J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(3): 853-866, 2024
Doi: 10.33483/jfpau.1448197

ORIGINAL ARTICLE / ÖZGÜN MAKALE

PHENOLIC AND ANTIOXIDANT PROFILE: FTIR AND LC-MS ANALYSES OF *SERAPIAS ORIENTALIS*

FENOLİK VE ANTİOKSİDAN PROFİLİ: SERAPIAS ORIENTALIS'İN FTIR VE LC-MS ANALİZLERİ

Erdi Can AYTAR[1,2]*, Yasemin Özdener KÖMPE[1]

[1]Ondokuz Mayıs University, Faculty of Sciences, Department of Biology, 55100, Samsun, Türkiye
[2]Usak University, Faculty of Agriculture, Department of Horticulture, 64200, Usak, Türkiye

ABSTRACT

Objective: *The objective of this study is to characterize the aerial parts of S. orientalis using Fourier Transform Infrared (FTIR) spectroscopy, evaluate the phenolic content and antioxidant activity of seeds, stems, and flowers, and conduct quantitative analysis of phenolic compounds using LC-MS/MS.*
Material and Method: *Fourier Transform Infrared (FTIR) spectroscopy was employed to characterize the aerial parts of S. orientalis. The analysis focused on identifying various functional groups such as -OH vibrations associated with polysaccharides, C-H vibrations from lipids and lignin compounds, and C=O vibrations related to cellulose derivatives. The total phenolic, flavonoid, flavanol, tannin, and proanthocyanidin contents of S. orientalis seeds, stems, and flowers were evaluated using standard analytical methods. DPPH radical scavenging activity was determined to assess antioxidant potential, with IC_{50} values calculated for each plant part. Quantitative analysis of phenolic compounds in the plant extract was conducted using LC-MS/MS. The abundance of various phenolic acids including P-coumaric acid, trans-ferulic acid, caffeic acid, and vanillic acid was determined. Additionally, other phenolic compounds such as gallic acid, chlorogenic acid, salicylic acid, (+) taxifolin, rutin hydrate, ellagic acid, quercetin dihydrate, and apigenin were also detected and quantified.*
Result and Discussion: *The evaluation of phenolic content showed differences among different plant parts, with flowers exhibiting the highest total phenolic and proanthocyanidin content. Seeds demonstrated superior DPPH radical scavenging activity. Quantitative analysis of phenolic compounds using LC-MS/MS highlighted the abundance of various phenolic acids and other phenolic compounds in S. orientalis. These findings underscore the potential of S. orientalis as a valuable source of natural antioxidants. Overall, the results suggest that S. orientalis possesses significant phenolic diversity and antioxidant activity, which could contribute to its potential applications in various industries, including pharmaceuticals and nutraceuticals.*
Keywords: *FTIR, LC-MS analysis, plant secondary metabolites, quantitative analysis*

ÖZ

Amaç: *Bu çalışmanın amacı, S. orientalis'in topraküstü kısımlarını Fourier Dönüşümü Kızılötesi (FTIR) spektroskopisi kullanarak karakterize etmek, tohumların, sapların ve çiçeklerin fenolik içeriğini ve antioksidan aktivitesini değerlendirmek ve LC-MS/MS kullanarak fenolik bileşiklerin nicel analizini yapmaktır.*

* **Corresponding Author / Sorumlu Yazar:** Erdi Can Aytar
e-mail / e-posta: erdicanaytar@gmail.com, **Phone / Tel.:** +905072291015

Submitted / Gönderilme : 06.03.2024
Accepted / Kabul : 07.06.2024
Published / Yayınlanma : 10.09.2024

Gereç ve Yöntem: *S. orientalis'in topraküstü kısımlarını karakterize etmek için Fourier Dönüşümü Kızılötesi (FTIR) spektroskopisi kullanıldı. Analiz, polisakkaritlerle ilişkilendirilen -OH titreşimleri, lipitlerden ve lignin bileşiklerinden kaynaklı C-H titreşimleri ve selüloz türevleriyle ilişkilendirilen C=O titreşimleri gibi çeşitli fonksiyonel grupları tanımlamaya odaklandı.S. orientalis tohumlarının, saplarının ve çiçeklerinin toplam fenolik, flavonoid, flavanol, tanin ve proantosiyanidin içeriği standart analitik yöntemler kullanılarak değerlendirildi. Antioksidan potansiyeli değerlendirmek için DPPH radikal temizleme aktivitesi belirlendi ve her bitki kısmı için IC_{50} değerleri hesaplandı. Bitki ekstraktındaki fenolik bileşiklerin nicel analizi LC-MS/MS kullanılarak gerçekleştirildi. P-kumarik asit, trans-ferulik asit, kafeik asit ve vanilinik asit gibi çeşitli fenolik asitlerin bol miktarda bulunduğu belirlendi. Ayrıca, galik asit, klorojenik asit, salisilik asit, (+) taksifolin, rutin hidrat, ellajik asit, kuersetin dihidrat ve apigenin gibi diğer fenolik bileşikler de tespit edildi ve nicel olarak belirlendi.*

Sonuç ve Tartışma: *Fenolik içeriğin değerlendirilmesi, farklı bitki parçaları arasında farklılıklar gösterdi, çiçekler en yüksek toplam fenolik ve proantosiyanidin içeriğini sergiledi. Tohumlar üstün DPPH radikal temizleme aktivitesi gösterdi.LC-MS/MS kullanarak fenolik bileşiklerin nicel analizi, S. orientalis'te çeşitli fenolik asitlerin ve diğer fenolik bileşiklerin bol miktarda bulunduğunu vurgulanmaktadır. Genel olarak, sonuçlar S. orientalis'in önemli bir fenolik çeşitliliğe ve antioksidan aktiviteye sahip olduğunu göstermektedir, bu da onun farmasötik ve nutrasötikler dahil çeşitli endüstrilerde potansiyel uygulamalarına katkıda bulunabileceğini düşündürmektedir.*

Anahtar Kelimeler: *Bitki ikincil metabolitleri, FTIR, LC-MS analizi, nicel analiz*

