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# Chemical Composition, Antioxidant, and Anti-Diabetic Activities of *Scorzonera phaeopappa* Boiss

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**Abstract:** Wild edible plants have attracted increasing interest from researchers because of their richness in biologically active phytochemicals. These are found to be a potential remedy for oxidative-stress-mediated diseases such as diabetes mellitus. In this study, total phenol, total terpene, and total flavonoid content as well as antioxidant and antidiabetic activities of *Scorzonera phaeopappa* Boiss from Lebanon were determined. Using dichloromethane, dichloromethane ammonia, methanol, acetone, and ethanol, extracts were prepared from the edible leaves. The antioxidant activity and the antidiabetic activity were determined by 2,2-diphenyl-1-picrylhydrazyl scavenging, $Fe^{2+}$-chelating and α-amylase/α-glucosidase inhibitory assays, respectively. Dichloromethane ammonia was found to have the highest extraction capacity on phenols (2.73 mg GAE /100 mg extract) and terpenes (232.42 mg LE/100 mg extract). Methanol was found to have the highest extraction capacity on total flavonoids (63.05 mg QE/100 mg extract). The methanol extract exhibited the highest DPPH scavenging activity ($IC_{50}$ 0.07 mg/mL) and the highest chelating activity as compared to other extracts (0.08 mg/mL, chelating activity 50%). The acetone extract was two times more active than acarbose against α-amylase enzyme and was the most active against α-glucosidase (6.3 mg/mL). Significant positive correlations were observed between total phenol content and α-glucosidase inhibitory assay and total terpene content and α-glucosidase inhibitory assay.

**Keywords:** *Scorzonera phaeopappa* Boiss; antioxidant activity; DPPH scavenging; chelating activity; antidiabetic activity; α-amylase; α-glucosidase



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## 1. Introduction

Plants are a major source of medicinal compounds; over half of the pharmaceutical drugs are made from plant extracts and about 60% of the total world population use herbs to treat some diseases [1]. As a result, edible plants have attracted considerable research interest owing to their role in natural home remedies and their role as important sources of nutrients in plant-based traditional foods/dishes and beverages. People living in underdeveloped and remote areas, i.e., rural areas, are economically disadvantaged, have access to wild edible plants (WEPs), and, therefore, are more familiar with the uses and health benefits of WEPs and are reliant on them to meet their daily needs of vegetables and fruits [2].

The genus *Scorzonera*, a member of the Asteracea family, is mainly distributed in Asia, Europe, and Northern Africa. *Scorzonera* includes about 170 species, nine of which are found in Lebanon. These nine species are *Scorzonera capitata*, *Scorzonera cana* (C.A. Mey) O.Hoffm, *Scorzonera jaccuiniana* (W. Koch.) Boiss, *Scorzonera labinotica* Boiss, *Scorzonera mack meliana* Boiss, *Scorzonera phaeopappa* Boiss, *Scorzonera mollis* M. Beib, *Scorzonera rigida* Aucher, and *Scorzonera papposa* DC [3]. The abundance of the genus *Scorzonera*, its edibility,

and its uses in traditional medicine have attracted the interest of researchers, the latter interested in finding its active secondary metabolites extracted from different parts, mainly leaves and stems. Several studies have been conducted to assess the chemical composition of the different species within the genus *Scorzonera*. The most common extracted secondary metabolites isolated from the different species include dihydroisocoumarins, stilbenes, lignans, lactones, phenolic derivatives [2], phtalides [4], coumarins, sesquiterpenes [5], triterpenes [6], and flavonoids [5].

*Scorzonera* species are consumed as food in Europe, Turkey, and Lebanon. For example, the young leaves of the *Scorzonera hispanica* L., also known as Black Salsify, Spanish salsify, and black oyster, are used to make salads, whereas its roots are consumed as cooked vegetables as part of the European cuisine [6].

Several studies have been conducted on roots, leaves, and aerial parts of *Scorzonera* species, demonstrating their biological activities such as anticarcinogenic, antihypertensive, antiinflammatory [7], analgesic, antinocecptive, antimicrobial, and antifungal [8], as well as anticholinesterase, antidiabetic, and antityrosinase activities [9].

Antioxidants are naturally occurring or synthetic chemicals in foods. They provide protection against free radicals and increase the shelf life of food products by retarding the process of lipid peroxidation, which is the main cause of food deterioration. Moreover, antioxidant compounds protect the human body from free radicals and reactive oxygen species, therefore enhancing immunity.

