CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTI-DIABETIC ACTIVITIES OF SCORZONERA PHAEOPAPPA BOISS

A Thesis

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Master of Science in Food Safety & Quality Management

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by

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Acknowledgment

I would like to express my sincere gratitude for Dr Leina Hosry for her patience, dedication, encouragements and her insights to this research. I would also like to thank Dr Joclyne Bou Mosleh , Dr elias BOu Maroun and Dr Micheal Afram for their support, comments and encouragement during the study and a thank you for Mr Khaled Sleem in collecting plant materials. An appreciation for Stuff and Colleagues at Notre Dae university-Louaize for their support and time. Finally this work won't be completed without the understanding, support and encouragement I received from my husband and family.

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List of abbreviations

2,2’ –azino-bis(3-3thylbenzothiazoline-6-sulphonic acid …… ABTS

Column chromatography ……..…….. CG

Cupric ion reducing antioxidant capacity ……..……... CUPRAC

Dimethyl-4-phenylenediamine …….……DMPD

Diphenyl picrylhydrazyl assay…….. DPPH assay

Ferric reducing antioxidant power assay …….…… FRAP assay

Flame ionization detector ……... FID

Gas chromatography …….. GC

High pressure liquid chromatography……. HPLC

Methanol ……..….. MeOH

Phosphomolybdenum – reducing antioxidant power ……... PRAP

Saturated fatty acids ……..……... SFA

Superoxide radical scavenging assay ……..…… …SRS assay

Unsaturated fatty acids ……..……..UFA

Dry weight……. DW

Total Phenol content……TPC

Total terpene content…… TTC

Total flavonoid content …… TFC

Dichloromethane+amounia …….. DCMa

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Abstract

Wild edible plants have attracted an increasing interest from researchers as they represent important inexpensive sources of nutrients, minerals, antioxidants and vitamins, as well as natural treatments for diseases. The genus Scorzonera includes about 170 species distributed worldwide, many species of this genus are either consumed as raw vegetables or cooked. In addition to their edible properties, some of the species have been used in traditional medicine for various purposes. Nine species of the genus Scorzonera are found in Lebanon, with one of the nine species, *Scorzonera Pheopappa* Boiss, found in Lebanon and used (its leaves) in in the Lebanese cuisine. Oxidative stress has been implicated in various pathological conditions including diabetes. The presence or intake of antioxidants protect the human body from oxidative stress. The aim of this study is to determine the total phenol, total terpene and total flavonoid contents, and antioxidant and anti-diabetic activities of *Scorzonera Phaeopappa* Boiss. Using dichloromethane, dichloromethane (pretreated with NH_4OH), methanol acetone and ethanol, extracts were prepared from the edible leaves of the plant. The extracts were assessed for their total phenolic content using Folin-Ciocalteu method, total terpene content using Salkwoski test and total flavonoids content using aluminum chloride method. The antioxidant activity and the anti-diabetic activity were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging/ Fe^{2+}-chelating assays and alpha-amylase/ alpha-glucosidase inhibitory assays, respectively, acarbose, a pharmaceutical drug used for treatment of diabetes, was used as standard reference. Dichloromethane (pretreated with NH_4OH) was found to have

the highest extraction capacity on phenols (2.73 mg Gallic acid equivalent /100 mg leave extract) and terpenes (232.42 mg linalool equivalent/100mg leave extract)methanol was found to have the highest extraction capacity on total flavonoids (63.05 mg Quercetin equivalent/100mg leave extract). In addition, the methanol extract exhibited the highest DPPH scavenging activity (IC50 0.07 mg/mL) and the highest chelating activity compared to other extracts (0.08mg/ml, chelating activity 50%). Acetone extract (0.21mg/ml) was two times more active than acarbose (0.47mg/ml) against alpha amylase enzyme and was the most active against alpha glucosidase (6.3mg/ml). Significant positive strong correlations were observed between total phenol content and alpha glucosidase inhibitory assay (r: 0.900 p: 0.037) and total terpene content and alpha glucosidase inhibitory assay (r: 1.000 p>0.0001). Further investigation for the anti-diabetic activity should be performed in order to identify and isolate the bioactive metabolites responsible for the activity.

Keywords: *Scorzonera Phaeopappa* Boiss, antioxidant activity, phenolic content, terpene56s content, flavonoids content, DPPH scavenging, chelating activity, anti-diabetic activity, alpha-amylase, alpha-glucosidase.

Chapter I

I.1 Introduction

Asteraceae, also known as composite, is one of the largest plant families. It is composed of around 1302 genera and 25,000 species (Adams, 1963). This family belongs to the Asterales order and is divided into two subfamilies: the Asteroideae and Cichorioideae (Adams, 1963). The Asteraceae family includes herbs, shrubs, trees, epiphytes, vines, and succulents. This family is characterized by having a daisy lookalike flower (Adams, 1963).

The genus Scorzonera L. belongs to the Cichorioideae subfamily, and is distributed in Africa, Europe, Asia, as well as the east Mediterranean region. It is a genus of perennial herbs, shrubs, edible leaves, phyllaries, seeds that could come with or without hollow pedicels (M.Nourozi et al., 2016). It encompasses around 160 -170 species, 9 of which were found in lebanon, and they include: *Scorzoner Capitata, Scorzonera* cana *(C.A .Mey)*O.Hoffm, *ScorzoneraJaccuiniana(W.Koch.)*Boiss, *Scorzonera libanotica Boiss, Scorzonera mack meliana* Boiss, *Scorzonera Phaeopappa* Boiss, *Scorzonera rigida* Aucher,*Scorzonera papposa* DC and *Scorzonera mollis* M. Bieb (G. Tohme and H, Tohme. 2014).

The most common one is *Scorzonera Hispanacia* known as black salsify (Fig1). The flower is a single bisexual ligulate yellow or pink-violet placed at the tips of the stem and its collateral branches. The roots are many headed or tuberously thickened; leaves

are alternate, narrowly linear to ovate-lanceolate (fig1), they are edible and safe (M.Nourozi et al., 2016).

Figure 1: Example of *Scorzonera Hispinacia* plant (supplier: Alpeflora, France)

I.2 *Botanical description and distribution of the species Scorzonera Phaeopappa Boiss*

Family: Asteraceae

Genus*: Scorzonera*

Species: *Scorzonera Phaeopappa* Boiss

Common synonyomus name*: podospermum phaeopappa*

Arabic name: Al meshe, المشي

Scorzonera Phaeopappa Boiss is distributed in the eastern lebanese cities and villages of Baalbak, Brietel, Rachya, Yanta, Mimes, Aayta from where the plant material for the present work was collected. It could also be found under Pinus,Quercus scub and calcaeuros slope.(Mouterde, 1986)

Scorzonera phaeopappa Boiss is a short, caulescent perennial, and glabrous plant. It has a thick cylindrical and vertical rootstock with a simple stem of 20-40cm height.(G. Tohme and H, Tohme 2014; Mouterde 1986). Its Leaves are linear more or less dilated at the base; the size of the leaves is 7-15*0.3-0.5cm; the lamina is arachnoid to glabrous, with a 1-4cm petiole, expanded at base and amplexicaul; the margin of the leaves are either plain or undulated.(G. Tohme and H, Tohme 2014; Mouterde 1986) .The stem holds 1-3 caputila 20-40 cm long with a diameter of 3-4 cm that distinguishes it from its variety *scorzonera phaeopappa* minor Boiss (diameter of 2cm) .(G. Tohme and H, Tohme 2014; Mouterde 1986) . The flower of *Scorzonera phaeopappa* Boiss is pinkish-mauve to purple with a long peduncle and its Achenes is a small dry, one seeded fruit that doesn't open to release the seed, it is of 18*1.5 mm, narrowly cylindrical, faintly ridged, smooth transevrsely lamellate-rugulose, glabours; pappus yelowish-white;and plumose hairs with monomorphic barbellate ends. *Scorzonera phaeopappa* Boiss flowering season is between April and May.(G. Tohme and H, Tohme 2014; Mouterde 1986)

Fig2: Example of *Scorzonera Phaeopappa* Boiss (G. Tohme and H, Tohme 2014)

I.3 Phytochemical constituents of genus *Scorzonera*

The abundance of the genus *Scoroznera*, its edibility and its uses in traditional medicine have attracted the interest of researchers to explore its active secondary metabolites . All parts of the species underwent chemical analysis; different secondary metabolites were extracted and identified depending on the studied parts. According to the literature, the most common extracted metabolites are: benzylphthalides, coumarins, flavonoids, lignans, kava lactones, phenolic acids, quinic acid derivatives and caffeic acid derivatives, sesquiterpenoids, stilbenes, sterols and triterpenoids (fig3).

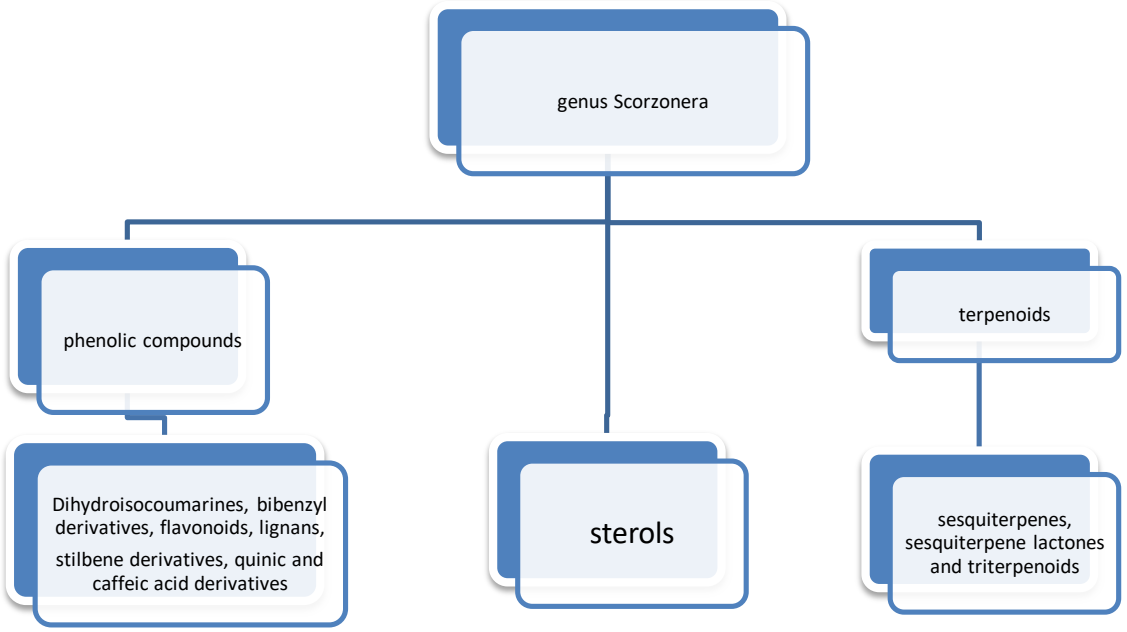


Fig3: major chemical constituents of the genus Scorzonera reported from different species.

I.3.1 Phenolic compounds

Phenolic compounds are secondary metabolites produced by the plant. They contain a benzyl ring, with one or more hydroxyl groups and range from simple phenols to highly polymerized compounds; including flavonoids, phenolic acids, lignans, stilbenes, tannins. These compounds are responsible for plant's organoleptic characteristics such as color and taste they are also known to have a high antioxidant properties (Lin et al., 2016).

I.3.1.1 Flavonoids

Flavonoids are phenolic compounds that have the C6–C3–C6 (Ring A, C, and B) general structural backbone in which the two C6 units are of phenolic nature (fig.4). Due to various substitutions of the C ring, flavonoids can be further divided into sub-groups such as flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids (Gülçin, 2012). Flavonoids are usually associated with a sugar moiety, and are commonly found in nature as glycones but when processed they form aglycones (Liu, 2004). Flavonoids are known to possess a broad spectrum of biological activities; their regular consumption is associated with high antioxidant potency, antibacterial, and antimicrobial activities and with a reduced risk of chronic diseases including cancer, cardiovascular disease and neurodegenerative disorders (kozlowska et al., 2014, Yao et al., 2004). Benzylphthalides and Bibenzyl derivatives belong to flavonoids and are a rare group of natural phytochemicals (Aynur, 2010).Some flavonoids that were isolated from *Scorzonera* species is summarized in table 1.