INTRODUCTION

Since the early 21st century, there has been significant interest in studying the origin of oxidation processes triggered by free radicals and the broader role of antioxidants. This fascination arises from the fact that free radicals, despite being neutral, are highly reactive and unstable molecules with notable impacts on human biological systems. Notably, even crucial lipid derivatives such as aldehydes can pose health risks, especially as they can naturally form due to thermal processes during food processing [1]. The potency of radicals' hinges on the presence of an unpaired electron within an atom, driving them to incessantly seek bonding with other atoms or molecules due to the deficiency of outer-shell electrons. Despite the existence of antioxidant defense mechanisms, human cells remain susceptible to damage, which can hasten the aging process and contribute significantly to the onset of various disease [2,3]. Extensive oxidative modifications to biological macromolecules, including lipids, proteins, and DNA, can culminate in tissue damage [4]. Understanding and preventing these processes are crucial for attaining a better quality of life. Oxidative stress emerges from an imbalance between prooxidant and antioxidant species responsible for maintaining cellular equilibrium. Insufficiencies in antioxidant defenses or breakdowns in preserving the cellular redox balance system result in the excessive production of reactive oxygen species, leading to oxidative stress and diverse alterations in biomolecules that foster disease development [5]. Previously known as the 'disruption of the prooxidant-antioxidant balance, 'oxidative stress has been redefined as the 'disruption of redox signaling and control and/or molecular damage' [6].

In recent years, the adverse effects associated with synthetic antioxidants, including butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, and citric acid, have raised concerns due to their demonstrated toxic and mutagenic effects [7]. Due to the long-term toxic effects associated with synthetic antioxidants, there has been a significant increase in demand for alternatives to replace their use in the food industry. This is especially important given the potential adverse effects of prolonged use, including carcinogenicity [8,9]. Phenolic compounds have become synonymous with health benefits associated with the consumption of high amounts of fruits and vegetables. These beneficial effects are at least partially attributed to their antioxidant activities [10,11]. These compounds can be a determining factor in the antioxidant potential of foods and, therefore, hold significance as natural sources of antioxidants [3].

Maceration is a commonly employed traditional extraction method for obtaining phenolic compounds from plants. In this method, plant samples are typically processed using solvents such as ethanol or methanol [12]. Traditional extraction relies on the principle that the target chemicals dissolve

in solvents with similar structures. In maceration extraction, techniques like shaking and agitation in an incubator are frequently employed to increase the contact surface area between samples and the solvent, thus enhancing the extraction rate and yield. Additionally, temperature control is crucial to enhance the effectiveness of the extraction process [13]. The advantages of this method include its ease of application, cost-effectiveness, and suitability for use on a wide variety of plant samples. Furthermore, being a tradition-based approach is indicative of its long-standing use for obtaining plant compounds [14].

Serapias orientalis (Greuter) Bauman & Künkele is a plant that thrives in grasslands with high water tables in the Middle and Eastern Black Sea regions, moist forest edges, and hazelnut orchards. The *S. orientalis* plant can reach a height of 15-30 cm and has thick, light green roots. Its flowers are red. This plant species is rarely found in moist meadows and open pine forests and typically grows in alkaline or calcareous soils. There is no study available regarding the antioxidant activity of *S. orientalis*. There is no information on the presence of antioxidants in this species. Therefore, the is study aims to evaluate the total phenolic, flavonoid, flavanol, proanthocyanidin, and tannin contents of extracts obtained from the seeds and *ex vitro*-produced stems and flower parts of the *S. orientalis* plant using the maceration method with methanol. Additionally, the study seeks to determine their antioxidant activities. Furthermore, characterization of the characteristic peak regions of the aerial parts of the plant will be conducted through FTIR analysis, and the identification of phenolic compounds will be carried out using the LC-MS technique.

MATERIAL AND METHOD

Plant Material and Extraction

In this study, mature flowering specimens of *S. orientalis* (2 years old) were collected during the flowering season in May for the aerial parts (stem and flower), while the seeds were collected after maturation in July (Ondokuz Mayıs Unıversity Campus, Samsun, Turkey). Subsequently, flowers, stems (leaves were considered as part of the stem), and seeds were dried in an oven at 40°C. The dried samples were ground into a fine powder and stored at +4°C until further analysis. For the maceration method, 0.5 g of the powder was extracted using 80% methanol at 35°C for 24 hours [15].

FTIR Analysis of *S. orientalis* in The Aerial Part

The FTIR spectra of *S. orientalis* aerial part were obtained by using the PERKIN ELMER Spectrum TWO FTIR Spectrometer equipped with a diamond attenuated total reflectance (ATR) accessory and a $LiTaO_3$ detector. Spectral measurements were conducted in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. The samples were directly analyzed without any prior treatment. The Perkin Elmer Spectrum Two FTIR software was employed to determine peak frequencies.

Total Phenolic Content

In this study, equal volumes of the sample and diluted Folin-Ciocalteu reagent were mixed. After incubating at room temperature for 3 minutes, 1 ml of 2% sodium carbonate solution was added. The mixture was then left to incubate in the dark at room temperature for 1 hour, followed by measuring the absorbance at 760 nm using a UV spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g extract). All measurements were performed in triplicate [16] .

Total Favonoid Content

This method was assessed using the $AlCl_3$ method with minor adjustments, following the procedure by [17]. Extracts were mixed with distilled water, followed by $NaNO_2$ (5%) addition and a standing period. Subsequently, $AlCl_3$ (10%) was added, and the solution underwent incubation. NaOH (1M) was then introduced, and the solution was left at room temperature. Absorbance was measured using a UV spectrophotometer, and total flavonoid content was quantified as quercetin equivalent (QE) per gram of dried extract (mg QE/g). All measurements were performed in triplicate.

Total Flavanols Content

The total flavonoid content was determined using the aluminum chloride method [18]. Briefly, 1 ml of the extracts was mixed with $AlCl_3$. Subsequently, 3 ml of sodium acetate solution was added. The mixture was then kept at room temperature in a dark environment for 30 minutes. After the incubation period, the absorbance of the sample was measured at 415 nm using quercetin as the standard. The total flavanols was expressed as quercetin equivalents (mg QE/g).