Oxidative stress is a condition in which there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize or eliminate them, leading to potential damage to cells and biomolecules. Oxidative stress is associated with a wide range of diseases and health conditions such as cardiovascular diseases, neurodegenerative diseases, cancer, diabetes, rheumatoid arthritis, and age-related diseases. Plants have long been a source of drugs and compounds with antioxidant properties that can help mitigate the effects of oxidative stress.

Diabetes mellitus is a set of chronic metabolic diseases characterized by hyperglycemia, resulting from insufficient amounts of insulin secretion or ineffective use of insulin by the body. According to the WHO, about 150 million people have diabetes mellitus worldwide and this number is expected to double by 2025. One of the most worrying features of this rapid increase is the emergence of type 2 diabetes in children, adolescents, and young adults. Two digestive enzymes are responsible for food metabolism and glucose blood levels: the $\alpha$-amylase enzyme in pancreatic juices, which breaks down fats and carbohydrates into absorbable molecules [10], and the $\alpha$-glucosidase enzyme in the small intestine, which catalyzes the end step of the digestion of starch and disaccharide [11]. Thus, the inhibition of these enzymes has been found to be effective in lowering the level of blood glucose [12].

Medicinal plants and herbal extracts that are rich in polyphenols have been reported to demonstrate antioxidant and potential antidiabetic activities [12]. However, to the best of our knowledge, only a few recent studies have been conducted to determine the antidiabetic activity of any of the *Scorzonera* species [13–17], despite the fact that *S. semicana* DC has been used to treat diabetes mellitus in Turkish traditional medicine [18].

*Scorzonera phaeopappa* Boiss is an edible plant that is widely used as a low-cost food and as traditional medicine by people living in rural areas in Lebanon; however, it has not been studied yet. Therefore, the objectives of this study were to investigate the chemical composition of *Scorzonera phaeopappa* Boiss and to evaluate the antioxidant and antidiabetic activities of the obtained extract. To achieve these objectives, (1) crude extracts using solvents of different polarity were prepared, (2) total phenolic compounds using the Follin Ciocalteu method, total flavonoid content using the Aluminum chloride method, and total terpene content using the Salkowski test were determined, (3) the antioxidant activity of the extracts using DPPH radical scavenging and ferrous ion chelating assays was assessed, and (4) the antidiabetic activity of the extracts using $\alpha$-amylase and $\alpha$-glucosidase assays was determined.

## 2. Results and Discussion

### *2.1. Extraction Yield, Total Phenol, Total Flavonoid, and Total Terpene Contents*

Wild food plants (WFPs) have been an essential component of traditional diets throughout human history, as they are free and are a rich source of nutrients such as vitamins, minerals, and other antioxidant compounds. In recent years, WEPs have been one of the top trends in healthy eating, emerging in several European countries and other developed countries [1]. The Mediterranean region harbors a wide variety of wild edible plants, including queen-of-the-Alps (Qors aane), cheeseweed (Khebbayze), asparagus, and *S. phaeopappa* (Al Meshe)—the plant that is the object of our study—which have always been an important food source for people living in rural areas [19]. Thus, WEPs have been attracting the interest of researchers not only because of their health benefits, but also because of their affordability and the role they play in the provision of food security.

Table 1 presents the extraction yields of the extracts obtained from the leaves of *Scorzonera phaeopappa* using five solvents with varying polarities: DCM, DCM-a, acetone, MeOH, and EtOH. The results showed that acetone has the highest extraction yield (41.3%), followed by DCM-a (23.5%), EtOH (13.5%), MeOH (12.2%), and DCM (9.5%). The yield of extraction depends on the extraction conditions (pH, temperature, extraction time) as well as on the polarity of the solvent and the phytochemical compounds found in the extract, with the last two factors being the most important determinants of the extraction yield under the same extraction conditions [20]. Due to the phytochemical diversity in plant species, it is not possible to extract all phytochemicals using one solvent, where polar/non-polar phytochemicals are extracted using a polar/non-polar solvent, respectively. The use of polar and non-polar solvents, therefore, is favorable to extract different phytochemical compounds [21]. Previous studies conducted on several *Scorzonera* species to determine its phytochemical composition used acetone and DCM for the isolation of phenolic compounds and terpenoids, respectively [22].

**Table 1.** Extraction yields of *Scorzonera phaeopappa* leaf extracts using solvents with different polarities. Mean ± SD (standard deviation).

| Solvent | Extraction Yield % Mean ± SD |
|---|---|
| Acetone | 41.3 ± 0.0 |
| DCM-a | 23.5 ± 0.0 |
| DCM | 9.5 ± 0.0 |
| MeOH | 12.2 ± 0.0 |
| EtOH | 13.5 ± 0.1 |

*Scorzonera* species are highly rich in flavonoids, terpenoids, monoterpenes, sesquiterpene lactones, triterpenes, and phenolic acids [2,22].