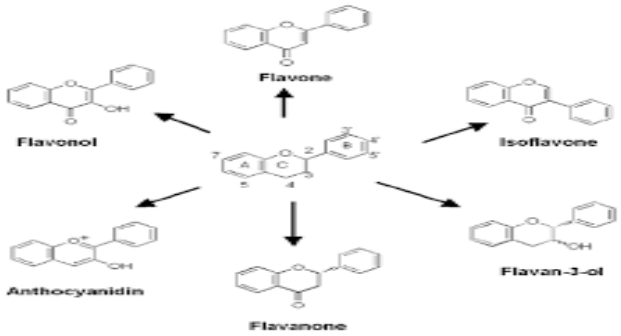
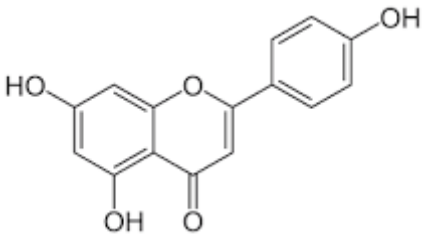
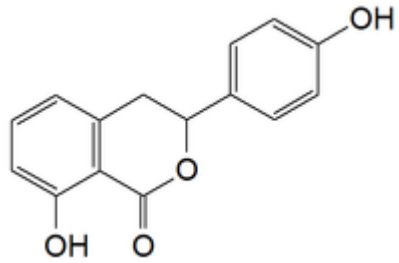
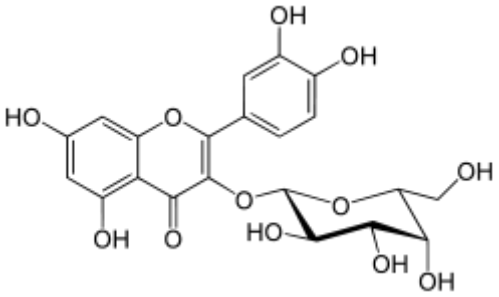
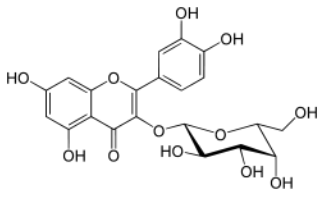
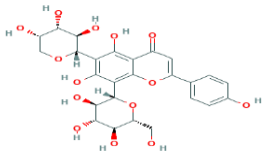
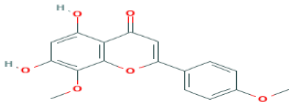
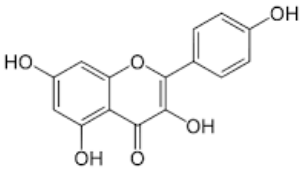
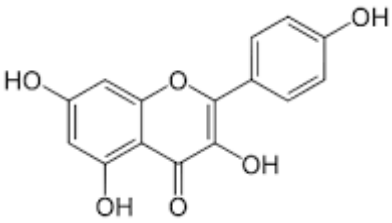
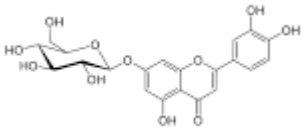


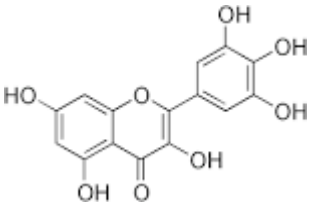
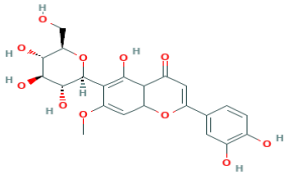
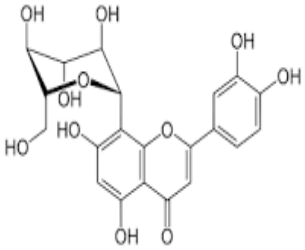
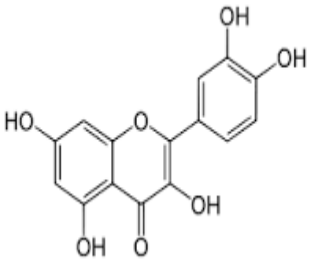
Fig4: Flavonoid structures (liu, 2004)

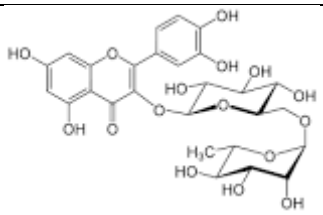
Table 1: Flavonoids from several *Scorzonera* species.

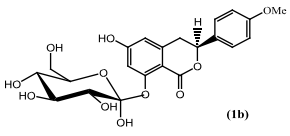
Phytoconstituents	Species	Reference
Apeginin 	*S. austriaca Willd. var. angustifolia; S. crispatula Boiss; S. graminifolia L.; S. hirsuta L.; S. hispanica L.; S. laciniata L.; S. mollis M.Bieb.; S. pseudolanata Grossb.; S. pusilla Pall; S. alexandrina*	Abd El Raheim. M. Donia,2013; Erden et al., 2013
Hydrangenol-8-O-glucoside 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
Hyperoside	*S. acuminate Boiss., S. argyria Boiss., S. aucherana DC., S. boissieri Lipschitz, S. cana (C.A.*	Senol et al., 2014, and Akkol et al., 2012, Acikara et al.,2013; Acikara et al.,2015

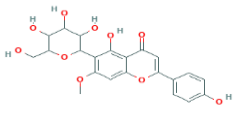
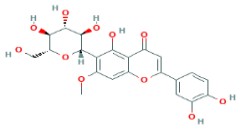
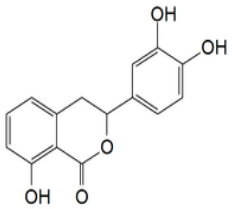
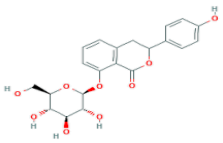
	*Meyer) Hoffm. var. alpina (Boiss.)Chamberlain, S. cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamberlain, S. cana (C.A. Meyer) Hoffm. var.radicosa (Boiss.) Chamberlain, S. sericea DC., S. cinerea Boiss., S. elata Boiss., S. ekimi A. Duran, S. eriophora DC., S. gokcheoglui O.U¨nal & R.S. Go¨ktu¨rk, S. incisa DC.,S. kotschyi Boiss., S. lacera Boiss. &Bal., S. laciniata L.subsp. laciniata, S. latifolia (Fisch. & Mey.) DC., S. mirabilis Lipschitz, S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain, S. parviflora Jacq., S. pisidica Hub-Mor., S.pseudolanata Grossh., S. suberosa C. Koch subsp. suberosa, S. suberosa C. Koch subsp. cariensis (Boiss.) Chamberlain, S.sublanata Lipschitz, S. tomentosa L. S. sublanta, S. cinera*	
Isoorientin 	*S. papposa, S. judaica*	Millela et al., 2013 and Bader et al.,2011
Isoschaftoside	*S. papposa, S. judaica*	Millela et al., 2013 and Bader et

		al., 2011
Galangustin 	*Scorzonera undulata spp deliciosa*	Harakti et al., 2013
Kaempferol 	*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*	Erden et al., 2013
Luteolin 	*S. alexandrina ;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*	Abd El Raheim. M. Donia,2013; Erden et al.,2013
Luteolin-7-glucoside 	*S. claciniata ssp. Laciniata, S. parviflora, S. incisa, S.eriophora, S.cinerea, S.cana var.radicosa, S.cana var.jacquiniana, S.acuminata S. alexandrina*	Akkol et al., 2011, Abd El Raheim. M. Donia,2013, Akkol et al.,2012

Myricetin 	*S. suberosa, S. laciniata and S. latifolia*	Yavuz Erden and Sevda Kırbag, 2013
7-O-methylisoorientin 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, S. parviflora*	Acikara et al. 2015
Orientin 	*S. papposa , S. judaica*	Millela et al.2013 andBader et al., 2011
Quercetin 	*S. suberosa, S. laciniata ,S. latifolia ;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*	Yavuz Erden and Sevda Kırbag, 2013; Erden et al., 2015
Rutin	*S. suberosa, S. laciniata and S.*	Senol et al 2014, Akkol etal

	*latifolia S.acuminataBoiss., S. argyria Boiss., S. aucherana DC., S. boissieri Lipschitz, S. cana (C.A. Meyer) Hoffm. var. alpina (Boiss.)Chamberlain, S. cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamberlain, S. cana (C.A. Meyer) Hoffm. var.radicosa (Boiss.)Chamberlain, S. sericea DC., S. cinerea Boiss., S. elata Boiss., S. ekimi A. Duran, S. eriophora DC.,S. gokcheoglui O.U¨ nal & R.S. Go¨ktu¨rk, S. incisa DC.,S. kotschyi Boiss., S. lacera Boiss. &Bal., S.laciniata L.subsp. laciniata, S. mirabilis Lipschitz, S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain,S. parviflora Jacq., S. pisidica Hub-Mor., S. pseudolanata Grossh., S. suberosa C. Koch subsp. suberosa, S. suberosa C. Koch subsp. cariensis (Boiss.) Chamberlain, S. sublanata Lipschitz,S. Incisa, S. tomentosa L.*	2011
Scorzotomentosin-4-O-b-glucoside (dihydrocoummarin)	*S. acuminata Boiss., S. argyria Boiss., S. aucherana DC., S. boissieri Lipschitz, S. cana (C.A. Meyer) Hoffm. var. alpina (Boiss.) Chamberlain, S. cana (C.A. Meyer) Hoffm. var. jacquiniana*	Senol et al., 2014

	(W. Koch) Chamberlain, S. cana (C.A. Meyer) Hoffm. var. radicosa (Boiss.) Chamberlain, S. sericea DC., S. cinerea Boiss., S. elata Boiss., S. ekimi A. Duran, S. eriophora DC., S. gokcheoglui O.U¨nal & R.S. Go¨ktu¨rk, S. incisa DC., S. kotschyi Boiss., S. lacera Boiss. & Bal., S. laciniata L. subsp. laciniata, S. latifolia (Fisch. & Mey.) DC., S. mirabilis Lipschitz, S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain, S. parviflora Jacq., S. pisidica Hub-Mor., S. pseudolanata Grossh., S. suberosa C. Koch subsp. suberosa, S. suberosa C. Koch subsp. cariensis (Boiss.) Chamberlain, S. sublanata Lipschitz, S. tomentosa L.	
Scorzocreticoside I  (1b)	*S.Cretica*	Saeed 2006

Swertisin 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, S. parviflora*	Acikara et al. 2015
Swertiajaponin 	*S. papposa, S. judaica*	Millela et al.2013 and Bader et al.,2011
Thunberginol G, 	*S. papposa, S. judaica*	Millela et al.2013 and Bader et al., 2011
Hydrangenol-8-*O*-*β*-glucoside 	*S. cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamberlain S. latifolia (Fisch. & Mey.) DC.; S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain; S. parviflora Jacq.; S. tomentosa L.*	Özbek, et al., 2017
Scorzoveratrin	*S. veratrifolia.*	Aynur, 2010
Scorzoveratrozit	*S. veratrifolia.*	Aynur, 2010
Tyrolobibenzyl A,D,E & F	*S. humilis L S.Aristata, S.austriaca, S.baetica S. hispanica, S.parviflora, S.purpurea, S.trachysperma,*	Zidorn et al., 2002

	S.rosea	

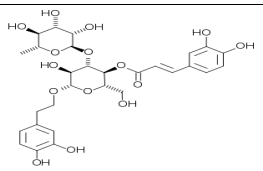
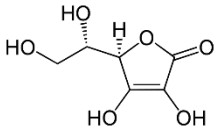
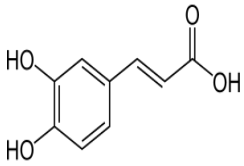
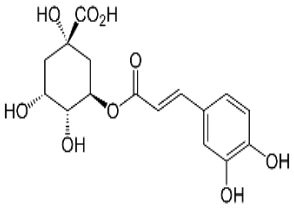
I.3.1.2 Phenolic Acids

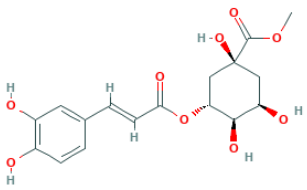
Phenolic acids are present in a variety of plant-based foods, their basic chemical structure is made of a phenol ring attached to it a carboxylic acid (-COOH) (Liu, 2004). Phenolic acids can be divided into two main groups: hydroxycynnamic acids and hydroxybenzoic acid. Hydroxycinnamic acid derivatives are present in bound form in the plant's cell walls; while the Hydroxybenzoic acid derivatives are usually present in bound form in complex structures such as hydrolyzable tannins and lignin (Liu, 2004).