Total Tannin Content

Total tannin content was assessed using the Folin-Ciocalteu reagent as described in [19]. A calibration curve was prepared using different concentrations of gallic acid in methanol. Samples were mixed with diluted Folin–Ciocalteu reagent in water and an aqueous sodium carbonate solution. After incubation in darkness at room temperature, absorbance was measured at 760 nm. Total tannin content was quantified as gallic acid equivalent (GAE) per gram of dried extract (mg GAE/g). All measurements were performed in triplicate.

Total Proanthocyanin Content

Total proanthocyanidins content was determined using the butanol-acid assay [20] Diluted phenolic extract was mixed with n-butanol/HCl reagent, followed by the addition of ferric ammonium sulfate in HCl. After boiling and cooling, the absorbance of the solutions was measured at 550 nm. Total proanthocyanidins content was expressed as catechin equivalent (CAE) per gram of dried extract. All measurements were performed in triplicate.

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay

The DPPH assay was employed to evaluate the free radical scavenging potential of the extracts, with modifications based on [21]. Extracts at various concentrations were mixed with a methanol solution of DPPH radical, and incubated in the dark at room temperature, and absorbance was measured at 517 nm using a UV spectrometer against a blank. Ascorbic acid served as a reference standard.

Determination of Phenolic Compound Contents by LC-MS Analysis

Phenolic standard stock solutions were prepared at a concentration of 5000 ppm in 100% methanol (hypergrade, Merck, Darmstadt, Germany). Each molecule's stock solution was then prepared at a concentration of 1000 ppm. Calibration curves were constructed using a diluted mixture with concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 ppm. Standard phenolic compound concentrations were determined by extrapolation from these calibration curves, which were generated using linear regression equations based on peak areas in the extracts (R^2 = 0.99). All dilutions were carried out using 50% methanol.

For the analysis, a Zorbax SB-C18 column (2.1 mm x 50 mm x 1.8 µm) was employed, maintained at a temperature of 35°C. A binary mobile phase consisting of water (solvent A) containing 0.05% formic acid + 5 mM ammonium formate, and methanol (solvent B) was used. These mobile phases were applied at a flow rate of 0.5 ml/min, resulting in a total runtime of 13 minutes. Phenolic compounds were analyzed using a methanol gradient, with a five µL injection volume for all samples.ESI ionization was conducted with the following settings: Nebulizer gas (nitrogen) temperature at 350°C, gas flow rate of 10 ml/min, nebulizer gas pressure at 45 psi, sheath gas temperature at 350°C, sheath gas flow rate of 9 ml/min, the capillary voltage at 4000 V (+, -), and nozzle voltage at 500 V (+, -). Detection and quantification were performed using Agilent G3793AA Mass Hunter Optimizer software. This software was utilized for both the identification and quantification of the selected phenolic compounds.

RESULT AND DISCUSSION

ATR- FTIR Profile Aerial Part

The FTIR peak details of the aerial parts of *S. orientalis* are illustrated in Figure 1. Based on these findings, the peak at 3291 cm^{-1} corresponds to the -OH vibration associated with polysaccharides

[22]. Peaks ranging from 2919 to 2845 cm^{-1} are attributed to the C-H vibrations originating from lipids and lignin compounds [23]. Peaks at 1738 cm^{-1} are indicative of the C=O vibrations related to cellulose derivatives [24]. Peaks at 1612 cm^{-1} are associated with the amid 1 vibrations of proteins. The presence of peaks at 1411 cm^{-1} is attributed to the C=C and C-OH vibrations stemming from glycosylated phenols and uronic acid [25,26]. Peaks at 1369 and 1312 cm^{-1} represent the asymmetric CH_2 bond vibrations of cellulose and lignin [27]. Peaks at 1218 cm^{-1} signify the planar -OH vibration characteristic of cellulose and lignin [28]. Peaks at 1139 cm^{-1} are associated with the C-H vibrations of phenolic acids [29]. Peaks at 1101 cm^{-1} are linked to the C-H vibration of lignin [30]. Peaks at 1020 cm^{-1} correspond to the primary alcohol -OH vibration present in polysaccharides, lignin, and cellulose, while peaks at 837 cm^{-1} indicate out-of-plane C-H vibrations observed in aromatic lignin, terpenes, and alkenes [31]. Specifically, peaks at 1139 cm^{-1} are associated with the C-H vibrations of phenolic acids, and the peaks at 837 cm^{-1} indicate out-of-plane C-H vibrations observed in aromatic lignin, terpenes, and alkenes. These points can potentially provide preliminary information, particularly about phenolic components and, consequently, antioxidant content. Such information could serve as a foundation for understanding and identifying potential antioxidants in the *S. orientalis*.

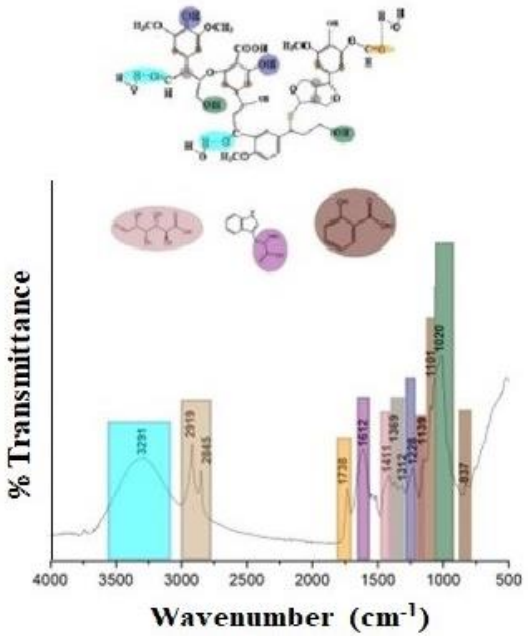


Figure 1. FTIR peaks of *S.orientalis* aerial part

Variation in the Content of Some Groups of Phenolic Compounds and Antioxidant Activity of *S. orientalis*

Based on the results obtained, the total phenolic content of seeds, stems, and flowers were quantified as follows: 100.14±1.58, 56.58±5.10, and 122.33±4.02 mg GAE/g, respectively, using the gallic acid standardization method. Similarly, the total flavonoid content, determined using the quercetin standardization method, was estimated as 177.84±8.75, 67.11±14.73, and 226.02±65.73 mg QE/g crude extract for seeds, stems, and flowers, respectively. Flavonol content, also determined using the quercetin standardization method, yielded values of 177.84±8.75, 67.11±14.73, and 226.02±65.73 mg QE/g crude extract for seeds, stems, and flowers, respectively.