The total phenolic (TPC), total flavonoid (TFC), and total terpene (TTC) content of *Scorzonera phaeopappa* was determined by the Folin–Ciocalteau method, aluminum chloride method, and Salkowski test, respectively, using five different solvents (acetone, DCM-a, DCM, MeOH, and EtOH). Results were expressed in GAE/100 mg DW, QE/100 mg DW, and LE/100 mg DW, respectively (Table 2).

**Table 2.** Total phenolic content, total flavonoid content, and total terpene content of the five extracts prepared using solvents with different polarities. Mean ± SD (standard deviation).

| | Total Phenolic Content | Total Flavonoid Content | Total Terpene Content |
|---|---|---|---|
| **Extract** | **mg GAE/100 mg DW** | **mg QE/100 mg DW** | **mg LE/100 mg DW** |
| Acetone | 1.69 ± 0.00 | 61.0 ± 0.6 | 11.3 ± 0.5 |
| DCM-a | 2.70 ± 0.00 | 14.0 ± 1.1 | 51.0 ± 1.4 |
| DCM | 2.04 ± 0.00 | 25.0 ± 0.7 | 232.0 ± 22.6 |
| MeOH | 1.83 ± 0.00 | 63.0 ± 1.4 | 28.3 ± 0.2 |
| EtOH | 1.46 ± 0.04 | 53.0 ± 1.1 | 8.7 ± 1.9 |

The DCM-a solvent showed the highest extraction yield of phenolic compounds followed by DCM, MeOH, acetone, and, lastly, by EtOH. These findings were supported by the findings of several other studies. Addai et al. [23] revealed that the extraction yield of phenolic compounds differed among solvents of different polarities (acetone, EtOH, and MeOH), with MeOH exhibiting the highest extraction yield followed by acetone and then EtOH. Sun et al. [24] reported that MeOH is an effective solvent for the extraction of phenolic compounds. Milella et al. [2] reported that MeOH has the highest extraction yield of phenolic content in *Scorzonera undulata* (80.7 mg GAE/g of extract). In addition, Bellassouad et al. [7], in a study conducted on *Scorzonera* species, reported that the extraction yield of phenolic compounds is greatly dependent on the solvent polarity and the time of maceration. Similarly to the results obtained as part of our study, it was also shown that the extraction of phenolic compounds was poor with EtOH as extracting solvent.

Plant polyphenols, i.e., antioxidative compounds, were shown to play a role in various biological activities such as antioxidant, antiinflammatory, antidiabetic, antimutagenic, and neuroprotective activities [25].

MeOH and acetone showed the highest extraction capacity for the total flavonoid content (63 and 61 QE/100 mg, respectively), followed by EtOH (53 QE/100 mg), DCM (25 QE/100 mg), and DCM-a (14 QE/100 mg). Results from several previous studies support these findings. Phytochemical studies conducted on several *Scorzonera* species to determine their total flavonoid content have demonstrated that MeOH is an effective solvent for flavonoid extraction [26]. Other researchers have also shown that MeOH is a better solvent for extraction of flavonoids than other solvents such as EtOH, acetone, and DCM [27]. In addition, in a comparative study conducted by Ghasmezadeet et al. [28] on the total flavonoid content of *Scorzonera* species the extraction capacity of MeOH was shown to be higher than that of acetone. Another study conducted to isolate and identify the flavonoids contained in nine taxa of *Scorzonera* species (*S. austriaca* Willd. var. angustifolia, *S. crispatula* Boiss., *S. graminifolia* L., *S. hirsuta* L., *S. hispanica* L., *S. laciniata* L., *S. mollis* M.Bieb., *S. pseudolanata* Grossb., *S. pusilla* Pall.) using MeOH as a solvent showed that all of the taxa contained high amounts of the most common flavonoids, quercetin and kaempferol, despite the fact that the content of flavonoid differs among different plant species and parts [29].

DCM and DCM-a showed the highest extraction capacity for the total terpene content (51 and 232 LE/100 mg, respectively), followed by MeOH (28.3 LE/100 mg), acetone (11.3 LE/100 mg), and EtOH (8.7 LE/100 mg). These findings could be explained by the fact that DCM is known to be an effective solvent for the extraction of volatile (non-polar) compounds such as terpenes because of its non-polar properties. In addition, in a study conducted by Wu et al. [30], DCM was used to isolate and extract triterpenes from *Scorzonera austrica* because of its high extraction capacity with respect to terpenes.