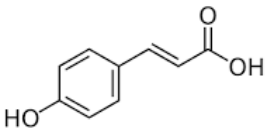
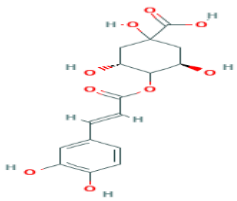
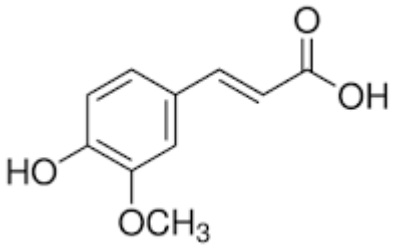
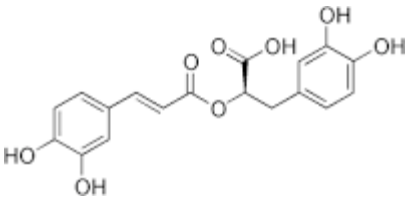
Chlorogenic acid is a hydoxycinnamic acid derivative (Liu, 2004). It is the most extracted phenolic acid from several *Scorzonera* species; several studies showed that it has antioxidant, antinflammatory, antinociceptive, hepatoprotective and neuroprotective activities (Senol et al., 2014; Özbek, *et al.*, 2017; Akkol et al.,2011). Table 2 summarizes phenolic acids and phenolic compounds extracted from several *Scorzonera* species.

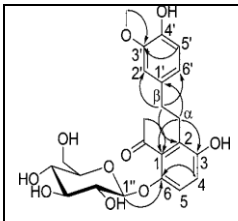
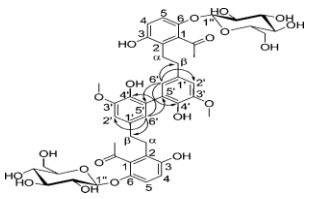
Table 2: phenolic acids and other phenolic compounds extracted from *Scorzonera* species

Phytochemicals	Species	Reference
Acteoside	*Scorzonera undulata spp deliciosa*	Brahim et al., 2013

		
Ascorbic acid (Vit. C not phenolic acid) 	*Scorzonera laciniata*	Zidron et al., 2004
Caffeic acid 	*Scorzonera laciniata, S. tomentosa*	Zidron et al 2004, Ozlem acikara et al.2013
Chlorogenic acid 	*S. acuminate Boiss., S. argyria Boiss., S. aucherana DC., S. boissieri Lipschitz, S. cana (C.A. Meyer) Hoffm. var. alpina (Boiss.)Chamberlain, S. cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamberlain, S. cana (C.A. Meyer) Hoffm. var. radicosa (Boiss.) Chamberlain, S. sericea DC., S. cinerea Boiss., S. elata Boiss., S. ekimi A. Duran, S. eriophora DC., S. gokcheoglui*	Senol et al., 2014; Akkol et al., 2012, Zidorn et al., 2004; Aynur 2010, Zidorn et al., 2002; Acikara et al., 2013;

	O.U¨ nal & R.S. Go¨ktu¨rk, S. incisa DC., S. kotschyi Boiss., S. lacera Boiss. &Bal., S. laciniata L.subsp. laciniata, S. latifolia (Fisch. & Mey.) DC., S. mirabilis Lipschitz, S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain, S. parviflora Jacq., S. pisidica Hub-Mor., S. pseudolanata Grossh., S. suberosa C. Koch subsp. suberosa, S. suberosa C. Koch subsp. cariensis (Boiss.) Chamberlain, S. sublanata Lipschitz, S. tomentosa L., and Scorzonera parviflora, Scorzonera veratrifolia, S.baetica , S.trachysperma, S.rosea	
Chlorogenic acid methyl ester 	*S. veratrifolia;*	Aynur 2010

Coumaric Acid 	*S. Tomentosa*	Ozlem acikara et al., 2013
Cryptochlorogenic acid 	*S.veratrifolia;*	Aynur 2010
Feurlic Acid 	*S. Tomentosa*	Ozlem acikara et al., 2013
Rosmarinic Acid 	*S. Tomentosa*	Ozlem acikara et al., 2013
Scorzoihydrostilbenes A	*S. radiate*	Wang et al.,2009

		
Scorzoihydrostilbenes E 	*S. radiate*	Wang et al.,2009

I.3.1.3 Coumarins and dihydrocoumarins

Coumarins belong to the benzopyrone family .They occur abundantly in fruits and vegetables, such as carrots, parsnip, and celery, and are classified into furanocoumarins, pyranoucoumarins and pyrone-substituted Coumarins (Tiwari et al., 2013). Coumarins that were extracted from *Scorzonera* species are summarized in table 3. Fig.5 shows the basic structure of Coumarin.

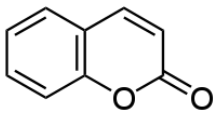
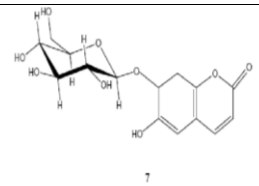
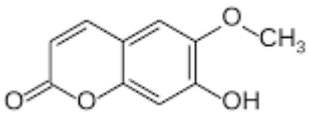
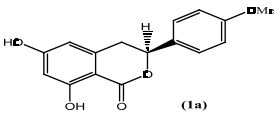
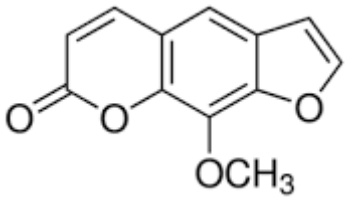


Fig.5 Basic structure of Coumarins

Table3 Coumarins and dihydrocoumarins from *Scorzonera* species

Phytoconstituents	Species	References
Coumarin-o-beta-glycoside	*S. undulata subsp. deliciosa (Guss.) Maire*	Harkati et al., 2010 and Harakati et al., 2013

		
Hyrangenol	*S. Latifolia*	O¨Bahadir Acikara et al., 2011
scorzotomentosin-40-O-glucopyranoside	*S. Latifolia*	O¨Bahadir Acikara et al., 2011
Scopoletin 	*S. Alexandrina*	Abd El Raheim. M. Donia,2013
Scorzocreticin (isocoumarin) 	*S. cretica*	Saeed 2004
scorzotomentosin-4'-*O*-*β*-glucoside	*S. cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamberlain S. latifolia (Fisch. & Mey.) DC.; S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain; S. parviflora Jacq.; S. tomentosa L.*	Özbek, et al., 2017
Xanthotoxin 	*S. alexandrina*	Abd El Raheim. M.Donia,2013
(6-trans-p-coumaroyl)-3-O-b-D-	*S. papposa , S. judaica*	Millela et al.,2013 andBader et al.,

glucopyranosyl-(5-acetyl)-2-deoxy-D-riburonic acid		2011
(6-trans-p-coumaroyl)-3-O-b-D-glucopyranosyl-2-deoxy-D-riburonic acid methyl ester	*S. papposa, S. judaica*	Millela et al., 2013 and Bader et al., 2011

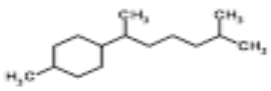
I.3.1.4 Quinic acid and Lignans Derivatives

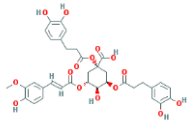
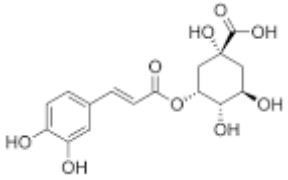
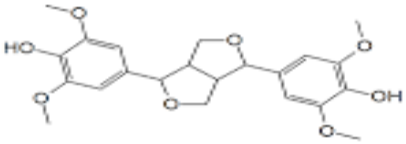
Lignans are phenylpropanoid dimers that are linked by a C-C bond between carbons 8 and 8 "prime". They are divided into several subgroups based on their linkage inside the chain. Lignans are mainly found in 55 different plants that belong to Gymnosperms and Angiosperms (Tiwari et al., 2013).

A novel new quinic acid derivative, called podospermic acid, was isolated from a *S. Lacinata* that has potent antioxidant properties compared to other compounds (zidron et al., 2004).

Table 4 summarizes Quinic and lignans derivatives extracted from several *Scorzonera* species.

Table 4: Quinic acid derivatives from *Scorzonera* Species

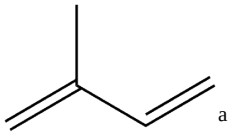
Phytochemicals	Species	Reference
Bisabolane 	*S. hispinacia*	Granica et al.,2014
Feruloylpodospermic acids A and B	*S. divaricate*	Tsevegsuren et al., 2007

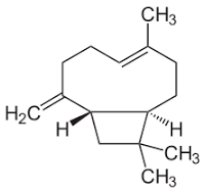
		
Podospermic acid (1,3,5-tridihydrocaffeoylquinic acid), 	*S. Lacinata*	Zidron et al., 2004
Syringaresinol 	*S. hispinacia*	Granica et al.,2014
(–)–1,4-di-O-feruloyl-3-Odihydrocaffeoylquinic Acid	*S. divaricate*	Yang et al., 2012
3,5-O-dicaffeoyl-quinic acid	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, S. parviflora and S.veratrifolia;*	Acikara et al., 2015 and Aynur, 2010
4,5-O-dicaffeoyl-quinic acid	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, S. parviflora and*	Acikara et al., 2015 and Aynur, 2010

	S.veratrifolia;	
(–)–1-O-feruloyl-4-O-dihydrocaffeoylquinic acid	*S. divaricate*	Yang et al., 2012
(–)–3,5-di-O-feruloylquinic Acid	*S. divaricate*	Yang et al., 2012
(–)–1-O-feruloyl-3-O-dihydrocaffeoylquinic acid	*S. divaricate*	Yang et al., 2012
(–)–1-O-feruloyl-5-O-dihydrocaffeoylquinic	*S. divaricate*	Yang et al., 2012

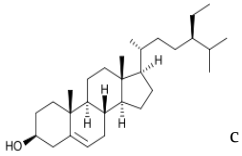
I.3.3 Terpenes

Terpenes originate from turpentine. Turpentine, also known as the rein of pine trees, is the viscous pleasant smell that flows upon cutting new wood of pine trees. Turpentine is made up of hydrocarbons and resin acid and turpentine refers to terpenes (Tiwari et al., 2013). Terpenes are found in thousands of plant species and responsible for several fragrances and flavours. Terpenes are made up of isoperene subunit (fig6,a). Terpenes are the largest group of natural compounds, they are composed around 36000 terpenes phytochemicals. They are classified based on the number of isoprenoid unit in their structure. The largest groups are made up of two (monoterpene), three (sesquiterpene), four (diterpenes), five (sesterpenes), six (Triterpenes), and eight (tetraterpenes) isopernoids units (Tiwari et al., 2013). Known terpenes that were extracted from Scorzonera species belong to Sequestriterpene (Fig. 6, b) and Triterpene (Fig. 6, c) are summarized in below tables.


a



b



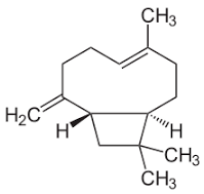
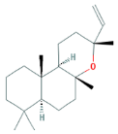
c

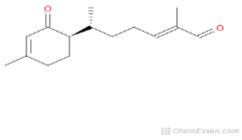
Fig6: a) isoprene unit (Tiwari et al., 2013); b) Sesquiterpene (Ugur et al., 2010); c) Triterpene (Acikara et al., 2015)

I.3.3.1 Sequestriterpene

Two novel sequestriterpene; Biguaiascorzolides A and B have been isolated and identified from *Scorzonera Asturiaca* (zhu et al., 2009). Sequestriterpene that were isolated from *Scorzonera* species are summarized in table 5.