Furthermore, the total tannin content, assessed via the gallic acid standardization method, was estimated as 2.36±0.16, 3.92±0.44, and 3.45±0.46 mg GAE/g crude extract for seeds, stems, and flowers, respectively. Finally, the total proanthocyanidin content, determined using the catechin standardization method, was found to be 133.42±39.82, 126.52±5.73, and 171.97±8.59 mg CAE/g crude extract for seeds, stems, and flowers, respectively.

These findings highlight the richness of phenolic compounds across different parts of the plant, with detailed results presented in Table 1. Additionally, Table 1 includes the DPPH radical scavenging activity of *S. orientalis* seeds, stems, and flowers, along with the presence of the well-known natural antioxidant, ascorbic acid. The results reveal significant antioxidant properties in seeds, stems, and flowers, with IC_{50} values of 54.37±14.73, 71.77±6.46, and 75.48±7.07, respectively.

In summary, seeds exhibit the highest DPPH activity, reflecting their potent radical scavenging effect. The flower part shows the highest total flavonoid, total phenolic, and total proanthocyanidin content, while seeds have the highest total flavanol content, and stems exhibit the highest total tannin content.

Table 1. Bioactive components and antioxidant activity of *S. orientalis* seed, stem and flower

Plant Name	DPPH (IC_{50} mg/ml)	Total Flavonol Compound (mg QE/g extract)	Total Flavonoid Compound (mg QE/g extract)	Total Phenolic Compound (mg GAE/g extract)	Total Proanthocyanidin content (mg CAE/g extract)	Total Tanen Content (mg GAE/g extract)
S. orientalis-Seed	54.37 ± 14.73	27.21 ± 3.72	177.84 ± 8.75	100.14 ± 1.18	133.42 ± 39.82	2.36 ±0.16
S. orientalis-Stem	71.77 ± 6.46	7.98 ± 3.66	67.11 ± 3.57	56.58 ± 5.10	126.52 ± 5.73	3.92±0.44
S. orientalis-Flower	75.48 ± 7.07	20.66 ± 6.85	226.02 ± 65.73	122.33 ± 4.02	171.97 ± 8.59	3.45 ±0.46

Phenolic Compound Contents by LC-MS analysis

The LC-MS/MS analysis was employed for the quantitative determination of total phenolic compounds in the aerial parts of *S. orientalis*. The sample was subjected to quantitative analysis of twenty-one distinct compounds, and the findings are presented in Table 2. As anticipated, the extract was observed to contain trace amounts of phenolic acids such as epigallocatechin-3-gallate, syringic acid, (-) epicatechin, coumarin, trans-resveratrol, hesperidin, and myricetin.

As indicated in Table 2, substantial quantities of p-coumaric acid (9.29 mg/g), trans-ferulic acid (3.84 mg/g), caffeic acid (2.548 mg/g), and vanillic acid (1.401 mg/g) were identified. Furthermore, gallic acid, chlorogenic acid, salicylic acid, (+) taxifolin, rutin hydrate, ellagic acid, quercetin dihydrate, apigenin, were also detected in the sample. Comprehensive compound structures were illustrated using the ChemDraw program.

Table 2. The LC-MS analysis of *S. orientalis* aerial parts and their molecular structures

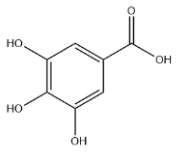
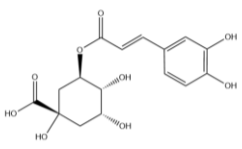
Phenolic Compound	Molecular Structure	*S. orientalis* Aerial Part of Methanol Extract (mg/g)
Gallic acid		0.081 ± 0.0003
Chlorogenic acid		0.193 ± 0.0002

Table 2 (*continue*). The LC-MS analysis of *S. orientalis* aerial parts and their molecular structures

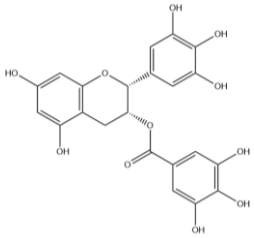
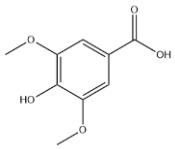
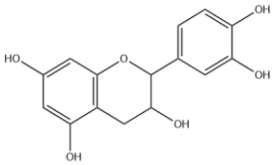
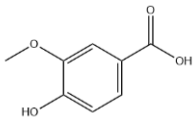
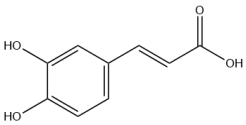
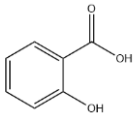
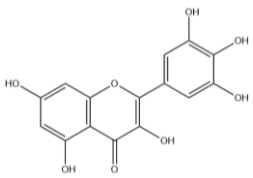
Phenolic Compound	Molecular Structure	*S. orientalis* Aerial Part of Methanol Extract (mg/g)
Epigallocatechın-3-gallate		≤ 0.02
Syringic acid		≤ 0.02
(-) epicatechin		≤ 0.1
Vanillic acid		1.401 ± 0.0979
Caffeic acid		2.548 ±0.0370
Salicylic acid		0.025 ± 0.0010
Myricetin		≤ 0.02

Table 2 (*continue*). The LC-MS analysis of *S. orientalis* aerial parts and their molecular structures