### *2.2. Antioxidant Activities of Scorzonera phaeopappa Extracts Using DPPH and $Fe^{2+}$ Chelating Assays*

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical assay is a widely accepted method to measure the ability of plant extracts to act as free radical scavengers or hydrogen donors and, therefore, to evaluate the antioxidant activity of plant extracts. DPPH is a stable free radical characterized by a deep violet coloration. It is dissolved in EtOH to form a DDPH solution with spectrophotometric absorption at about 520 nm. When a solution of DPPH is mixed with a hydrogen donor, such as an antioxidative agent, DPPH is converted to its reduced form and its deep violet color turns to yellow, indicating the pairing of the odd electron from the DPPH radical with the hydrogen from a free radical scavenging antioxidant.

The DPPH scavenging activity exhibited by *S. phaeopappa* extracts was expressed as IC50, which is defined as the concentration of substrate that causes 50% loss of the DPPH activity. These values were determined using the regression equations obtained from concentration–activity curves (Table 3).

**Table 3.** DPPH scavenging activity and $Fe^{2+}$ chelating activity of five *S. phaeopappa* leaf extracts using solvents with varying polarities.

| Extract | $IC_{50}$ DPPH Assay (mg/mL) | $Fe^{2+}$ Chelating Activity | |
|---|---|---|---|
| | | Concentration (mg/mL) | Inhibitory Percentage (%) |
| Acetone | 0.50 ± 0.07 | 0.10<br>0.15 | 45 ± 2<br>54 ± 1 |
| DCM-a | 1.05 ± 0.10 | 0.15<br>0.2 | 52 ± 1<br>62 ± 1 |
| DCM | 0.38 ± 0.03 | 0.15<br>0.2 | 52 ± 2<br>62 ± 1 |
| MeOH | 0.07 ± 0.02 | 0.06<br>0.08 | 21 ± 1<br>50 ± 1 |
| EtOH | 0.39 ± 0.02 | 0.10<br>0.15 | 37 ± 2<br>45 ± 1 |

Our results showed that the MeOH extract has the highest DPPH scavenging activity (0.07 mg/mL), followed by the DCM extract (0.38 mg/mL), EtOH extract (0.39 mg/mL), and acetone extract (0.50 mg/mL), while the DCM-a extract has the lowest DPPH scavenging activity (1.05 mg/mL). Thus, the MeOH extract was found to show the most potent scavenging activity on DPPH. Comparing our results with ascorbic acid, the commonly used reference compound known to exhibit a DPPH radical scavenging activity at an IC50 of 0.003 mg/mL, we found that all extracts exhibited an activity 23–350 times lower than that of ascorbic acid. Previous studies have shown that the methanolic extracts from different *Scorzonera* species exhibit inhibitory activities against DPPH. In one study, for example, feruloyl pedospermic acids A and B, new quinic acid derivatives isolated from *S. divarcata*, showed potent antioxidant activities against DPPH [31]. Another study, conducted by Wang et al. [6], also showed that scorzodiyhdrostilbenes A-E, isolated from *S. radiata*, exhibit a stronger antioxidant activity than resveratrol against DPPH. Similar results were also obtained in another study in which chlorogenic acid, a phenolic acid extracted from 27 different *Scorzonera* species, showed a potent antioxidant activity against DPPH [9].

Ferrous ions ($Fe^{2+}$) could catalyze the Fenton-type reactions in a biological system, resulting in the generation of hydroxyl radicals (OH•). Thus, the minimization of the $Fe^{2+}$ concentration by a chelating agent provides protection against oxidative damage. Ferrozine has largely been used for the determination of chelating activity, forming a colored complex with $Fe^{2+}$ that has an absorption peak at 562 nm. Other chelators, such as phytochemicals extracted from plants, can also make a complex with ferrous ions, thus competing with ferrozine, inhibiting the reaction of ferrozine with ferrous ion, and reducing the color intensity of the solution. Measurement of the color reduction allows estimation of the chelating activity of the tested antioxidant [32].

