$^{e}$ | 1.64$^{k}$ | 35.14$^{e}$ |

* Homoscedasticity among cultivars was tested through the Levene test. Different letters in each column indicate mean values that differ significantly ($P < 0.05$). These differences were classified using Tamhane's T2 tests, since the homoscedasticity requirement was not fulfilled.

## DISCUSSION

It is well known that orchids probably contain a range of chemical and biochemical compounds, such as terpenes, steroids, saponins, and polyphenols [26]. Observed antimicrobial activity can be attributed to the contents of the plants. Some orchids' antimicrobial activities have been reported, although detailed investigations are still studied with this plant continues [27]. It is known that many medicinal orchids are reported to contain alkaloids. The study results also show similar findings with previous studies on the various antimicrobial activities of orchids [28]. It has been confirmed that the presence of alkaloids in the structure of secondary compounds contained in orchids can be the cause of this antimicrobial activity. Paul and coauthors [29] reported that the acetone extract of *Aerides odorata* inhibited the drug-resistant growth of *E. coli* strains. Sandrasagaran and coauthors [30] showed that *Dendrobium crumenatum* exhibited potential antimicrobial activity due to the presence of alkaloid and flavonoid compounds**.** Similar to our findings, it was also reported that the *Coelogyne stricta* (leaf) and *Dendrobium amoeneum* were shown good activity against *K. pneumonae* and *S. aureus* strains [31].

Gas Chromatography and Mass Spectrometry (GC-MS) analysis is an important technique to identify general chemical profile of plant extracts [32]. In this work, ethanolic extracts of the plant and tuber parts of different species belonging to *the Orchidaceae* family [16] were investigated to determine their chemical constituents. According to the GC-MS results, each species of *the Orchidaceae* family exhibited a similar chemical profile with different relative abundances and several chemical constituents. Aromatic substituted amine and alcohol functionalities proved alkaloids, while alkenes and unsaturated alcohol organic compound classes indicated the existence of terpenoids in the extracts. The results showed that phenolics were found in the extracts solely or in the different organic component classes, as expected. In general, an aromatic amine compound: N-[(2-fluorophenyl)methyl]-1*H*-purin-6-amine as an alkaloid with antitumor activity [33], 2-propenoic acid, hexadecyl ester, and 1,2-benzene dicarboxylic acid, diethyl ester were detected as the major chemical structures (Figure 3) while the other ester compounds were found as the second major identified class of the components.

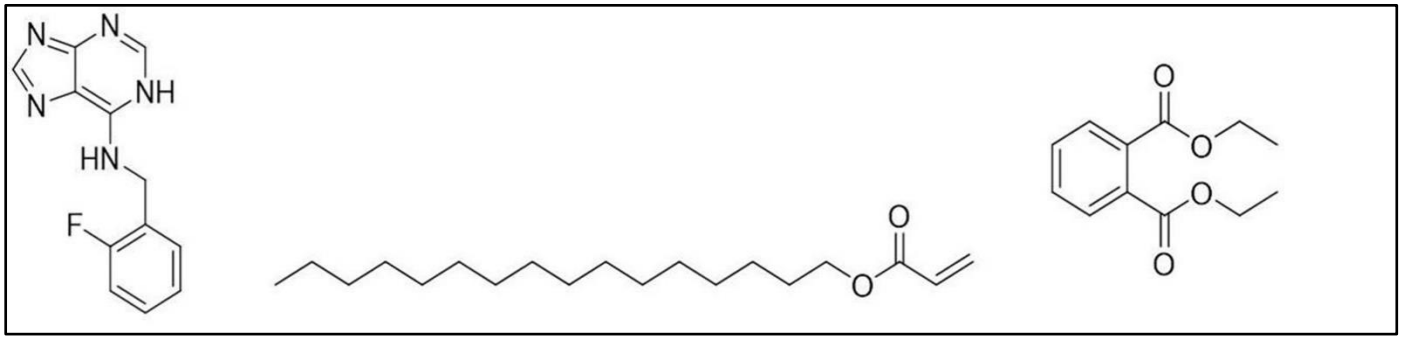


**Figure 3.** N-[(2-Fluorophenyl)methyl]-1*H*-purin-6-amine; 2-Propenoic acid, hexadecyl ester; 1,2-Benzenedicarboxylic acid, diethyl ester, left to right respectively