Table 5 Sequestriterpene from *Scorzonera* species

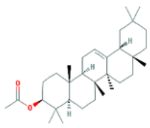
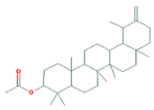
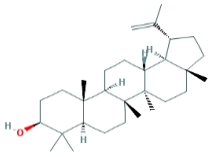
Phytoconstituents	Species	References
Biguaiascorzolides A and B	*S. Austriaca*	Zhu et al., 2008
Caryophyllene 	*S. Sandrasica*	Ugur et al.,2010
C-6 trans-fused a-methylene-c-lactone;	*S. divaricate*	Yang et al., 2016
Manoyl oxide 	*S. Sandrasica*	Ugur et al.,2010
Oxygenated sequestriterpene	*S. Sandrasica*	Ugur et al.,2010
Puliglutone	*S.hipnacia*	Granica et al., 2014

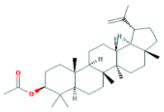
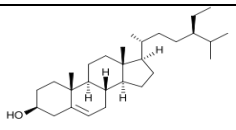
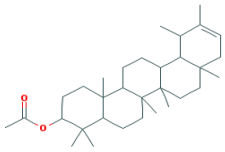
		
Ptilostemonol	*S.hipnacia*	Granica et al.,2014
Sulfoscorzonin D (1) and Sulfoscorzonin E	*S. divaricate*	Quan-Xiang Wu et al., 2018
(6-trans-p-coumaroyl)-3-O-b-D-glucopyranosyl-2-deoxy-D-riburonic Acid	*S. papposa , S. judaica*	Millela et al., 2013 andBader et al., 2011
(6-cis-p-coumaroyl)-3-O-b-D-glucopyranosyl-2-deoxy-D-riburonic acid	*S. papposa, S. judaica*	Millela et al., 2013 and Bader et al., 2011
(6-cis-p-coumaroyl)-3-O-b-D-glucopyranosyl-2-deoxy-D-ribono-c-lactone	*S. papposa, S. judaica*	Millela et al.,2013 and Bader et al.,2011
1-Oxo-bisabola-(2,10E)-diene-12-carboxylic acid methyl Ester	*S.hipnacia*	Granica et al., 2014
1-Oxo-bisabola-(2,10E)-diene-12-ol	*S.hipnacia*	Granica et al.,2014
1-Oxo-bisabola-(2,10E)-diene-12-carboxylic acid Methyl	*S.hipnacia*	Granica et al., 2014
a,b-saturated 6,7-trans-lactone;	*S. divaricate*	Yang et al., 2016
scorzodivaricin A	*S. divaricate*	Yang et al., 2016
Scorzodivaricin D	*S. divaricate*	Yang et al., 2016
Scorzodivaricin	*S. divaricate*	Yang et al., 2016

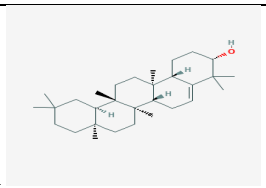
I.3.3.2 Triterpene

Table 6. Summarizes Triterpenes that were isolated from *Scorzonera* species.

Table 6 Triterpene from *Scorzonera* species

Phytochemical	Species	Reference
Beta acetate amyrin 	*S. undulata ssp deliciosa (Guss)*	Harakti et al., 2013
Taraxasterol acetate 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
Lupeol 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
olean-12-en-11-one-3-acetyl	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
3beta-dodecanoyl erythrodiol	*S.mongolica*	Wang et al., 2009
3beta-tetradecanoyl erythrodiol	*S.mongolica*	Wang et al., 2009

Lupeol Acetate; 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
Sitosterol 	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
3-_-hydroxy-fern-8-en-7-one-acetate	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
urs-12-en-11-one-3-acetyl	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
3-_-hydroxy-fern-7-en-6-one-acetate	*S. latifolia, S. cana var. jacquiniana, S. tomentosa, S. mollis ssp. szowitsii, S.eriophora, S. incisa, S. cinerea, and S. parviflora*	Acikara et al., 2015
Taraxasteryl acetate, 	*S. latifolia*	Citoglu et al.,2010
Taraxasteryl myristate	*S. latifolia*	Citoglu et al.,2010
fern-7-en-3-_-ol	*S. latifolia*	Citoglu et al.,2010
fern-7-en-3-_-one	*S. latifolia*	Citoglu et al.,2010
Tirucallane Triterpene;	*S. divaricate*	Yang et al., 2016

oleanolic acid	*S. divaricate*	Yang et al., 2016
3β-acetoxyglutin-5(10)-en-6-oxo.;	*S. austriaca*	Zhu et al., 2010
Glutinol 	*S. austriaca*	Zhu et al., 2010
β-amyrin-3(3′-methylbutanonate),	*S. austriaca*	Zhu et al., 2010
β-amyrin-3-acetyl	*S. austriaca*	Zhu et al., 2010
3β-acetyl-11α, 12α-oxidotaraxerol	*S. austriaca*	Zhu et al., 2010
α-amyrin-3-acetyl	*S. austriaca*	Zhu et al., 2010
α-amyrin-3-acetyl-11-oxo	*S. austriaca*	Zhu et al., 2010
D-friedours-14-en-3β-acetyl-11α,12α-epoxy taraxasterol	*S. austriaca*	Zhu et al., 2010
ψ-taraxasteryl-3 (3′-methyl-butanonate)	*S. austriaca*	Zhu et al., 2010
3-_-hydroxy-fern-7-en-6-one-acetate	*S. Latifolia*	Bahadir Acikara et al., 2011

I.4 Traditional uses of *Scorzonera* species in Folk medicine

Traditionally, different species of Scorzonera have been used in folk medicine. In European countries, they have been used as treatment for pulmonary diseases, colds, wounds as well as for their gastrointestinal, urinary, inflammatory, galactagogue and appetizing effects (Zidron et al., 2000; Zidron et al., 2003). Mongolian traditional medicine used Scorzonera for the treatment of diarrhea, lung edema, parasitic diseases, and fever caused by bacterial, and viral infections (Tsevegsuren et al., 2007; wang et al., 2009). In Indian folk medicine, *Scorzonera divaricata* Turcz and *Scorzonera virgata* DC have been used to treat rheumatism and jaundice respectively (Gairola et al., 2014). In

Turkish folk medicine *Scorzonera semicana* DC was used in the treatment of diabetes mellitus (C.Durmuskahaya & M.Ozturk 2013). Azerbaijan/Iran has approved the use of decoction of the roots of *Scorzonera cinerea* Boiss, in Azerbaijan/Iran as a laxative and its uses was approved by the Urmia drug and food administration (Bahmani et al., 2014). In Lebanon, the decoction of the aerial parts of *Scorzonera Libanotica* Boiss, *Scorzonera cana* (C. A. Mey.), *Scorzonera euphratica* and *Scorzonera phaeopappa* are used to treat headache (Arnold et al., 2015; Baydoun et al., 2015)

I.5 Pharmacological studies on several *Scorzonera* species

Plants are a major source of medicinal compounds; over half of pharmaceutical drugs are made from plant extracts and almost 60% of the people worldwide use plants for the treatment of many diseases (S.Nabi et al 2016, Nasseri MA et al.2014). The importance of *scorzonera* species in folk medicine leads to further investigation for their effective pharmacological application. Several studies have been done on roots, leaves and aerial parts of *Scorzonera* species that demonstrated their biological effects. They were shown to have anti-carcinogenic properties, anti-hypertensive, anti-inflammatory analgesic, antinocecptive, antimicrobial and antifungal, anticholinesterase and anti-tyrosinase activities (*K,Athmouni,* 2015; Yavuz Erden & Sevda Kırbag,2013; Acikara et al., 2013; Senol et al., 2014; Bahdir et al., 2010; Citoglu et al., 2008;).

Two *Scorzonera* species *S. papposa* and *S. cana* (C.A. Meyer) Hoffm that are known to be found in Lebanon have been studied. The roots and leaves of *S. papposa* have been shown to have an antioxidant capacity through radical scavenging activity. This is due to the presence of 9 different phenolic compounds; these compounds act synergistically to

enhance the activity *(*L.Millela *et.al,* 2013*).* Also the roots and aerial parts of *S.cana (C.A. Meyer)* Hoffm were shown to have antinflamatory and antinociceptive activities due to its richness in flavonoids *(*O.B.Acikara *et al.*2013*).*

Table 7 summarizes various pharmacological studies that have been reported for different *Scorzonera* species.

Table 7 summarizes pharmacological studies done on *Scorzonera* species

Species	Identified compound	Phamrmacological use	Reference
S. latifolia	taraxasteryl acetate	Analgesic , Antinocecptive	Bahadır et al. 2010, Citoglu et al., 2008, Yavuz Erden Sevda Kırbag, 2013, Akkol et al.,2011 ; Senol et al., 2014
	total phenolic and flavonoids	Anti microbial, antioxidant	
	chlorogenic acid, hyperoside and luteolin-7-O-glucoside	Wound healing , anti-inflamatory	
	Rutin	Anticholinesterase, anti-TYRO, antioxidant activity	
S.suberosa ssp. Suberosa	NA	Antinocecptive	Citoglu et al., 2008
S. mollis ssp. szowitsii	NA	Antinocecptive	Citoglu et al., 2008; Akkol et al., 2011 ; Acikara et al. 2015 ; Senol et al., 2014
	Total flavonoids; phenolic and tritepenes Hyperioside, chlorogenic acid and Rutin	Wound healing and anti-inflamatory	
		Anticholinesterase, anti-TYRO, and activity	
S. tomentosa	NA	Antinocective	Citoglu et al., 2008
S. Papposa	Dihydrocoumarins	Antioxidant activity	Millela et al., 2013

S., judaica	Phenolic contents	Antioxidant activity	Badder et al.,2011
S. austriaca	biguaiascorzolides A (1)	Anticancer against human erythroleukaemia	Zhu et al., 2008
S. suberosa	Total flavonoids and phenolic acids	Antifungal, antimicrobial and antioxidant	Yavuz Erden Sevda Kırbag, 2013
S. Lacinata	Total flavonoids and phenolic acids Podospermic	Antioxidant and Antimicrobial	Yavuz Erden Sevda Kırbag, 2013; Acikara et al., 2013 ; Zidron et al., 2004
S. sandrasica	Caryophyllene oxide	Antimicrobial	Ugur et al., 2010
S. cinerea	Total flavonoids; chlorogenic acid and triterpenes	Wound healing ,anti-inflamatory,antinocecptive, and antioxidant	Akkol et al.,2011, Akkol et al., 2012; Acikara et al., 2013 ; Acikara et al., 2015 ;
S.incisa	Total flavonoids; chlorogenic acid and triterpenes. Hyperioside, Rutin	Wound healing,anti-inflamatory,antinocecptive and antioxidant Anticholinesterase, anti-TYRO, and antioxidant activity	Akkol et al.,2011; Akkol et al., 2012; Acikara et al., 2013 ; Acikara et al., 2015 ; Senol et al., 2014
S. parviflora	Total flavonoids; chlorogenic acid and triterpenes Hyperioside, Rutin	Wound healing, anti-inflamatory, antinocecptive and antioxidant Anticholinesterase, anti-TYRO, and antioxidant activity	Akkol et al.,2011; Akkol et al., 2012; Acikara et al., 2013 ; Acikara et al. 2015 ; Senol et al., 2014
S. tomentosa	Total flavonoids and phenolic	Wound healing and anti-inflamatory Hepatoprotection against acute hepatotoxicity induced by carbon tetrachloride	Akkol et al.,2011; Özbek, et al., 2017 ; Acikara et al. 2015 ; Citoglu et al., 2008 ; Senol et al., 2014