As shown in Table 3, all the extracts exhibited $Fe^{2+}$ chelating activity in a concentration-dependent manner. At very low concentrations, the methanolic (0.06 mg/mL and 0.08 mg/mL), ethanolic (0.10 mg/mL and 0.15 mg/mL), and acetone (0.10 mg/mL and 0.15 mg/mL) extracts exhibited iron chelating activities of 21% and 50%, 37% and 45%, and 45% and 54%, respectively. At higher concentrations, however, DCM-a and DCM extracts (0.15 mg/mL and 0.20 mg/mL, respectively) exhibited iron chelating activities of 52% and 62%, respectively. It is well-known that compounds with structures containing two or more functional groups such as -OH, -SH, -COOH, $-PO_3H_2$, C=O, $-NR_2$, -S, and -O- can exhibit metal chelating activity [33]. Phytochemical studies showed that *Scorzonera* species are rich in such functional groups, such as chlorogenic acid, rutin, hyperoside, and scorzotomentosin-4-glucoside, which have a moderate $Fe^{2+}$ chelating activity as compared to EDTA [9]. In addition, Senol et al. [9] showed that the methanolic extract of

27 different *Scorzonera* species exhibited a low-to-moderate $Fe^{2+}$ chelating activity. In conclusion, all extracts have a promising chelating activity.

### *2.3. Antidiabetic Activities of Scorzonera pheoppapa Leaves Extracts Using α-Amylase and α-Glucosidase Inhibitory Assays*

The antidiabetic activity of *S. phaeopappa* extracts obtained with five different solvents (acetone, DCM-a, DCM, EtOH, and MeOH) was determined using the α-amylase and α-glucosidase inhibitory assays. Results were expressed as IC50 (Table 4) and the trade names for acarbose, a known antidiabetic drug, Glucobay and Precose, were used as the reference standard with IC50 values of 0.47 ± 0.03 mg/mL and 0.21 ± 0.06 mg/mL, respectively.

**Table 4.** Results of α-amylase and α-glucosidase inhibitory assays of five *S. phaeopappa* leaf extracts using solvents with varying polarities.

| Extract | $IC_{50}$ α-Amylase (mg/mL) | $IC_{50}$ α-Glucosidase (mg/mL) |
|---|---|---|
| Acetone | 0.21 ± 0.00 | 6.30 ± 0.21 |
| DCM-a | 3.43 ± 2.10 | 12.55 ± 1.10 |
| DCM | 0.97 ± 0.01 | 16.80 ± 1.13 |
| EtOH | 0.56 ± 0.05 | 5.46 ± 0.32 |
| MeOH | 2.06 ± 0.06 | 9.01 ± 0.36 |
| Acarbose | 0.42 ± 0.03 | 0.28 ± 0.06 |

The data obtained showed that the acetone extract is the most active against α-amylase (IC 50 = 0.21 mg /mL) and is more than two times more potent than acarbose (0.42 mg/mL). All other extracts exerted α-amylase inhibitory activities ranging between 0.56 mg/mL and 3.43 mg/mL, levels that were 1.2 to 7.29 times less effective than acarbose. All other extracts exerted inhibitory α-glucosidase activities ranging between 5.46 mg/mL and 16.8 mg/mL, values 26 to 80 times less effective than acarbose (0.21 mg/mL).

Idoudi et al. (2023) tested the antidiabetic activity of the different parts (flowers, leaves, and tubers) of *Scorzonera undulata* using anti-alpha-glucosidase and anti-alpha-amylase essays, using acarbose (50 µg/mL) as a reference. The results showed that the best inhibitory results of α-glucosidase were those of the aqueous flower extract obtained by ultrasound, with the percentage ranging from 0% to 9.77%, while for the α-amylase the inhibition percentage of the aqueous tuber extract obtained by maceration (50 µg/L) was the highest and ranged from 0% to 31.34% [15].

Spearman's correlation coefficients between TPC, TFC, and TTC, on one hand, and α-amylase inhibitory activity and α-glucosidase inhibitory activity, on the other, are shown in Table 5.

**Table 5.** Correlations between total phenolic, total flavonoid, and total terpene content, on one hand, and antidiabetic activity (α-amylase and α-glucosidase) on the other.

| | | α-Amylase Inhibitory Activity | α-Glucosidase Inhibitory Activity |
|---|---|---|---|
| Total Phenol content | Correlation coefficient | 0.800 | 0.900 * |
| | *p* value | 0.104 | 0.037 |
| | N | 5 | 5 |
| Total Flavonoid content | Correlation coefficient | −0.400 | −0.500 |
| | *p* value | 0.505 | 0.391 |
| | N | 5 | 5 |
| Total Terpene content | Correlation coefficient | 0.600 | 1.000 ** |
| | *p* value | 0.285 | 0.112 |
| | N | 5 | 5 |

*: correlation is significant at the 0.05 level (two-tailed); **: correlation is also significant at the 0.01 level (two-tailed).