In the literature, there are many examples of GC-MS work to detect bioconstituents of *Orchidaceae* species. Jakubska-Busse and co-workers investigated the floral extract of *Epipogium aphyllums Sw*. (*Orchidaceae*) and they found some alkane and alkene compounds such as 9-tricosene, nonadecane, 1-nonadecene, and nonacosane in the floral extracts of the plant, like in this study [34]. In another interesting study, Manzo and co-workers demonstrated volatile fingerprint of Italian populations of three orchid species using solid-phase microextraction and GC coupled with MS Unlike our study; they concluded that hydrocarbons, aldehydes, alcohols, and terpenes were the major constituents of *in vivo* orchid scents [35].

Although it is generally known that orchid species contain a wide variety of phytochemicals that are thought to provide biological activity [36], the orchid species that are listed in Table 1 are not widely studied in terms of antioxidant activity. Therefore, in the present study, evaluating the whole plant and tuber extracts from this perspective will make a valuable contribution to the literature.

Tuber, stem, leaf, and flower parts of the *Dactylorhiza chuhensis* endemic orchid from Eastern Anatolia were investigated in terms of the phenolic content. According to the Folin–Ciocalteu method, the plant's tuber part was the poorest in phenolic terms [36]. However, when the results of the present study are evaluated, it can be easily seen that such a generalization cannot be made.

The samples' antioxidant activities, which were thought to have remarkable phenolic content, were also tested by DPPH free radical scavenging and FRAP assays. To evaluate DPPH free radical scavenging activity, $SC_{50}$ value (sample concentration sufficient to sweep half of the DPPH radical in the reaction medium) of each sample was determined, and obtained results were compared with $SC_{50}$ value of ascorbic acid. The calculated $SC_{50}$ value for ascorbic acid is fortunately 6.17 µg/mL, higher than the value obtained for most of the samples. In other words, most of the samples are more effective at scavenging DPPH radicals than the standard antioxidant known as ascorbic acid.

Obtained values for DPPH radical scavenging activity are higher than similar samples presented in the literature. For example, DPPH radical scavenging activity of the *Eulophia macrobulbon* was calculated around 10% for 100 µg/mL [37]. Furthermore, $IC_{50}$ of essential oil of Tunisian *Anacamptis coriophora* subsp. fragrans as an indicator of DPPH free radical scavenging activity was calculated as 1.3 mg/mL [38].

In addition to DPPH assay, ferring reducing activities were also measured. All values obtained for FRAP test are higher than the FRAP values reported for different parts of *Dactylorhiza chuhensis* in the literature [36]. Like the DPPH test results, the average of the FRAP values calculated for the extracts is higher than the value found for the iron-reducing power of the standard antioxidant. In this context, we can say that most of the extracts are better antioxidants than standard antioxidant ascorbic acid.

A negative strong linear correlation was found among DPPH scavenging activity and TPC ($r_S$=-0.939, $p$=0.01) and among FRAP and DPPH ($r_S$=-0,911, $p$=0.01). Also a positive strong linear correlation was found between FRAP and TPC ($r_S$=0.948, $p$=0.01). There were similar strong correlations reported in the literature [39-41].

## CONCLUSION

The present study was concluded that the tuber and whole plant extracts of eight orchids plant had moderate antimicrobial activity. We can say that tuber orchid extracts were generally more effective than whole plant extracts for eliminating microorganisms. Orchid tubers typically have a great relationship with bacteria and fungi. Since these plants are difficult to grow from seed, they necessarily develop from a tuber. The present study's findings can also provide an essential clue to isolate new antibiotic substances to control the infectious diseases caused by various bacterial and fungal pathogens. The medicinal plants are still constituting one of the significant sources of a drug in modern and traditional medicines. Among the

medicinal plants that are abundant throughout the world, but only small amounts have yet been investigated for its biological activity.

The GC-MS results showed the rich phytoconstituent content in six different *Orchidaceae* species from Bartın city of Turkey. The results did not show distinctive differences in composition between orchid species. All these identified phytoconstituents with their known bioactive properties could contribute to the plant's medicinal and cosmetic importance thanks to their measured antioxidative and antimicrobial activities. However, isolation of these structures and subjecting them to the biological activity works would give more specific results. In addition to all this, it is a striking result that samples belonging to a plant species that can be used for beverage preparation show higher levels of antioxidative than ascorbic acid used as standard antioxidant.