	Chlorogenic acid ; triterpenes Hyperioside, Rutin	Antinociceptive Anticholinesterase, anti-TYRO, and antioxidant activity	
S. cana var. jacquiniana,	Chlorogenic acid Total phenolic compounds	Anti inflammatory Antioxidant activity	Akkol et al. 2011; Acikara et al., 2013
S.eriophora,	Chlorogenic acid and triterpenes Hyperioside, ruitn	Anti inflammatory and antioxidant Anticholinesterase, anti-TYRO, and antioxidant activity	Akkol et al. 2011; Acikara et al., 2013 ; Acikara et al. 2015, Senol et al., 2014
S. undulata ssp deliciosa (Guss)	Acteoside and galangustin	Anti oxidant	Harakti et al.,2013
S. Alexandrina	Luteolin and luteolin-7-O-glucoside	Hepatoprotective and anti-ulcerogenic effect	Abd El Raheim. M.Donia,2013
S. cana (C.A. Meyer) Hoffrn. var. *alpina*, (Boiss.) Chamb.	Total phenolic compounds Chlorogenic acid, Hyperoside, and rutin	Antioxidant Anticholinesterase, anti-TYRO, and antioxidant activity	Acikara et al., 2013 and Senol et al., 2014
S. cana (C.A. Meyer) Hoffm. var. *radicosa* Erzurum, (Boiss.) Chamb. (SCVR)	Total phenolic compounds Chlorogenic acid, Hyperoside, and rutin	Antioxidant Anticholinesterase, anti-TYRO, and antioxidant activity	Acikara et al., 2013 and Senol et al., 2014
S. cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamberlain.	Chlorogenic acid rutin and hyperioside	Hepatoprotection against acute hepatotoxicity induced by carbon tetrachloride Anticholinesterase, anti-TYRO, and antioxidant activity	Özbek, *et al.*, 2017; Senol et al., 2014
S. latifolia (Fisch. & Mey.) DC	Chlorogenic acid	Hepatoprotection against acute hepatotoxicity induced by carbon tetrachloride	Özbek, *et al.*, 2017

S. mollis Bieb. subsp. szowitsii (DC.) Chamberlain	Chlorogenic acid	Hepatoprotection against acute hepatotoxicity induced by carbon tetrachloride	Özbek, *et al.*, 2017
S. parviflora Jacq	Chlorogenic acid	Hepatoprotection against acute hepatotoxicity induced by carbon tetrachloride	Özbek, *et al.*, 2017
S. divaricate		Antioxidant activity	Tsevegsuren et al., 2007
S. radiate	scorzodihydrostilbenes A–E	Antioxidant activity	Wang et al., 2009
S. acuminate;;	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S.divaricata	Triterpene and sequestriterpenoids Feruloylpodospermic acids A and B (–)–1,4-Di-O-feruloyl-3-O-dihydrocaffeoylquinic Sulfated sesquiterpenoid salt alkaloid	Cytotoxic activities against four human cancer cell lines (HL60, HeLa, HepG2, and SMMC-7721 Antiradical effect against DPPH moderate cytotoxic activity against Hep-G2 cell lines Cytotoxicity Antibacterial activity	Yang et al., 2016; Tsevegsuren et al., 2007 ; Yang et al., 2012 ; Quan-Xiang Wu et al., 2018
	ψ-taraxasteryl-3 (3′-methyl-butanonate); lupeol;(23Z)-cycloart-23-ene-3β, 25-dihydroxy;9β,19-cyclolanostane- 24-en-3-oxo; β-sitosterol; stigmast-4-en-3-one; β-amyrin-3-Acetyl; glutinol	inhibit human tumor HL-60 and BEL-7404 cell lines	Quan-Xiang Wu et al.,2011
S. a rgyrea	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
	chlorogenic acid, hyperoside,	Anticholinesterase, anti-TYRO, and	Senol et al., 2014

S. aucheriana	and rutin	antioxidant activity	
S. boissieri	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. cericea	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. elata	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. ekimii;	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. gokceoglui	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. kotschyi	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. lacera	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. laciniata subsp. Laciniata	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. mirabilis	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. pisid ica	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. pseudolanata;	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. suberosa subsp. Cariensis	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S.sublanata	chlorogenic acid, hyperoside, and rutin	Anticholinesterase, anti-TYRO, and antioxidant activity	Senol et al., 2014
S. hispanacia	syringaresinol and bisabolane	active against myeloma cell lines, colon cancer cell line	S.Granica et al. 2014
S. paradoxa	Fatty acids methyl ester(oleic and arachidonic), total phenolic compound , flavonoid and tanin	Good source of natural antioxidant, And good antidiabetic properties	Nasseri MA et al.2015

S. magnolia	3beta-dodecanoyl erythrodiol (1) and 3beta-tetradecanoyl erythrodiol	Anti tumor Mild cytotoxicity on A-549 cell line	Wang et al.,2009
S. ammophila	Total phenolic compounds, tanin, flavonoids,alkaloids	Antifungal and antibacterial activities	Najb Sabi et al.2016

I.6 Antioxidant studies done on *Scorzonera* species

Antioxidants are naturally occurring or synthetic chemicals in foods that prevent the harmful effects of free radicals and therefore increase the shelf life of foods reversing the process of lipid peroxidation which is the main cause of food deterioration (Halliwell et al., 2009; Gülçin, 2012). Various epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality rate from chronic diseases due to their richness in antioxidant molecules (Gülçin, 2012). The major bioactive compounds responsible for this activity are phenolic compounds and flavonoids (Gülçin, 2012). In this context, several studies have been done on Scorzonera genus that showed its high antioxidant activity.

A study was done on the sub-aerial parts of *S. lacinata* , collected from Italy, . To isolate a new natural polyphenolic compound from *S. lacinata* and to determine its antioxidant activity using DPPH- Radical scavenging activity. As a result, 1,3,5-Tridihydrocaffeoylquinic acid (podospermic acid) was isolated from the methanolic extract of the subaerial parts of *S. lacinata* by silica gel 60 CC using a gradient of CH_2CL_2 and MeOH and two successive Sephadex LH-20 CCs using MeOH as an eluent.

Podospermic acid was shown to have a potent antioxidant capacity compared to chlorogenic acid, ascorbic and caffeic acid (Zidron et al., 2004).

Yang et al. conducted a study on *S. divaricata* roots were collected from China, to determine phytochemical constituents of *S. divaricata* and the antioxidant activity of each of the isolated compounds. The plant materials were extracted using 95% ethanol aqueous medium at 25^{o}C, the isolation was done using silica gel CC followed by RP-HPLC. The antioxidant activity was determined using DPPH-radical scavenging. As a result, seven new quinic acid derivatives were isolated from the roots of *S. divaricata* and all the isolated compounds exhibited strong antioxidant activity against DPPH and ABTS (Yang et al., 2012*)*. Similar results were shown by Tsevegsuren et al. they conducted a study on the aerial parts of *S. divaricate* , that were collected from Mangolia,;two quinic acid derivatives were isolated from the aerial parts by maceration using MeOH at room temperature and showed antioxidant activity against DPPH radical (Tsevegsuren et al., 2007).

In another study done in Mangolia in which the Aerial parts of *S. Radiata* were collected and studied to isolate and evaluate the antioxidant activities of five new natural diyhdrostilbenes derivatives. As a result, the diyhdrostilbenes A-E compounds were isolated and identified using HPLC-DAD and LC-MS, a strong antioxidant activity were shown for all derivative, but diyhdrostilbenes A and E were more active in comparison to the reference group resveratrol (Wang et al., 2009). The plant materials were extracted by maceration using MeOH at room temperature and the antioxidant activity was determined using DPPH- radical scavenger assay.

In 2013, Millela et al. conducted a study on the aerial parts and tuberous roots of *S. papposa* that were collected from Jordan. The aim of the study was to assess the antioxidant activities of the extracted phytochemicals. The plant materials were collected from Jordan and extracted by solvents with increasing polarity n-hexane, $CHCl_3$, $CHCl_3$–MeOH (9:1) and MeOH using exhaustive maceration, then purified using CC, and separated by RP-HPLC. The total phenolic content was determined using the Folin–Ciocalteu method. The antioxidant activities was determined by: Ferric reducing antioxidant power (FRAP) assay, β-Carotene bleaching inhibition assay, DPPH radical-scavenging activity. As a result, fractions that shows positive antioxidant activities on FRAP and BCB undergoes further analysis. The phytochemical analysis of positive fractions yield for the isolation nine compounds from the aerial parts and roots of *S. papposa* of which four compounds were new. The isolated flavonoid compounds, thunberginol G and isooreintin, obtained the highest antioxidant activity in comparison to the other remaining flavonoids and Coumarins compounds. Adding to that, it has been concluded that the antioxidant activity of *S. papposa* extracts and fractions may be due to the presence of a combination of compounds acting synergistically thus enhancing its biological activity. Further investigation of the antioxidant activity of phtalides and dihydroisocoumarins was suggested. (Millela et al., 2013).

In another study done by Harakti et al., on the roots of *S. undulata ssp deliciosa,* that were collected from Eastern Algeria, a new flavonoid, Galngustin, and an acteoside were isolated and both showed a potent antioxidant activity using DPPH and CUPRAC testing. The plant materials were extracted by maceration using CH_2CL_2 and MeOH as solvents

three times during 24 hrs at room temperature, and then the soluble extract was purified and isolated using column chromatography. The antioxidant activity was determined using DPPH radical-scavenging activity and CUPRAC. (Harakti et al., 2013).

In 2013, plant samples from *S. suberosa, S. laciniata and S. latifolia* were collected from the city of Elazig in Turkey to determine their phytochemical composition using HPLC and antioxidant activity using DPPH free radicals. The aerial parts were dissolved in a ratio 1:5 with 80 % aqueous MEOH and then the total polyphenols and flavonoids were measured using Folin–Ciocalteau assay. The results showed that the total extracted polyphenols and flavonoids have potent antioxidant capacity compared to the control group. Additionally, these species were found to be able to detoxify yeast culture and increase cells viability (Yavuz Erden Sevda Kırbag, 2013).

A study was done be Acikara et al., 2013 on the aerial parts and roots *S. cana* (C.A. Meyer) Hoffman. var. *alpina* (Boiss.) Chamberlain. (SCVA), S. *cana* (C.A. Meyer) Hoffman. var. *jacquiniana* (W. Koch) Chamberlain. (SCVJ), *S. cana* (C.A. Meyer) Hoffman. var. *radicosa* (Boiss.) Chamberlain. (SCVR), *S. cinérea* Boiss, *S. eriophora*., *S. incisa* DC. , *S. laciniata* L. ssp. *Laciniata, S. parviflora* Jocq. (SP) collected from different parts of turkey. Extracts were prepared using 80% aqueous MEOH at room temperature they were left to macerate for 3 h by continuous stirring. The isolation of compounds were done using HPLC and the antioxidant activity was determined using Superoxide radical scavenging assay. The results showed that all extracts exhibited significant scavenger activity against Superoxide anion radical. The highest inhibitory

activity was observed with *S. parviflora* root extract. The flavonoids Hyperoside and rutin were found to be in the extracts from the aerial parts and the phenolic acid compound chlorogenic acid was detected in all investigated extracts (Acikara et al., 2013).

Also, a collective study on 27 different Scorzonera species collected from different areas in turkey was done by Senol et al., in 2014. Each plant sample was extracted using 80% MEOH aqueous medium at room temperature for 24 hrs with continuous stirring. The antioxidant activity was determined using DPPH radical scavenging activity, DMPD radical scavenging activity, PRAP, FRAP, NO radical scavenging activity and Metal-chelation capacity by Fe^{2+}-ferrozine test system. The results showed that the aerial parts of some *Scorzonera* species exerted antiradical activity towards DPPH, and high Scavenging properties toward NO radical due to their richness in chlorogenic acid, rutin, and hyperoside, whereas the extracts and compounds tested possessed either no or low to moderate activity in methal-chelation capacity, PRAP, and FRAP (Senol et al., 2014).