Significant strong positive correlations were observed between TPC and TTC, on one hand, and α-glucosidase inhibitory activity, on the other (r = 0.900, $p$ = 0.037 and

r = 1.000, $p$ = 0.112, respectively). These significant correlations could be explained by the presence of phenolic compounds and terpenoids that play a potential role in α-glucosidase inhibitory activities.

Our findings are consistent with previous studies. Loizzo et al. [34] studied 27 extracts from nine Lebanese medicinal plants to identify their phytochemical profile and determine their inhibitory activity against α-amylase and α-glucosidase enzymes. This study shows that TTC has significant inhibitory effects on α-amylase and α-glucosidase enzymes that are attributed to the presence of monoterpenes, sequisterpene, steroids, triterpenoids, and fatty acid extracts. In addition, Russo et al. showed that extracts rich in terpenes, flavonoids, and phenolics possessed significant antidiabetic activities against both α-amylase and α-glucosidase enzymes, with further analysis on a methanolic extract from yacon leaves showing that the concentrations of chlorogenic acid, quinic acid, and caffeic acid are directly correlated with α-amylase and α-glucosidase inhibitory activity [12].

Many studies have been conducted to isolate and identify the phytochemicals that are responsible for the antidiabetic activity of different medicinal plants. In a study performed by Narita et al. [35] on different *Helichrysum* species, the authors identified chlorogenic acid as an active metabolite against α-amylase and α-glucosidase enzymes. In addition, hydroalcoholic extracts from two *Juniperus* plants rich in coumarins, sterols, terpenes, and ligannas were shown to play an antidiabetic role mainly by inhibiting the α-glucosidase enzyme [36]. The phytochemicals chlorogenic acid, caffeic acid, ferulic acid, syringic acid, apigenin, apigenin-7-glucoside, and hesperidin, alongside luteolin, naringenin, quercetin, and resveratrol were reported to be present in different *Scorzonera* species [37]. The observed inhibitory effect of *Scorzonera phaeopappa* extracts could, therefore, be related to the presence of phytochemicals such as chlorogenic acid, rutin, hyperoside, apigenin-7-glucosidase, and sequestiterpenes.

## 3. Conclusions

The screening of different extracts from the leaves of *Scorzonera phaeopappa* for total phenolic, total flavonoid, and total terpene content estimation and antidiabetic and antioxidant role assessment was performed. Acetone was found to have the highest extraction capacity. In addition, acetone, together with EtOH extracts, was found to exhibit the highest antidiabetic activity using α-amylase and α-glucosidase inhibitory assays, noting that acetone extract was a more effective α-amylase inhibitor than acarobse. MeOH extract, on the other hand, exhibited the highest antioxidant activity, as determined by DPPH free radical scavenging and $Fe^{2+}$ ion chelating assays. Moreover, total flavonoids were found to be strongly correlated with antioxidant and antidiabetic activities.

The present findings can serve as a building block for future research studies, which should aim to validate the antidiabetic role of the total phenolic and total terpene content using in vivo methods and perform a bio-guided fractionation in order to determine the bioactive molecules that are responsible for this activity.

## 4. Materials and Methods

### *4.1. Plant Material*

Leaves of *Scorzonera phaeopappa* Boiss were collected in May 2017 from Ayta al foukhar, a village located in the Bekaa Valley in the Rachaya region of Lebanon (altitude 1600 m). The plant was identified by Dr. Tanos Hage, Associate Professor of Plant Biochemistry and Molecular Biology at Notre Dame University (NDU), main campus, Lebanon. The voucher specimen was deposited in the NDU herbarium, Shouf campus, Lebanon.

### *4.2. Chemicals*

Ethanol, methanol, acetone, dichloromethane, ammonia, Folin–Ciocalteu's phenol reagent, sodium carbonate ($Na_2CO_3$), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, quercetin, ethylene-diamine-tetra-acetic acid (EDTA), 3-[2-pyridyl]-5, 6-diphenyl-1,2,4-triazine-4,4′-disulfonic acid monosodium salt hydrate (Ferrozine), sodium

nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), α-amylase and α-glucosidase enzymes, potassium tartrate, sodium phosphate, sodium chloride, 4-nitrophenyl-β-D-glucopyranoside (P-NPG), chloroform, sulfuric acid, linalool, starch and acarbose were purchased from Sigma-Aldrich Co (Steinheim, Germany). $FeSO_4 \cdot 7H_2O$ was purchased from Merck (Darmstadt, Germany). Ultra-pure water was used for the ferrous ion chelating assay. All other reagents and organic solvents used were of analytical grade.