**Funding:** This research received no external funding.
**Conflicts of Interest:** The authors declare no conflict of interest

## REFERENCES

1. Singh DK. Morphological diversity of the orchids of Orissa/Sarat Misra, In: Pathak P, Sehgal RN, Shekhar N. Sharma M. Sood A, editors. Orchids: Science and Commerce, New Delhi, India: BSMPS; 2001. p. 35.
2. Bose TK, Bhattacharjee SK, Das P, Basak UC. Orchids of India (2nd Ed) Kolkata, India: Naya Prakash. 1999.
3. Baytop T. Türkiye'de Bitkiler ile Tedavi, Geçmişte ve Bugün [Herbal Treatment in Turkey, Past and Present] (2.baskı), Nobel Tıp Kitabevleri, İstanbul, 1999. 480 sayfa.
4. Schippmann U, Leaman DJ, Cunnigham ABA. Comparison of Cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. In: Bogers RJ, Craker LE, Lange D, editors. Medicinal and Aromatic Plants. Springer. Printed in the Netherlands; 2006; 17:75-95.
5. Gutiérrez RMP. Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. J Med Plant Res. 2010 Apr; 4(8):592-638.
6. Joshi G, Tewari LM, Lohani N, Upreti K, Jalal JS, Tewari G. Diversity of orchids on Uttarakhand and their conservation strategy with special reference to their medicinal importance. Rep Opin. 2009 Jan; 1(3):47-52.
7. Jalal JS, Kumar P, Pangtey YPS. Ethnomedicinal Orchids of Uttarakhand, Western Himalaya. Ethnobot Leaflets. 2008 Dec; 12: 1227-30.
8. Lüning B. Alkaloid content of Orchidaceae. In: Withner CL, editor. The orchids: scientific studies. New York: London. John Wiley and Sons. 1974.
9. Kaushik P. Ecological and anatomical marvels of the Himalayan orchids. Progress in Ecology. Today and Tomorrow's Printers and Publishers. New Delhi, India. 1983.
10. Kreutz CAJ. Orchidaceae. In Flora of Turkey and The East Aegean Islands. (Güner A, Özhatay N, Ekim T, Başer KHC. Eds). Vol. 11, Edinburgh University Press, Edinburgh. 2000.
11. Pant B. Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. Afr J Plant Sci. 2013 Oct; 7(10):448-67.
12. Hürkul MM, Çift, RB, Köroğlu A. Investigation of salep and salep containing products in view of food and pharmacy. Biodivers Conserv. 2020 Aug; 13(2): 144-52.
13. Danial M, Saghal G, Mubbarakh SA, Sundarasekar J, Subramanıam S. Antibacterial studies on In vivo plant parts of medicinally important Eurycoma longifolia (Tongkat Ali). Pak J Bot. 2013 Apr; 45(5): 1693-700.
14. Bulpitt CJ, Li Y, Bulpitt PF, Wang J. The use of orchids in chinese medicine. J R Soc Med. 2007 Dec; 100(12): 558-63.
15. Davis PH. Flora of Turkey and the east Aegean islands. (1966–1988): Vol. 1–10. Edinburgh University Press, Edinburgh.
16. Turkis S, Erturk O. Distribution of Orchid species in urban and meadow areas of Bartın city (Turkey). Biol Divers Conserv. 2015 Dec; 8 (3): 147-52.
17. Ertürk Ö. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia. 2006 Jun; 61(3): 275-8.
18. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016 Apr; 6(2): 71-9.
19. Clinical Laboratory Standard Institute, Performance Standard for antimicrobial susceptibility testing, M100, 2018, 29th ed.
20. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7. Wayne, PA: Clinical and Laboratory Standards Institute, 2018, 11th ed.
21. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2. Wayne, PA: Clinical and Laboratory Standards Institute, 2018, 13th ed.
22. Vanden Berghe DA, Vlietinck AJ. Screening methods for antibacterial and antiviral agents from higher plants. In: Dey PM, Harbone JD, editors. Methods in Plant Biochemistry, Academic Press, London, 1991. p: 47–69.
23. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965 Jan; 16(3): 144-58.