In 2015, Nasseri et al., conducted a study on the leaves and roots of *S. paradoxa* that were collected from Iran, to evaluate the amount of fatty acids, total phenols, flavonoids and tannins as well as to evaluate its antioxidant properties. The amount of fatty acids was determined by the methylation of fatty acids and GC-FID; the total phenols by using tannins as a standard; total tannins and total flavonoids content were analyzed using uv-vis spectrophotometry. The antioxidant activity was determined using DPPH-radical scavenger activity. It was concluded that *S. paradoxa* contains a significant amount of UFA and SFA. The most available UFA are oleic and arachidonic acids which were mainly abundant in the leaves. The most available SFA is stearic acid; it is mainly

abundant in the roots as compared to the leaves. The root extract showed lower antioxidant activity than the leaves and this is mainly due to the presence of high amounts of phenolic compounds in the leaves (Nasseri MA et al. 2015).

I.7 Anti diabetic studies done on *Scorzonera* species

According to the WHO, 150 million people have diabetes mellitus world-wide and this number is expected to double by 2025 (WHO, fact sheets). Diabetes mellitus is a set of chronic metabolic diseases characterized by hyperglycemia, resulting from insufficient or inefficient amounts of insulin secretion (WHO, Fact sheets). The alpha amylase enzyme in pancreatic juices breaks down fats and carbohydrates into absorbable molecules (Gupta et al., 2003); on the other hand, the alpha glucosidase enzyme in the small intestine catalyzes the end step of the digestion of starch and disaccharide (Annam et al., 2009). Thus the inhibition of these enzymes has been found as an effective way to lower the level of blood glucose (Russo et al., 2015). Different Scorzonera species have been used in Turkish folk medicine for the treatment of diabetes mellitus (Durmuskahya & Ozturk 2013). Up to our knowledge no studies have been done on the anti-diabetic activity of the genus *Scorzonera.*

I.9 Food recipes for *Scorzonera* species

Scorzonera species are mainly used as a vegetable in Europe as well as in Turkey. *Scorzonera hispanica* L. also known as Black Salsify, Spanish salsify, black oyster plant, is the most recognized species that grows naturally and widely in Europe and has been cultivated since the seventeenth century as a food. The long black roots are boiled,

steamed, baked, batter-fried, put into soups and stews or roasted as a coffee substitute (Tsevegsuren et al., 2007; Wang et al., 2009). In Turkey several Scorzonera species are used as vegetables (Baytop., 1999)

Herein, are some *Scorzonera* recipes :

1- A black salsify with parsely sauce recipe from the 17th century and it is still used until nowadays (New National food art of cooking, 1797) :

- Collect the roots of the black salsify,
- Peel and cut them into small pieces then boil them until they turn red,
- Wash, clean and stew them with water and a piece of butter
- When they are done, add butter with flour, add nutmeg, salt and chopped parsley

Fig7 Black salsify with parsely (New National food art of cooking, 1797)

2- In the rural areas of Lebanon, *Meshe (Scorzonera Pheoppapa* Boiss) is a popular kind of food that is prepared by boiling the leaves of *Scorzonera phaeopappa* and then adding to it some garlic and lemon juice.

3- Black salsify soup recipe:

- Peel the salsify, chop into one inch sections and add water with a squeeze of lemon.
- Roast the garlic, for 20 minutes with olive oil.

- Add the carrots and potato for a couple of minutes. Then, add stocks, bay leaves, thyme, garlic cloves, lentils and salsify.
- Bring to boil and simmer for about 20 minutes or until the salsify is tender. Remove the thyme sprigs and bay leaves and blitz in a blender. Season to taste with salt & pepper.
- Serve with some thyme leaves as garnish.

Fig.8 Soup with black salsify

Based on the literature review presented above, we can now say that the Genus *Scorzonera* is a rich source of phytochemicals, and possesses antioxidant and anti-cholinesterase potentials. Adding to that, *Scorzonera* has been used in traditional medicine as an anti-diabetic agent. But no studies have been conducted on the Genus *Scorozonera* to test its anti-diabetic properties. The species *Scorzonera Pheoppapa* Boiss. has long been used in Lebanese food recipes. However, up to our knowledge this species hasn't been studied yet; therefore, the objective of our work is to determine the flavonoids, Phenolics, and Terpenes contents of *Scorzonera Phaeoppapa* Boiss*;* and to study its possible antioxidant, anti-diabetic, and anti-cholinesterase potentials.

To determine the antioxidant activity of *Scorzonera Phaeoppapa* Boiss; DPPH radical scavenging assay, and the ferrous ion chelating assay will be used. DPPH

radical scavenging is a rapid, simple, highly reproducible, inexpensive; it is widely used method to evaluate the antioxidant activity of phenolic compounds extracted from plants (Russo et al., 205). This method is based on the reduction of ethanol DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H. Adding the extracts to the medium will be able to reduce the stable radical DPPH and changing the color to a yellow colored diphenylpicrylhydrazine (Thangaraj, 2016). The inhibition capacity is directly proportional to the concentration of the antioxidant (Russo et al., 2015). Another method will be used to determine the antioxidant activity is the ferrous ion chelating activity; where Fe^{2+} is a powerful pro-oxidant involved in many oxidative stress related pathways. An effective ferrous ion chelator will prevent or inhibit the oxidation by removing iron from the medium (Auezova et al., 2013).

For the anti-diabetic activity, inhibition activity against α-Glucosidase and α-Amylase enzymes assay will be performed. Since one therapeutic approach for treating diabetes is to decrease post-prandial hyperglycemia. This is done by inhibiting the absorption of glucose through the inhibition of α-amylase and α-glucosidase, in the digestive tract.

Chapter II

1. Introduction

Plants are a major source of medicinal compounds; over half of pharmaceutical drugs are made from plant extracts and almost 60% of the world uses plant herbs for different medicinal disease (Nasseri MA et al.2014). Nowadays, wild edible plants attracts the attention of researchers since they are an important source of food, beverages and natural remedies. People living in the rural areas are in contact with natural sources and have a good knowledge in edible, aromatic and medicinal plants (Milella et al.,2014).

The Genus *Scorzonera*, a member of the Asteracea family, mainly distributed in Asia, Europe, and Northern Africa. Scorzonera includes about 170 species, 9 of which were found to be in lebanon, that includes: *Scorzoner Capitata,Scorzonera cana (C.A .Mey) O.Hoffm; Scorzonera Jaccuiniana(W.Koch.) Boiss; Scorzonera labinotica Boiss; Scorzonera mack meliana Boiss; Scorzonera Phaeopappa Boiss; Scorzonera rigida Aucher; Scorzonera papposa DC* (G. Tohme and H, Tohme. 2014). The abundance of the genus *Scoroznera*, its edibility and its uses in traditional medicine have attracted the interest of researchers to find the active secondary metabolites . All parts of the species underwent chemical analysis; different secondary metabolites were extracted and identified depending on the studied parts. According to the literature, the most common extracted metabolites are: dihydroisocoumarins, stilbenes, lignans, phenolic derivatives (Bader et al., 2011), phtalides (Sariet al., 2007), coumarins, kavalactones (Jiang et al., 2007), sesquiterpenes (Zidorn, 2008), triterpenes (Wang et al., 2007), and flavonoids (Zidorn, 2010).

Scorzonera species are mainly used as a vegetable in Europe as well as in Turkey. Scorzonera hispanica L. also known as Black Salsify, Spanish salsify, black oyster, the young leaves of the plant are used as salads and the roots are consumed as cooked vegetables in the European cuisine (Wang et al., 2009). In Turkey several Scorzonera species have also been conusmed as vegetables (Baytop., 1999).

Moreover different species of Scorzonera have been used in traditional folk medicine; in the European countries they have been used as against pulmonary diseases, colds, for the treatment of wounds as well as for their stomachic, diuretic, galactagogue, antipyretic, and appetizing effects (Zidron et al., 2003); in Mongolian traditional medicine for the treatment of diarrhea, lung edema, parasitic diseases, and fever caused by bacterial, and viral infections (wang et al., 2009); in the Indian folk medicine *Scorzonera divaricata Turcz* and *Scorzonera virgata DC,* have been used Rheumatism and Jaundice respectively (Gairola et al., 2014); in the Turkish folk medicine several *Scorzonera semicana* DC were used in treatment of diabetes mellitus (Durmuskahya & Ozturk 2013); in the Urmia drug and food administration in Azerbaijan/Iran the decoction of the roots of *Scorzonera cinerea* Boiss, is used as a food laxative (Bahmani et al., 2014); in Lebanon the decoction of the aerial parts of *Scorzonera Libanotica Boiss; Scorzonera cana* (C. A. Mey.); *Scorzonera euphratica* and *Scorzonera phaeopappa* are used to treat headache (Baydoun et al., 2015) .

Furthermore, Several studies have been done on roots, leaves and aerial parts of *Scorzonera species* that demonstrated its biological effect such as ant carcinogenic properties, anti hypertensive, anti inflammatory (*K,Athmouni,* 2015); analgesic,

antinocecptive (Bahdir et al., 2010; Citoglu et al., 2008); antimicrobial and antifungal (Yavuz Erden Sevda Kırbag,2013; Acikara et al., 2013); and anticholinesterase and anti-tyrosinase activities (Senol et al., 2014).

Antioxidants are naturally occurring or synthetic chemicals in foods they provide protection against free radical compounds and increase the shelf life by retarding the process of lipid peroxidation which is the main cause of food deterioration (Halliwell et al., 2009; Gülçin, 2012). Moreover antioxidants compounds protect the human body from free radicals and Reactive oxygen species (Gülçin, 2012). According to the WHO, 150 million people have diabetes mellitus worldwide and this number is expected to double by 2025 (WHO, Facts sheets, 2015). One of the most worrying features of this rapid increase is the emergence of type 2 diabetes in children, adolescents, and young adults.

Medicinal plants and herbel extracts that are rich in polyphenols have been reported to demonstrate potential antidiabetic activity (Russo et al., 2015). Diabetes mellitus is a set of chronic metabolic diseases characterized by hyperglycemia, resulting from insufficient or inefficient amounts of insulin secretion (WHO, Fact sheets). 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 (Gupta et al., 2003); and the alpha glucosidase enzyme in the small intestine which catalyzes the end step of the digestion of starch and disaccharide (Annam et al., 2009). Thus the inhibition of these enzymes has been found as an effective way to lower the level of blood glucose (Russo et al., 2015). Up to our knowledge, no studies have been done to determine the antidiabetic acitvity of any of *Scorzonera* species, knowing that in

traditional turkish medicine *S.papposa* was used to treat diabetes mellitus (Durmuskahya & Ozturk 2013).

One of the interesting edible plants is *Scorzonera Phaeopappa* Boiss; however, it hasn't been studied yet. Therefore, the objectives of this study is to investigate the chemical composition content of *Scorzonera Phaeopappa Boiss.* and to evaluate the antioxidant, anti-diabetic activity of the obtained extract. To achieve the objective of our work the following steps will be done: 1) Prepare crude extracts using solvents of different polarity, 2) determine the total phenolic compounds using Follin Ciocalteu index, total flavonoids using Aluminum chloride method and total terpenes using salkowski test 3) determine the antioxidant activity of the extracts using DPPH-radical scavenger assay, and ferrous iron chelating capacity, 4) determine the anti-diabetic activity of the extracts using alpha- amylase and alpha- glucosidase assay.

2. Material and Methods

Raw Material and Reagents

Plant material:

Leaves of *Scorzonera Phaeoppapa* Boiss. were collected in May 2017 from Ayta al foukhar in bekaa lebanon (altitude 1600 m). The plant was identified by Dr Antoine Haj, Associate professor at NDU main campus, Lebanon. The voucher specimen was deposited in the herbarium of NDU University Chouf or main campus, Lebanon.

The plant parts were shade dried and pulverized using an electric blender.

Extraction Methods

The plant parts will be shade dried and powdered using an electric blender. The MeOH, , EtOH, acetone, dichloromethane + ammonia and dichloromethane extracts will be prepared as follows: 20 g of plant powder were macerated in 200 mL in five different solvents (acetone, methanol, ethanol, dichloromethane and dichloromethane+amounia) under constant magnetic agitation for 24 h at 25 °C. The mixture was then filtered, condensed at 40 °C under reduced pressure.