### *4.3. Extraction Methods*

The leaves of the plant were shade dried and pulverized using an electric blender. The methanol (MeOH), ethanol (EtOH), acetone, and dichloromethane (DCM) extracts were prepared as follows: 20 g of plant powder were macerated in 200 mL of each of the four different solvents (MeOH, EtOH, acetone, and DCM) under constant magnetic agitation for 24 h at 25 °C. The mixture was then filtered and the solvent eliminated under reduced pressure at 40 °C using a rotary evaporator (IKA RV 10 BASIC, Boutersem, Belgium).

The dichloromethane pretreated with ammonium extract (DCM-a) was prepared as follows: 20 g of plant powder was moistened with ammonium ($NH_4OH$) solution for 2 h, followed by the addition of 200 mL of DCM. The mixture was then macerated under constant magnetic stirring for 24 h at 25 °C, filtered, and the solvent was eliminated under reduced pressure at 40 °C using a rotary evaporator (IKA RV 10 BASIC, Boutersem, Belgium). The obtained extracts were then kept in dark jars at 25 °C.

All the extracts were lyophilized twice in order to remove any remaining extracting solvent and they were later tested for residues, with none containing any detectable extracting solvent.

### *4.4. Total Phenolic Content for Leaves Extracts*

Total phenolic content in the extracts was assessed by the modified Folin–Ciocalteu method. In a typical experiment, 0.5 mL of the sample solution, prepared by dissolving the extract in EtOH, was mixed with 0.5 mL of 1N Folin–Ciocalteu reagent. The mixture was allowed to stand for 3 min, after which 1 mL of 20% sodium carbonate ($Na_2CO_3$) was added. Samples were left in the dark at room temperature for 45 min, then centrifuged at 2400× *g* for 5 min. The absorbance of the supernatants was measured at 730 nm using a Jenway 6405 UV/Vis spectrophotometer (Essex, United Kingdom). Total phenol content was expressed as mg of gallic acid equivalents (GAE) per 100 mg of extract. All measurements were performed in duplicates.

### *4.5. Total Flavonoid Content*

Total flavonoid content was assessed by the modified aluminum chloride method [38]. For that, 1.5 mL of the sample solution, prepared by dissolving the extract in EtOH (3 mg/mL for the acetone, EtOH, DCM-a, and DCM extracts and 1 mg/mL for the MeOH extract) was mixed with 0.75 mL of 5% sodium nitrite ($NaNO_2$) and 0.15 mL of aluminum chloride ($AlCl_3$). The mixture was left to stand for 5 min, after which 0.5 mL of 1 M sodium hydroxide (NaOH) was added and the volume was increased to 5 mL with distilled water. The absorbance of the mixture was measured at 510 nm using a Jenway 6405 UV/Vis spectrophotometer. Total flavonoid content was expressed as mg of quercetin equivalents (QE) per 100 mg of extract. All measurements were performed in duplicates.

### *4.6. Total Terpenes Content*

To determine the total terpene content of the plant extracts, two tests were performed. First, for terpenoids screening, the Salkowski test was carried out. Then, 5 mL of each extract was mixed with 2 mL of chloroform and 3 mL of 98% concentrated sulfuric acid ($H_2SO_4$) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids [39].

After that, the total terpenoid content of the plant extracts was assessed. For that, linalool was used as standard. An aliquot of the reaction mixture obtained after the

Salkowski test was transferred into a colorimetric cuvette. The absorbance was measured at 538 nm against 95% (*v*/*v*) MeOH as blank using a Jenway 6405 UV/Vis spectrophotometer. For the standard curve, 200 µL of linalool solution in MeOH was added to 1.5 mL chloroform and serial dilutions (100 µg/200 µL to 1 mg/200 µL of linalool) were prepared in which total volume was increased to 200 µL with the addition of 95% (*v*/*v*) MeOH. The calibration curve of linalool was plotted and the total terpenoids content was expressed as milligrams of linalool equivalents per gram of dry weight (mg LE/g DW) and was determined using the regression equation. Samples were analyzed in duplicates.