24. Sánchez Moreno C, Larrauri JA, Saura-Calixto F. A Procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric. 1998 Feb; 76(2): 270-6.
25. Oyaizu M. Studies on products of browning reaction – Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986; 44: 307–15.
26. Okhale SE, Ugbabe GE, Bamidele O, Ajoku GA, Egharevba HO. Phytochemical and antimicrobial studies on extractives of Calyptrochilum emarginatum (S.W.) Schltr (Orchidaceae) growing in Nigeria. J Med Plant Res. 2014 Jan;8(4):223-8.
27. Khasin SM, Rao PRM. Medicinal Importance of Orchids. The Botanica. 1999;49:86-91.
28. Sahaya SB, Chitra DB, Moin S, Wesley S. Evaluation of bioactive potential of Coelogyne nervosa a. rich. - an endemic medicinal orchid of Western Ghats, India. Asian J Pharm Clin Res. 2013 Mar; 6(S-1):114-8.
29. Paul P, Chowdhury A, Nath D, Bhattacharjee MK. Antimicrobial efficacy of orchid extracts as potential inhibitors of antibiotic resistant strains of Escherichia Coli. Asian J Pharm Clin Res. 2013 Jan;6 (3):108-11.
30. Sandrasagaran UM, Subramaniam S, Murugaiyah V. New perspective of dendrobium crumenatum orchid for antimicrobial activity against selected pathogenic bacteria. Pak J Bot. 2014 Apr; 46(2): 719-24.
31. Marasini R, Joshi S. Antibacterial and Antifungal Activity of Medicinal Orchids Growing in Nepal. J Nepal Chem Soc. 2012 Dec;29:104-9.
32. Iordache A, Culea M, Gherman C, Cozar O. Nuclear characterization of some plant extracts by GC-MS. Nucl Instr Meth Phys Res B. 2009 Jan;267(2):338-42.
33. Subbaiyan B, Samydurai P, Karthik Prabu M, Thangapandian V. Gas Chromatography and Mass Spectrum Analysis of *Catharanthus Pusıllus* Murray G. Don (*Apocyanaceae*). Int Res J Pharm App Sci. 2014; 4 (2): 48-52.
34. Jakubska-Busse A, Jasıcka-Mısıak I, Polıwoda A, Swıęczkowska S, Kafarskı P. The chemical composition of the floral extract of *Epıpogium aphyllum* sw. (*Orchıdaceae*): A clue for their pollination biology. Arch Biol Sci Belgrade. 2014 Jan;66(3):989-98.
35. Manzo A, Panseri S, Vagge I, Giorgi A. Volatile fingerprint of Italian populations of orchids using solid phase microextraction and Gas Chromatography coupled with Mass Spectrometry. Molecules. 2014 Jun;19(6):7913-36.
36. Dalar A, Guo Y, Esim N, Bengu AS, Konczak I. Health attributes of an endemic orchid from Eastern Anatolia, Dactylorhiza chuhensis Renz &Taub. In vitro investigations. J Herb Med. 2015 Jun;5(2): 77–85.
37. Schuster R, Zeindl L, Holzer W, Khumpirapang N, Okonogi S, Viernstein H, et al. Eulophia macrobulbon –an orchid with significant anti-inflammatory and antioxidant effect and anticancerogenic potential exerted by its root extract. Phytomedicine. 2017 Jan;24:157–65.
38. El Mokni R, Hammami S, Dall'Acqua S, Peron G, Faidi K, Braude JP, et al. Chemical composition, antioxidant and cytotoxic activities of essential oil of the inflorescence of Anacamptis coriophora subsp. fragrans (Orchidaceae) from Tunisia. Nat Prod Commun. 2016 Jun;11(6):857-60.
39. Fidrianny I, Rizkiya A, Ruslan K. Antioxidant activities of various fruit extracts from three solanum sp. using DPPH and ABTS method and correlation with phenolic, flavonoid and carotenoid content. J Chem Pharm Res. 2015 Jan;7(5):666-72.
40. Fidrianny I, Suhendy H, Insanu M. Correlation of phytochemical content with antioxidant potential of various sweet potato (Ipomoea batatas) in West Java, Indonesia. Asian Pac J Trop Biomed. 2018 Jan;8(1):25-30.
41. Gan J, Feng Y, He Z, Li X, Zhang H. Correlations between antioxidant activity and alkaloids and phenols of maca (*Lepidium meyenii*). J Food Qual. 2017 Oct, Article ID 3185945, 10 pages, https://doi.org/10.1155/2017/3185945.