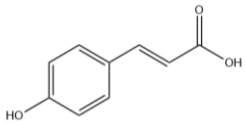
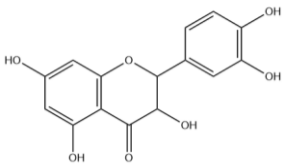
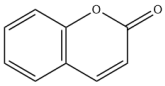
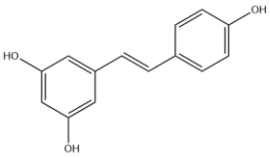
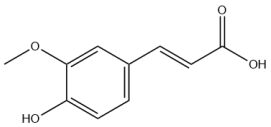
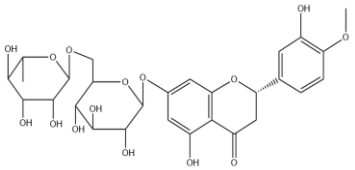
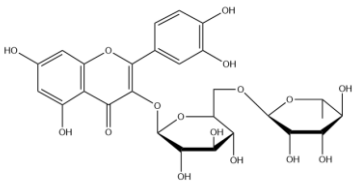
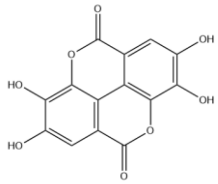
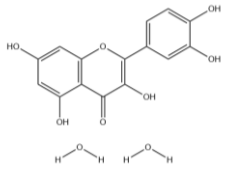
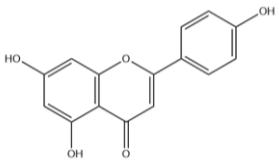
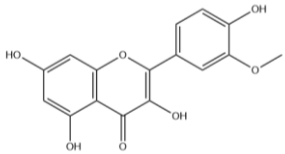
Phenolic Compound	Molecular Structure	*S. orientalis* Aerial Part of Methanol Extract (mg/g)
P-Coumaric acid		9.29 ± 0.0414
(+) Taxifolin		0.934 ± 0.0431
Coumarin		≤0.002
Trans- resveratrol		≤0.002
Trans-ferulic acid		3.84 ± 0.0873
Hesperidin		≤0.005
Rutin hydrate		0.096 ± 0.0074

Table 2 (*continue*). The LC-MS analysis of *S. orientalis* aerial parts and their molecular structures

Phenolic Compound	Molecular Structure	*S. orientalis* Aerial Part of Methanol Extract (mg/g)
Ellagic acid		0.661±0.0760
Quercetin dihydrate		0.495 ± 0.0039
Apigenin		0.031± 0.0002
Isorhamnetin		0.063 ± 0.0020

The excessive oxidation and reduction of cellular components can be harmful; hence, maintaining redox balance is of vital importance [32]. Plants, generally possessing widespread antioxidant capabilities, delay or prevent cell damage through effective antioxidants at low concentrations [33]. The diversity of antioxidants in plants includes water-soluble compounds such as ascorbate, glutathione, and phenols, as well as lipid-soluble ones like tocopherols, tocotrienols, and carotenoids [33,34].

Recent emphasis on the antioxidant potential of primary metabolites, polysaccharides, and glycoconjugates derived from natural sources highlights that plant species from various organs exhibit exceptional antioxidant potential [35]. The Orchidaceae, widespread worldwide and discovering approximately 200 new species annually, is particularly valuable for its diverse potential applications, including anti-inflammatory, anticancer, antimicrobial, antiviral, and immune-boosting properties[36]. While extensive research is available on tropical orchids, studies on terrestrial orchids are notably limited. In previous studies with orchids, Debnath and Kumari (2023) examined the accumulation of plant secondary metabolites in *Pholidota articulata* Lindl. and found that the accumulation was influenced by the polarity of different solvents used and various solvents (methanol, acetone, and chloroform) were effective in metabolite extraction. In this context, methanol was identified as the most effective solvent, providing the best results. In *P. articulata*, it was observed that phenols were the predominant secondary metabolites, followed by alkaloids, flavonoids, and tannins. Additionally, after treatment with 4 mg/L chitosan, the plants exhibited the highest accumulation of TPC (Total Phenolic Content) (81.73±0.32 mg/g dry weight), TFC (Total Flavonoid Content) (30.21±0.04 mg/g dry weight),

and TTC (Total Tannin Content) (33.10±0.10 mg/g dry weight) in leaf tissues [37]. However, when compared to *S. orientalis*, all parts of *S. orientalis* had higher phenolic content, but the results were reversed in terms of tannins. Furthermore, Hasnu et al. (2022) found that the methanolic leaf extract of *Vanilla borneensis* Rolfe exhibited a significant amount of phenolic content at 50 µg/ml and its flavonoid content was noted to be 73.87 µg/ml quercetin equivalent [38]. These results suggest that *V. borneensis* possesses high antioxidant activity, especially when compared to commercially cultivated Vanilla species. It is noteworthy to mention that species like *V. planifolia* Andrews, which are among the most cultivated, also have a remarkable total phenolic content. Additionally, natural *V. planifolia* extract has been found to have high DPPH radical scavenging activity and reduced power activity [39]. Another study determined that the dichloromethane extract of *Tridactyl tridentata* (Harv.) Schltr. roots exhibited a DPPH activity with an IC_{50} value of 0.02 mg/ml. However, it was observed that the ethanol extract showed low activity [40]. Similarly, a study conducted using methanol extracts of the wild *Rhynchostele rossii* (Lindly) plant found that the highest antioxidant activity was in the root extracts of the plant, with an IC_{50} value of 53.64±0.82 µg/ml [41]. In the study conducted by Ertürk et al. (2023), the antioxidant activity of *Serapias vomeracea* (Burm.f.) Briq., a member of the *Serapias* genus, was determined using the DPPH method [42]. The IC_{50} value obtained in the study was reported to be higher than that of the aerial part of *S. orientalis* (IC_{50}: 13.33). Consequently, it has been observed that tropical orchid species have more effective antioxidant properties compared to *S. orientalis*. However, this study also highlights that the *S. orientalis* plant possesses significant antioxidant potential and points to the potential use of different plant organs.

One advantage of attenuated total reflection Fourier-transform infrared spectroscopy is that it requires minimal sample preparation and shortens the analysis time by obtaining spectra on a wide range of samples, including powders, liquids, and pastes [43,44] In previous studies, amid I, II, and III peaks were prominent, and aromatic rings, geminal methyl, and ether linkages were commonly found in callus and leaf extracts. These observed peaks suggested the presence of flavanones and terpenoids. Terpenoids may also play a role in the reduction of metal ions, which can occur through the oxidation of aldehyde groups to carboxylic acids [45,46]. Similar observations were made in the case of *Carica papaya* Linn, callus culture [47]. In another study, low-intensity absorption bands were observed due to OH groups originating from gallic acid, quercetin, rutin, and tannic acid. These groups play a significant role in antidiabetic, antioxidant, and antibacterial activities. Therefore, FTIR has been used to determine different phytochemical components in plant samples [48,49]. According to the obtained FTIR spectral results, various functional groups such as carboxyl, hydroxyl, lipids, alcohols, amides, and phenolic compounds were found in the aerial parts of the *S. orientalis* plant.