The ammonia-dichloromethane extract was prepared as follows: 20g of plant powder were moistened for 2 h with NH4OH solution followed by dichloromethane addition (200 mL). The mixture will undergo maceration for 24 h under magnetic stirring and filtered. The organic phase will concentrate at 40 °C under reduced pressure and freeze dried. The obtained extracts will be cooled in dark containers. The rotary evaporator that will be used is IKA RV 10 BASIC. The obtained extracts will be kept in a cool place in dark containers.

Total Phenol Content for leaves extracts.

Total phenols in the extracts will be assessed by a modified Folin-Ciocalteu method (Koivikko et al., 2005). Briefly, 0.5 mL of the diluted samples of different concentrations will be mixed with 0.5 mL of 1N Folin-Ciocalteu reagent. The mixture will stand for 3 min, after which 1 mL of 20% sodium carbonate (Na2CO3) was added. Samples will be incubated in the dark at room temperature for 45 min then centrifuged (5 min at 2400 g). Absorbance of the supernatants will be measured at 730 nm using a Jenway 6405 UV/Vis spectrophotometer. Total phenol content will be expressed as mg of gallic acid

equivalents (GAE) per 100 mg of extract. All measurements will be performed in duplicate.

Total flavonoid content

Total flavonoids content will be assessed by a modified aluminum chloride method (Erden et al., 2015). Briefly, 1.5ml of the diluted samples with different concentration are mixed with 0.75 ml of 5% $NaNO_2$ and 0.15 ml of $ALCL_3$. The mixture will stand for 5 minutes, after which 0.5m of 1 M NaOH is added and then make up the volume to 5 ml distilled water. Absorbance of the mixture was measured at 510 nm using UV-visible spectrophotometer. Total flavonoid content expressed as mg of quercetin equivalents (QE) per 100 mg of extract. All measurements were performed in duplicates

Total terpenes content

Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids (Edeoga et al., 2005)

The total terpenoid content of the plant extracts was determined based on an assay described by Ghorai et al. (2012) with some modifications. Linalool was used as the standard for estimation. An aliquot of the reaction mixture obtained after Salkowski test employed for the qualitative analysis of terpenoids in the extract was transferred to colorimetric cuvette. The absorbance was measured at 538 nm against blank i.e., 95% (v/v) methanol. For the standard curve, 200 µl of linalool solution in methanol was added

with 1.5 ml chloroform and serial dilutions [dilution level-100 mg/200 µl to 1 mg/200 µl linalool Conc.] were prepared in which total volume of 200 µl was made up by the addition of 95% (v/v) methanol. Calibration curve of linalool was plotted and the total terpenoids content expressed as milligrams of linalool equivalents per gram of dry weight (mg linalool/g DW) was determined using the regression equation. Samples were analyzed in duplicates.

Biological activities

DPPH radical scavenging assay

The scavenging effects of the extracts for DPPH radical will be determined by the method of Yan and Chen (1995) with slight modifications. Serial dilutions of the extracts was prepared in EtOH. The basic procedure was to add an aliquot (1 mL) of test sample to 1 mL of DPPH 0.15 mM 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 read at 517 nm using a UV/Vis spectrophotometer, and the calculations of the scavenging activity (%) (SA) is as follows: SA (%): [1- (Asample - Asample blank) /Acontrol] x 100. Sample solution (1 mL) plus EtOH (1 mL) is used as a sample blank and DPPH solution (1 mL) plus EtOH (1 mL) is used as a negative control. Ascorbic acid are used as the positive controls. Stock solutions ascorbic acid (0.8 mg/mL) will be diluted with EtOH to give concentrations ranging from 1.5 to 20 µg/mL. All measurements was performed in duplicate or triplicate.

Ferrous ion chelating assay

The ferrous ion chelating activity was determined according to Lim et al (2007). Equal volumes of 0.12 mM FeSO4, test sample (at different concentrations), and 0.6 mM ferrozine was mixed. The solutions was allowed to stand for 10 min at room temperature, and the absorbance of Fe2+-ferrozine complex was measured at 562 nm using UV/Vis spectrophotometer. Ultra-pure water instead of sample solution will be used as a negative control. Ultra-pure water instead of ferrozine solution will be used as a blank, which is used for error correction because of unequal color of the sample solutions. EDTA-Na2 will be used as the positive control. The ability of the sample to chelate ferrous ions will be calculated by using the following formula. All measurements will be performed in duplicate.

Alpha-amylase and Alpha-glucosidase Inhibitory Activity

α-amylase inhibitory activity

α-amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification (Ademiluyi,& Oboh, 2013). In a 96-well plate, 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 extract and fractions (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, the 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate ELISA Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was

used as a standard. Without test (extract and fractions) substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = (1 − As/Ac) ×100

Where, As is the absorbance in the presence of test substance and Ac is the absorbance of control.

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification (Shai et al., 2011) In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying concentrations of extract and fractions (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 P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl $Na_2 CO_3$ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate ELISA Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = (1 − As/Ac) ×100

Where, As is the absorbance in the presence of test substance and Ac is the absorbance of control

Statistical analysis

The statistical analysis carried out in the present study included a general descriptive exploration of data, the determination of Pearson coefficients of correlation analyses. All the statistical analysis was carried out using the statistical software SPSS version 16

3. Results and Discussion

3.1 Extraction yield, total phenol, total flavonoid, and total terpene contents.

Wild edible plants are an important nutritional resource for humans' worldwide as they are an inexpensive source of nutrients, vitamins, antioxidants and minerals. In the Mediterranean region, different wild edible plants that grow spontaneously without being cultivated such as Queen-of-the alps (القرصعنة), cheeseweed (الخبيزة), asparagus (هليون) and S.Phaeopappa Al Meshe, the plant of our study, are still traditionally consumed and play an important role in the diet of local populations, in particular of those living in rural areas (Baydoun et al., 2015).

In the last years, Wild edible plants have been attracting the interests of researchers, not only because of their richness in phytochemicals and their health benefits, but also from the food industry and consumers, which are increasingly interested in sustainable and healthy foods. Moreover, across Europe and several developed countries, a new trend has been recently emerging in nutrition and cuisine, the uses of local wild plants in modern dishes not only as an element of cultural identity but also seeking their health benefits (Harumi et al., 2019, Geraci et al., 2018) Table.1 displays the extraction mean yields of

extracts obtained from the leaves of *Scorzonera Phaeoppapa* using five solvents with varying polarities: dichloromethane, dichloromethane pretreated with NH_4OH, acetone, methanol and ethanol. Our results showed that acetone has the highest extraction yield 41.3% followed by dichloromethane-NH_4OH (DCMa) 23.5%, ethanol 13.5%, methanol 12.2% and dichloromethane has the lowest extraction yield 9.5%. It was reported by Do et al., that the yield of extraction depends on the polarity of the solvent, as well as pH, temperature, extraction time, and phytochemical compounds of the sample. Under the same extraction time and temperature, solvent polarity and the phytochemical compounds of the sample are known as the most important determinants of the yield of extraction (Do et al., 2014). Due to the diversity in the nature of phytochemical compounds in a plant its hard and uncertain to extract all phytochemicals using one solvent, where a polar phytochemicals are extracted using polar solvent and the non-polar phytochemicals are extracted by a non-polar solvent, therefore using a polar and non-polar solvents is more favorable to obtain high extraction yield and different phytochemical compounds (Nawaz et al., 2018). Previous studies done on several *Scorzonera* species, to determine its phytochemical composition, used acetone and dichloromethane for the isolation of phenolic and terpenoids compounds respectively(Acikara et.,al 2015, Zidron et al., 2004, Harakati et al., 2010, Zidron et al., 2002).

The total phenolic (TPC), total flavonoid (TFC), and total terpenes content of *Scorzonera phaeoppapa* were determined by Folin-ciocalteau, Aluminium Choloride and Salkowski test, using five different solvents (acetone, dichloromethane+NH_4OH, dichloromethane, methanol and ethanol) and results were expressed in GAE/100mgDW, QE/100mg DW, and LE/100mg DW respectively.

Plant phenolic are diversified groups of compounds with varying degree of polymerization and thus a wide range of polarity. In addition, they may also exist as complexes with carbohydrates, proteins and other plant components. Thus, the extraction efficiency of phenolic is mainly dependent, under the same conditions of time, pH and temperature, the solvent polarity and the chemical composition of the sample (Naczk and Shahidi, 2004 and Do et al., 2014).

For the total phenolic content DCMa solvent showed the highest mean extraction yield of phenolic compounds (2.73mg GAE/100mg) followed by Dichloromethane (DCM) (2.04mg GAE/100mg), methanol (1.83mg GAE/100mgDW), acetone(1.69 mgGAE/100mg) and ethanol (1.46 mgGAE/100mg) table2. Polyphenols, as anti-oxidative agent, were shown to possess various biological activities such as antioxidant, anti-inflammatory, anti-diabetic, anti-mutagenic and neuroprotective activities (Tai et al., 2011).

These findings were supported by findings of several studies. Addai et al., revealed that the extraction yield of phenolic compounds differed with solvents with different polarities (acetone, ethanol and methanol) where methanol was shown to have the highest extraction yield followed by acetone while ethanol was found to possess the lowest extraction yield (Addai et al., 2013). Sun et al., (2011) reported that methanol is an effective solvent for phenolic extraction. Millela et al., reported that methanol has the highest extraction yield of phenolic content in *Scorzonera Undulata* (80.7 mg GAE/g of extract). In addition Athmouni et al., in a study done on *Scorzonera* species reported that the extraction of total phenolic is significantly affected by using solvents with different

polarities, and maceration period, also it was shown that the extraction of phenolic compounds was poor with ethanol solvents which is similar to our study results (Athmouni et al., 2015).

For the total flavonoid content, methanol, and acetone showed the highest extractive capacity 63 QE/100mg, 61 QE/100mg respectively) followed by ethanol 53.23 QE/100mg, where DCM and DCMa showed the lowest extractive capacity (25.02 QE/100mg DW and 14.41 QE/100mg respectively). Finding of several previous studies supported these findings. Phytochemical studies done on several *Scorzonera* species to determine their total flavonoid content on demonstrated that methanol is an effective solvent for flavonoid extraction (Erden et al., 2013; Erden et al., 2015; Donia, 2016). Other researchers also showed that methanol has the highest extractive capacity on flavonoids content as compared to other solvents such as ethanol, acetone and dichloromethane (Iloki-Assanga et al., 2015). In addition, in a comparative study done by Ghasmezadeet et al on the total flavonoid content, the extractive capacity of methanol was shown to be higher than that of acetone (Ghasemzadeet al.,2010).

Another study done to isolate and identify the extracted flavonoids in nine taxa of *Scorzonera* species: *(S. austriaca Willd. var. angustifolia; S. crispatula Boiss.; S. graminifolia L.; S. hirsuta L.; S. hispanica L.; S. laciniataL.; S. mollis M.Bieb.; S. pseudolanata Grossb.; S. pusilla Pall.)*; using methanol as a solvent, showed that all of the taxa contained a high amount of the most common flavonoids quercetin and kaempferol (Rees, 1984), this is despite the fact that the content of flavonoid differ from one plant to another and within the same plant parts (Justesen, 2000).