### *4.7. DPPH Radical Scavenging Assay*

The scavenging effects of the extracts for the DPPH radical were determined by using the method of Yen and Chen with minor modifications [40]. Serial dilutions of the extracts were prepared in EtOH. The basic procedure consisted of the addition of an aliquot (1 mL) of the extract solution to 1 mL of 0.15 mM DPPH in EtOH solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance was read at 517 nm using a Jenway 6405 UV/Vis spectrophotometer and the calculations of the scavenging activity (SA (%)) were performed as follows: SA (%): $[1 - (A_{sample} - A_{sample\ blank})/A_{control}] \times 100$. A total of 1 mL sample solution and 1 mL EtOH was used as the sample blank, whilst 1 mL DPPH solution and 1 mL EtOH was used as the negative control. Ascorbic acid was used as the positive control. A stock solution of ascorbic acid (0.8 mg/mL) was diluted with EtOH to obtain concentrations ranging from 1.5 µg/mL to 20 µg/mL. All measurements were performed in duplicates or triplicates.

### *4.8. Ferrous Ion Chelating Assay*

The ferrous ion chelating activity was determined according to Lim et al. [41]. Equal volumes of 0.12 mM ferrous sulfate ($FeSO_4$) solution, extract solution at different concentration (0.06 mg/mL and 0.08 mg/mL for MeOH extract, 0.10 and 0.15 for EtOH extract, 0.10 mg/mL and 0.5 mg/mL for acetone extract, 0.10 mg/mL and 0.15 mg/mL for DCM extract, 0.15 mg/mL and 0.20 mg/mL for DCM-a extract), and 0.6 mM ferrozine solution were mixed. The mixtures were allowed to stand for 10 min at room temperature, and the absorbance of the $Fe^{2+}$–ferrozine complex was measured at 562 nm using a Jenway 6405 UV/Vis spectrophotometer. A solution containing ultra-pure water, ferrous sulfate, and ferrozine, instead of the sample solution, was used as a negative control ($A_o$). A solution containing ultra-pure water, sample solution, and ferrous sulfate, instead of the ferrozine solution, was used as sample blank ($A_s$). Ethylenediaminetetracetic acid disodium salt (EDTA-$Na_2$) was used as the positive control. The ability of the sample to chelate ferrous ions was calculated with the following formula: % chelating activity = $A_o - ((A_s - A_{test})/A_o) \times 100$. All measurements were performed in duplicates.

### *4.9. α-Amylase Inhibitory Activity*

The α-amylase inhibitory activity of extracts were carried out according to the standard method with minor modifications [42]. In a 96-well plate, a reaction mixture containing 50 µL phosphate buffer (100 mM, pH = 6.8), 10 µL α-amylase (2 U/mL), and 20 µL of varying concentrations of the extracts (0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/mL) was pre-incubated at 37 °C for 20 min. Then, 20 µL of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and the mixture was incubated at 37 °C for 30 min. Following the incubation for 30 min, 100 µL of the 3,5-dinitrosalicylic acid (DNS) color reagent was added to the mixture, which was then boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a multiplate ELISA reader. Acarbose at various concentrations (0.1–0.5 mg/mL) was used as the positive standard. A blank was prepared with 100% enzyme activity and 200 µL of phosphate buffer was used instead of the sample extract; another blank was prepared for each extract following similar steps without adding the enzyme. Each experiment was performed in triplicates. The results were expressed as percentage of inhibition, which was calculated with the following formula:

inhibitory activity (%) = (1 − As/Ac) × 100 where As is the absorbance in the presence of the test substance and Ac is the absorbance of the positive standard.

### *4.10. α-Glucosidase Inhibitory Activity*

The α-glucosidase inhibitory activity of the extracts was carried out according to the standard method with minor modifications [43]. In a 96-well plate, a reaction mixture containing 50 µL phosphate buffer (100 mM, pH = 6.8), 10 µL α-glucosidase (1 U/mL), and 20 µL of varying concentrations of extract (0.4, 0.6, 0.8, 1, 2, 2.5, 3, 3.5, and 4 mg/mL) was pre-incubated at 37 °C for 15 min. Then, 20 µL of 4-nitrophenyl-β-D- glucopyranoside (P-NPG) (5 mM) was added as a substrate and the mixture was incubated at 37 °C for 20 min. After incubation, the reaction was stopped by adding 50 µL of sodium carbonate ($Na_2CO_3$ 0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a multiplate ELISA reader. Acarbose at various concentrations (0.1–0.5 mg/mL) was used as the positive standard. A blank was prepared with 100% enzyme activity without adding the extract, and another blank was prepared for each extract without adding the enzyme; each experiment was performed in triplicates. The results were expressed as percentage of inhibition, which was calculated with the following formula: inhibitory activity (%) = (1 − As/Ac) × 100 where As is the absorbance in the presence of the test substance and Ac is the absorbance of the positive standard.

### *4.11. Statistical Analyses*

Descriptive and correlation analyses of the data were performed. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software version 23.

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