According to the LC-MS results of *S. orientalis*, the revealed phenolic compounds exhibit strong antioxidant activities. Particularly, key components such as p-coumaric acid (9.29 mg/g), trans-ferulic acid (3.84 mg/g), caffeic acid (2.54 mg/g), and vanillic acid (1.401 mg/g) stand out. Previous studies have identified antioxidant activities in extracts of *Sasa quelpaertensis* Nakai plant, containing compounds such as p-coumaric acid and myricetin. The amount of p-coumaric acid was determined to be 3.92 µg per 1 mg of extract [50]. On the other hand, phenolic compounds like ferulic acid, salicylic acid, and p-coumaric acid were detected in all tested extracts of upland and wetland rice (*Oryza sativa* L.) samples from Malaysia. Especially, in the Bario variety of upland rice, ferulic acid (10.31 mg/100g) and p-coumaric acid (1.10 mg/100g) were found at higher levels [51]. In comparison to the above-ground parts of *S. orientalis*, p-coumaric acid is present at approximately 9 times higher levels. In another study related to orchids, the above-ground parts of *Dactylorhiza osmanica* (Kl) Soo var. *osmanica* were analyzed by RP-HPLC, revealing the presence of phenolic compounds such as benzoic acid (289.123 mg/l), protocatechuic acid (1006 mg/l), p-hydroxybenzoic acid (1472 mg/l), vanillin (0.694 mg/l), and p-coumaric acid (3133 mg/L) [52]. According to the UPLC-PDA results of *Malaxis acuminata*, prominent components such as syringic acid, p-coumaric acid, rutin, and quercetin were identified. Analysis of the aerial parts of *M. acuminata* revealed the presence of rutin at a concentration of 3.78 ± 0.051 µg/mg. In the leaf part of M. acuminata, phenolic compounds including p-coumaric acid (3.20 ± 0.031 µg/mg), rutin (1.12 ± 0.058 µg/mg), and quercetin (0.40 ± 0.002 µg/mg) were detected. Similarly, the pseudobulb of *M. acuminata* exhibited the presence of phenolic compounds such as p-coumaric acid (3.35 ± 0.06µg/mg), rutin (1.10 ± 0.084 µg/mg), and quercetin (0.24 ± 0.003µg/mg) [53].

The highest concentration of ferulic acid (432.68 µg/g DW) was detected in the conjugate phenolic leaf extracts of the Phalaenopsis hybrid Younghome Golden Leopard "Peachy," while the highest concentrations of sinapic acid (2232.81 µg/g) and p-coumaric acid (767.81 µg/g) were found in the root extracts of the Sogo Yukidian "V3" and Sakura Hime hybrids, respectively. Previously, phenolic acids such as ferulic, sinapic, p-coumaric, and ellagic acids have been reported to possess strong antioxidant activity [54] studies conducted on orchids, deficiencies regarding these compounds are observed, particularly indicating limited literature on research concerning black orchids. Furthermore, the limitations of these studies are clearly evident in our literature review. In conclusion, LC-MS analysis of *S. orientalis* demonstrates the presence of phenolic compounds and their strong antioxidant activities. Key components such as p-coumaric acid, Trans-ferulic acid, caffeic acid, and vanillic acid are present in high concentrations. These components have been observed to have positive effects on the antioxidant potential of plants, consistent with other studies.

Conclusion

In conclusion, our study has definitively demonstrated the rich phenolic content and potent antioxidant properties of *S. orientalis*. These findings underscore the potential significance of this plant as a valuable resource for health-related applications. Particularly, the high concentrations of phenolic compounds such as p-coumaric acid, trans-ferulic acid, caffeic acid, and vanillic acid significantly contribute to the plant's antioxidant activity. Therefore, it can be concluded that *S. orientalis* holds great potential for use in traditional medicine, as well as in the food and pharmaceutical industries. Future research efforts should aim to further investigate the effects of these compounds in greater detail to better understand their contributions to human health.

ACKNOWLEDGEMENTS

This study was prepared from Erdi Can Aytar's doctoral thesis “Germination of Some Orchid Species from Seed and Determination of Their Phytochemical Properties”. This work was supported by Ondokuz Mayis University Scientific Research Projects Coordination. (Project Number: PYO.FEN.1904.22.012).

AUTHOR CONTRIBUTIONS

Concept: E.A., Y.O.K.; Design: E.A., Y.O.K.; Control: E.A., Y.O.K.; Sources: E.A., Y.O.K.; Materials: E.A., Y.O.K.; Data Collection and/or Processing: E.A.; Analysis and/or Interpretation: E.A.; Literature Review: E.A.; Manuscript Writing: E.A.; Critical Review: E.A., Y.O.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Christodoulou, M.C., Orellana Palacios, J.C., Hesami, G., Jafarzadeh, S., Lorenzo, J.M., Domínguez, R., Moreno, A., Hadidi, M. (2022). Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals. Antioxidants, 11(11), 2213. [CrossRef]
2. Awan, S.S., Khan, R.T., Mehmood, A., Hafeez, M., Abass, S.R., Nazir, M., Raffi, M. (2023). Ailanthus altissima leaf extract mediated green production of zinc oxide (ZnO) nanoparticles for antibacterial and antioxidant activity. Saudi Journal of Biological Sciences, 30(1), 103487. [CrossRef]
3. Jakupović, L., Bačić, I., Jablan, J., Marguí, E., Marijan, M., Inić, S., Nodilo, L.N., Hafner, A., Zovko Končić, M. (2023). Hydroxypropyl-β-cyclodextrin-based Helichrysum italicum extracts: Antioxidant and cosmeceutical activity and biocompatibility. Antioxidants, 12(4), 855. [CrossRef]
4. Apak, R., Özyürek, M., Güçlü, K., Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 1.

Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. Journal of Agricultural and Food Chemistry, 64(5), 997-1027. [CrossRef]
5. Pisoschi, A.M., Pop, A., Iordache, F., Stanca, L., Predoi, G., Serban, A.I. (2021). Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry, 209, 112891. [CrossRef]
6. Mukherjee, K., Chio, T.I., Sackett, D.L., Bane, S.L. (2015). Detection of oxidative stress-induced carbonylation in live mammalian cells. Free Radical Biology and Medicine, 84, 11-21. [CrossRef]
7. Ebrahimabadi, A.H., Ebrahimabadi, E.H., Djafari-Bidgoli, Z., Kashi, F.J., Mazoochi, A., Batooli, H. (2010). Composition and antioxidant and antimicrobial activity of the essential oil and extracts of Stachys inflata Benth from Iran. Food Chemistry, 119(2), 452-458. [CrossRef]
8. Lorenzo, J.M., Pateiro, M., Domínguez, R., Barba, F.J., Putnik, P., Kovačević, D.B., Shpigelman, A., Granato, D., Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. Food Research International, 106, 1095-1104. [CrossRef]
9. Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. Archives of Toxicology, 94(3), 651-715. [CrossRef]
10. Luna-Guevara, M.L., Luna-Guevara, J.J., Hernández-Carranza, P., Ruíz-Espinosa, H., Ochoa-Velasco, C. E. (2018). Phenolic compounds: A good choice against chronic degenerative diseases. Studies in Natural Products Chemistry, 59, 79-108. [CrossRef]
11. Heim, K.E., Tagliaferro, A.R., Bobilya, D.J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry, 13(10), 572-584. [CrossRef]
12. Shi, L., Zhao, W., Yang, Z., Subbiah, V., Suleria, H.A.R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research, 29(54), 81112-81129. [CrossRef]
13. Zhang, Q.W., Lin, L.G., Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine, 13, 1-26. [CrossRef]
14. Chebaro, Z., Abdallah, R., Badran, A., Hamade, K., Hijazi, A., Maresca, M., Mesmar, J.E., Baydoun, E. (2023). Study of the antioxidant and anti-pancreatic cancer activities of Anchusa strigosa aqueous extracts obtained by maceration and ultrasonic extraction techniques. Frontiers in Pharmacology, 14, 1201969. [CrossRef]
15. Cai, Y., Luo, Q., Sun, M., Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences, 74(17), 2157-2184. [CrossRef]
16. Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158. [CrossRef]
17. Osuna-Ruiz, I., López-Saiz, C.M., Burgos-Hernández, A., Velázquez, C., Nieves-Soto, M., Hurtado-Oliva, M.A. (2016). Antioxidant, antimutagenic and antiproliferative activities in selected seaweed species from Sinaloa, Mexico. Pharmaceutical Biology, 54(10), 2196-2210. [CrossRef]
18. Mahmoudi, M., Boughalleb, F., Maaloul, S., Mabrouk, M., Abdellaoui, R. (2023). Phytochemical screening, antioxidant potential, and lc–esi–ms profiling of ephedra alata and Ephedra altissima seeds naturally growing in tunisia. Applied Biochemistry and Biotechnology, 1-13. [CrossRef]
19. Lou, S.N., Lin, Y.S., Hsu, Y.S., Chiu, E.M., Ho, C.T. (2014). Soluble and insoluble phenolic compounds and antioxidant activity of immature calamondin affected by solvents and heat treatment. Food chemistry, 161, 246-253. [CrossRef]
20. Porter, L.J., Hrstich, L.N., Chan, B.G. (1985). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 25(1), 223-230. [CrossRef]
21. Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M., Morelli, I. (2001). Antioxidant principles from bauhinia t arapotensis. Journal of Natural Products, 64(7), 892-895. [CrossRef]
22. George, T.S., Muhammadaly, S.A., Parambath Kanoth, B., Joseph, T., Chemmarickal Dominic, M.D., George, N., Balachandrakurupp, V., John, H. (2022). Isolation of high crystalline nanocellulose from Mimosa pudica plant fibres with potential in packaging applications. Packaging Technology and Science, 35(2), 163-174. [CrossRef]
23. Xin Lee, K., Shameli, K., Miyake, M., Kuwano, N., Bt Ahmad Khairudin, N.B., Bt Mohamad, S.E., Yew, Y.P. (2016). Green synthesis of gold nanoparticles using aqueous extract of garcinia mangostanafruit peels. Journal of Nanomaterials, 2016, 1-7. [CrossRef]
24. Martin, J.A., Solla, A., Woodward, S., Gil, L. (2007). Detection of differential changes in lignin composition of elm xylem tissues inoculated with Ophiostoma novo-ulmi using Fourier transform-infrared spectroscopy. Forest Pathology, 37(3), 187-191. [CrossRef]
25. Akhter, G., Khan, A., Ali, S.G., Khan, T.A., Siddiqi, K.S., Khan, H.M. (2020). Antibacterial and nematicidal properties of biosynthesized Cu nanoparticles using extract of holoparasitic plant. SN Applied

Sciences, 2, 1-6. [CrossRef]
26. He, J., Wang, J., Qi, G., Yao, L., Li, X., Paek, K.Y., Park, S.Y., Gao, W. (2022). Comparison of polysaccharides in ginseng root cultures and cultivated ginseng and establishment of high-content uronic acid plant synthesis system. Industrial Crops and Products, 186, 115155. [CrossRef]
27. Park, J., Park, Y. (2022). Hydrolyzed protein treatment to natural cellulosic lightweight and scattering fibers for improving the hydrophilicity. Sustainable Chemistry and Pharmacy, 29, 100814. [CrossRef]
28. Bouafia, A., Laouini, S.E. (2020). Green synthesis of iron oxide nanoparticles by aqueous leaves extract of Mentha Pulegium L.: Effect of ferric chloride concentration on the type of product. Materials Letters, 265, 127364. [CrossRef]
29. Jeyaram, S. (2022). Spectral, third-order nonlinear optical and optical switching behavior of β-carotenoid extracted from phyllanthus niruri. Indian Journal of Physics, 96(6), 1655-1661. [CrossRef]
30. Abdullah, H.M., Latif, M.H.A., Attiya, H.G. (2013). Characterization and determination of lignin in different types of Iraqi phoenix date palm pruning woods. International journal of biological macromolecules, 61, 340-346. [CrossRef]
31. Le, V., Sukhikh, A., Larichev, T., Ivanova, S., Prosekov, A., Dmitrieva, A. (2023). Isolation of the main biologically active substances and phytochemical analysis of ginkgo biloba callus culture extracts. Molecules, 28(4), 1560. [CrossRef]
32. Foyer, C.H., Noctor, G. (2016). Stress-triggered redox signalling: What's in pROSpect? Plant, Cell & Environment, 39(5), 951-964. [CrossRef]
33. Dumanović, J., Nepovimova, E., Natić, M., Kuča, K., Jaćević, V. (2021). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Frontiers in Plant Science, 11, 552969. [CrossRef]
34. Allen, J.F. (2015). Why chloroplasts and mitochondria retain their own genomes and genetic systems: colocation for redox regulation of gene expression. Proceedings of the National Academy of Sciences, 112(33), 10231-10238. [CrossRef]
35. Krishnaiah, D., Sarbatly, R., Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing, 89(3), 217-233. [CrossRef]
36. Hossain, M.M. (2011). Therapeutic orchids: traditional uses and recent advances-an overview. Fitoterapia, 82(2), 102-140. [CrossRef]
37. Debnath, S., Kumaria, S. (2023). Insights into the phytochemical accumulation, antioxidant potential and genetic stability in the in vitro regenerants of Pholidota articulata Lindl., an endangered orchid of medicinal importance. South African Journal of Botany, 152, 313-320. [CrossRef]
38. Hasnu, S., Deka, K., Saikia, D., Lahkar, L., Tanti, B. (2022). Morpho-taxonomical and phytochemical analysis of Vanilla borneensis Rolfe-a rare, endemic and threatened orchid of Assam, India. Vegetos,35, 1-11. [CrossRef]
39. Calva-Estrada, S.J., Mendoza, M.R., García, O., Jiménez-Fernández, V.M., Jiménez, M. (2018). Microencapsulation of vanilla (Vanilla planifolia Andrews) and powder characterization. Powder Technology, 323, 416-423. [CrossRef]
40. Chinsamy, M., Finnie, J.F., Van Staden, J. (2014). Anti-inflammatory, antioxidant, anti-cholinesterase activity and mutagenicity of South African medicinal orchids. South African Journal of Botany, 91, 88-98. [CrossRef]
41. Gutiérrez-Sánchez, A., Monribot-Villanueva, J.L., Cocotle-Ronzón, Y., Martínez-Cruz, N.S., Guerrero-Analco, J.A. (2020). Phenolic profile and antioxidant activity from wild and in vitro cultivated Rhynchostele rossii (Orchidaceae). Acta Botánica Mexicana, (127). [CrossRef]
42. Ertürk, Ö., Ayvaz, M. Ç., Çil, E., Bağdatlı, E. (2023). Gas chromatography-Mass spectrometry analysis and antimicrobial and antioxidant activities of some orchid (orchidaceae) species growing in Turkey. Brazilian Archives of Biology and Technology, 66, e23210265.
43. Canteri, M.H., Renard, C.M., Le Bourvellec, C., Bureau, S. (2019). ATR-FTIR spectroscopy to determine cell wall composition: Application on a large diversity of fruits and vegetables. Carbohydrate Polymers, 212, 186-196. [CrossRef]
44. Robic, A., Bertrand, D., Sassi, J.F., Lerat, Y., Lahaye, M. (2009). Determination of the chemical composition of ulvan, a cell wall polysaccharide from Ulva spp. (Ulvales, Chlorophyta) by FT-IR and chemometrics. Journal of Applied Phycology, 21(4), 451-456. [CrossRef]
45. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R., Sastry, M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B: Biointerfaces, 28(4), 313-318. [CrossRef]
46. Shankar, S.S., Rai, A., Ahmad, A., Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. Journal of Colloid and Interface

Science, 275(2), 496-502. [CrossRef]
47. Mude, N., Ingle, A., Gade, A., Rai, M. (2009). Synthesis of silver nanoparticles using callus extract of Carica papaya-A first report. Journal of Plant Biochemistry and Biotechnology, 18, 83-86. [CrossRef]
48. Lu, X., Wang, J., Al-Qadiri, H.M., Ross, C.F., Powers, J.R., Tang, J., Rasco, B.A. (2011). Determination of total phenolic content and antioxidant capacity of onion (Allium cepa) and shallot (Allium oschaninii) using infrared spectroscopy. Food Chemistry, 129(2), 637-644. [CrossRef]
49. Hamedi, S., Shojaosadati, S.A. (2019). Rapid and green synthesis of silver nanoparticles using Diospyros lotus extract: Evaluation of their biological and catalytic activities. Polyhedron, 171, 172-180. [CrossRef]
50. Pyeon, D.B., Lee, S. E., Yoon, J.W., Park, H.J., Oh, S.H., Lee, D.G., Park, S.P. (2022). Comparison of the improving embryo development effects of Sasa quelpaertensis Nakai extract, p-coumaric acid, and myricetin on porcine oocytes according to their antioxidant capacities. Theriogenology, 185, 97-108. [CrossRef]
51. Abd Latiff, N., Alam, S. A.Z., Hanapi, S.Z., Sarmidi, M.R. (2019). Quantification of polyphenol content, antioxidant properties and LC-MS/MS analysis in Malaysian indigenous rice cultivars (Oryza sativa L.). Agriculture and Natural Resources, 53(4), 402-409. [CrossRef]
52. Kotiloğlu, D., Acet, T., Özcan, K. (2020). Phytochemical profile and biological activity of a therapeutic orchid from Anatolia: Dactylorhiza romana subsp. georgica. Journal of Food Measurement and Characterization, 14(6), 3310-3318. [CrossRef]
53. Bhattacharyya, P., Lalthafamkimi, L., Sharma, T., Kumar, D., Kumar, A., Kumar, D., Kumar, S. (2022). Metabolic and tissue-specific expression profiling in micropropagated plants of Malaxis acuminata: An endangered medicinal orchid. Plant Cell, Tissue, and Organ Culture, 151(3), 535-549. [CrossRef]
54. Minh, T.N., Khang, D.T., Tuyen, P.T., Minh, L.T., Anh, L.H., Quan, N.V., Ha, P.T., Quan, N.T., Toan, N.P., Elzaawely, A.A., Xuan, T.D. (2016). Phenolic compounds and antioxidant activity of Phalaenopsis orchid hybrids. Antioxidants, 5(3), 31. [CrossRef]