Different studies demonstrated that flavonoid-rich plants have many biological activity such as anti-inflammatory, anti-microbial anti-tumor (Formica et al., 1995; Yang et al., 2001). Moreover, it was shown that flavonoids have antioxidant activities stronger than vitamin A and C (Sokol et al., 2007)

On the other hand, *Scorzonera* species are found to be highly rich in terpenes compounds: monoterpenes, sesquiterpene lactones,and triterpenes (Zhu et al., 2010, Bader et al., 2011, Millella et al., 2013 Acikara et al., 2015, and Yang et al., 2016), In this study, DCM and DCMa had the highest extraction capacity for total terpenes (TTC) 51 LE/100 mg and 232 LE/ 100mg respectively followed by methanol (28.33 LE/ 100mg) and acetone (11.33 LE/100mg), whereas ethanol (8.68 LE/100mg) had the least extraction capacity (Table 2), These finding could be explain by the fact that dichloromethane is known to be effective for the extraction of volatile (non-polar)

compounds such as terpenes because of its non-polar properties(Johnson & Lusas 1983). In addition, in a study done by Wu et al., dichloromethane was used to isolate and extract triterpenes from *Scorzonera austrica*, because of its high extraction capacity on terpenes (Wu et al.,2011). Moreover these studies revealed that the genus Scorzonera is highly rich in flavonoids, terpenoids and phenolic acids (Acikara et.,al 2015, Zidron et al., 2004, Harakati et al., 2010)

Solvents	Yield (mg)	% of Total Sum
Acetone	2.2700	41.3%
Dichloro+Amounnia	1.2900	23.5%
Dichloromethane	.5200	9.5%
Methanol	.6700	12.2%
Ethanol	.7400	13.5%

Table 8: Extract yield means of *Scorzonera Phaeopappa* leaves extracts using solvents with different polarities

Table 9: Total phenol contents, total flavonoids content and total terpene content of the 5 extracts using solvents with different polarities.

	Total phenol content		Total flavonoid content		Total terpenes content	
Solvents	Mean mgGAE/ 100mgDW	%	Mean mgQE/ 100mg DW	%	Mean Mg LE/100mg DW	%
Acetone	1.6900	17.3%	61.0500	28.2%	11.3300	3.4%
Dichloro+Amounnia	2.7300	28.0%	14.4100	6.6%	51.0200	15.4%
Dichloromethane	2.0400	20.9%	25.0200	11.5%	232.4200	70.1%
Methanol	1.8300	18.8%	63.0500	29.1%	28.3400	8.5%
Ethanol	1.4600	15.0%	53.2300	24.6%	8.6800	2.6%

3.2 Antioxidant activities of *Scorzonera Phaeoppapa* extracts using DPPH assay and Fe^{2+} chelating assays

DPPH assay is an easy and an accurate method widely accepted as a tool for estimating the radical scavenging activity of potential antioxidants (Sánchez-Moreno, 2002; Buenger et al., 2006). DPPH is a stable free radical characterized by a deep violet color, it is dissolved in ethanol to form a DDPH solution with a spectrophotometric absorption at about 520nm. When a solution of DPPH is mixed with a hydrogen donor such as anti-oxidative agent, it is converted to its reduced form; as a result of which, the deep violet color will fade indicating the antioxidant effect of a hydrogen donor.

The DPPH scavenging activity exhibited by *S. Phaeoppapa* extracts was expressed as IC50 (Table3) 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). Our results showed that ethanol extract has highest DPPH scavenging activity 0.07 mg/ml followed by DCM (0.38 mg/ml), ethanol (0.39 mg/ml) and ethanol (0.50mg/ml), while DCMa has the lowest activity 1.05 mg/ml. Thus methanol extract was found to be the most potent towards DPPH free radicals. Comparing our results with ascorbic acid the commonly used reference compound, that is known to exhibit DPPH radical scavenging activity at an IC50 0.003 mg/mL, we noted that all extracts exhibited an activity lower than the reference group. In previous studies it was shown that the methanolic extracts from different *Scorzonera* species exhibited an

inhibitory activity against DPPH. Among them, feruloylpedospermic acid A &B a new quinic acid derivatives isolated from *S.Divarcata* showed a potent antioxidant activity aginst DPPH (Tsevegsuren et al., 2007). In another study done by Wang et al., showed that scorzodiyhdrostilbenes A-E isolated from *S.radiata* were shown to exhibit a stronger antioxidant activity than resveratrol against DPPH (Wang et al., 2009). Similar results were also obtained from Chlorogenic acid (phenolic acid) extracted from 27 different *Scorzonera* species with a potent antioxidant activity against DPPH (Senol et al., 2014).

Ferrous ions (Fe^{2+}) could catalyze the Fenton-type reactions in a biological system, resulting in generation of hydroxyl radicals (OH•). Thus, the minimization of the Fe^{2+} concentration by a chelating agent provides protection against oxidative damage. Ferrozine has been largely used for the determination of the chelating activity; it forms with Fe^{2+} a colored complex measurable at 562 nm. Other chelators such as phytochemicals extracted from plants can also make a complex with ferrous ions competing ferrozine thus inhibiting the reaction of ferrozine with ferrous ion and therefore reducing its color. This allows estimating the chelating activity of the tested antioxidant (Soler-Rivas et al., 2000).

As shown in Table 3, all extracts exhibited Fe^{2+} chelating activity in a concentration dependent manner. At a very low concentration, the methanolic extract (0.06 mg/ml & 0.08 mg/ml) exhibited 21% & 50% of chelating activity ethanolic extract at a concentration 0.10 mg/ml & 0.15 mg/ml exhibited 37 % and 45 % of chelating activity, acetone at a concentration of 0.10 mg/ml & 0.15mg/ml exhibited 45% and 54% chelating activity, while at a higher concentrations DCMa and dichloromethane (0.15 mg/ml &

0.20 mg/ml) exhibited 52% & 62% of chelating activity. It is well-known that the compounds with structures containing two or more of functional groups such as -OH, -SH, -COOH, $-PO_3H_2$, C=O, $-NR_2$, -S, and -O- can show metal chelating activity (Yuan et al., 2005). Phytochemical studies showed that *Scorzonera* species are rich in such functional groups such as chlorogenic acid, rutin, hyperoside and Scorzotomentosin-4-glucoside have a moderate activity against Fe^{2+} metal chelating compared to EDTA (Senol el al., 2014). In addition, Senol et al., showed that the methanolic extract of 27 different *Scorzonera* species exhibited a low to moderate activity against Fe^{2+} metal chelating assay (Senol et al., 2014). In conclusion, all extracts have a promising chelating activity.

Table 10. DPPH scavenging activity and Fe^{2+} chelating activity of 5 *S. Phaeopappa* leaves extracts using solvents with varying polarities.

Solvent	IC50 DPPH assay mg/ml	Fe^{2+} chelating activity	
		Concentration mg/ml	Inhibitory percentage (%)
Acetone	0.50	0.10	45
		0.15	54
DCMa	1.05	0.15	52
		0.2	62
DCM	0.38	0.15	52
		0.2	62

Methanol	0.07	0.06	21
		0.08	50
Ethanol	0.39	0.10	37
		0.15	45

3.3 Correlation between phytochemical constituents and antioxidant activity.

Correlation coefficients between the assessed phytochemical constituents and both DPPH radical scavenging activity and Fe^{2+} metal chelating activity are reported in Table 4. Specifically a weak positive correlation was found between TPC and DPPH scavenging activity (r 0.200, p 0.744) expressed in IC50, and a negative weak/strong correlation was observed between TTC, TFC and DDPH scavenging activity (r:-0.100, p: 0.873 & r:-0.600 p: 0.285 respectively).

On the other hand a strong positive correlation was observed between TPC , TTC and Fe^{2+} chelating activity expressed in percentage (r: 0.667, p 0.219 & r:0.667 p:0.219 respectively), and a strong negative correlation between TFC and Fe^{2+} chelating activity (r:-0.872, p: 0.054).

Flavonoids seems to be involved in Fe^{2+} metal chelating activity since the p value is around 0.05(r:-0.872, p: 0.054), indicating the ability of these compounds to chelate $Fe^{2+,}$ reducing oxidative stress. However, in previous studies it was reported that the antioxidant activity is significantly correlated to the polyphenolic contents extracted from several *Scrozonera* species (Zidron et al., 2004; Wang et al., 2009; Millela et al., 2013, Yavuz Erden Sevda Kırbag, 2013; Harakti et al., 2013) and this correlation was mainly

related to the presence and the concentration of phenolic acids and flavonoids such as chlorogenic acid, coumarins, stilbenes, rutin hyperoside in different *Scorzonera* species (Yavuz Erden Sevda Kırbag, 2013, 2013 and Senol et al., 2014). Moreover, a novel phenolic acid known as pedospermic acid was extracted from *Pedospermum lacinata* and was shown to have a potent significant antiradical scavenging activity compared to chlorogenic acid, resveratrol and caffeic acid (Zidron et al., 2004).

In addition, Harakti et al., showed that diyhdrostilbenes extracted from *Scorzoner Undulata* exhibited a positive significant anti-radical scavenging activity (Harakti et al., 2013). On the other hand and up to our knowledge a very limited number of studies were done to determine the correlation of terpenes and antioxidant activity, where it was shown by Yang et al., that the two sequestriterpene Sulfoscorzonin A and C that were extracted from *Scorzonera Davicarta* have a moderate antiradical scavenging activity on ABTS (Yang et al., 2015).

As a conclusion and according to the literature, the antioxidant activity vary among species and it was determined to be highly related to the concentration of phenolic compounds and flavonoids present in the plant, where terpenes exhibited a low antioxidant activity.

Table 11. Correlation between total phenols, total flavonoids and total terpene contents and antioxidant activities (DPPH radical scavenging & Fe^{2+} chelating activities)

		DPPH radical scavenging activity	Fe^{2+} metal chelating activity
Total Phenol content	Correlation coefficient	.200	.667
	P value	.747	.219
	N	5	5
Total Flavonoid content	Correlation coefficient	-.600	-.872
	P value	.285	.054
	N	5	5
Total Terpene content	Correlation coefficient	-.100	.667
	P value	.873	.219
	N	5	5

*: correlation is significant at the 0.05 level (2-talied)

**: correlation is significant at the 0.01 level (2 tailed)

3.4 Anti-Diabetic activities of *Scorzonera Pheoppapa* leaves extracts using alpha-amylase and alpha glucosidase inhibitory assays.

The anti-diabetic activity of *S. Phaeopappa* extracts obtained from 5 different solvents (acetone, DCMa, dichloromethane, ethanol and methanol) was determined using the alpha-amylase and alpha-glucosidase inhibitory assay, results were expressed as IC50 (Table 5) and acarbose, a known anti-diabetic drug such as Glucobay and Precose, was used as the reference standard with IC50 values 0.47 mg/ml and 0.21 mg /ml respectively. Data obtained showed that acetone was the most active against alpha amylase (IC 50 0.21 mg /ml) and more than two times more potent than acarbose (0.47mg /ml).While all other extracts exerted inhibitory activities ranging between

0.56mg/ml and 3.43mg/ml for alpha amylase enzyme which were 1.2 to 7.29 lower than acarbose and an inhibitory activity ranging between 5.46mg/ml and 16.8 mg/ml for the alpha glucosidase enzyme which were 26 to 80 times lower than acarbose (0.21 mg/ml) (table 5).

Spearmen correlation coefficients between TPC, TFC and TTC and alpha amylase inhibitory activity and alpha glucosidase inhibitory activity are shown in table 6. Significant positive correlations were observed between TPC, TTC and alpha-glucosidase inhibitory activity (r: 0.900, p: 0.037 & r:1.000 $p<0.001$ respectively). In addition, moderate negative to strong positive correlations were found between TFC and alpha amylase inhibitory activity (r: -0.400 p: 0.505) and between TPC, TTC and alpha amylase inhibitory activity (r: 0.800, p: 0.104 & r: 0.600, p: 0.505 respectively) and a negative strong correlation between TFC and alpha glucosidase inhibitory activity r: -0.500, p: 0.391). This could be related to the presence of phenolic compounds and terpenoids that have a potential inhibitory activity alpha-glucosidase enzyme.

Loizzo et al., studied 27 extracts from nine Lebanese medicinal plants to identify their phytochemical profile and to determine their inhibitory activity against alpha amylase and alpha glucosidase enzymes, showing a significant inhibitory effect of TTC on alpha amylase and alpha glucosidase enzymes that is related to the presence of monoterpenes, sequisterpene, steroids, triterpenoids and fatty acids extracts (Loizzo et al., 2008). In addition Russo et al., showed that extracts rich in terpenes, flavonoids and phenolics possessed significant anti-diabetic activities against both alpha amylase and alpha glucosidase enzymes, where further analysis on a methanolic extract from yacon leaves

which showed that the concentration of chlorogenic acid, quinic and caffeic is directly correlated with alpha amylase and alpha glucosidase inhibitory activity (Russo et al., 2015). Where it was reported by Albayrak et al., the presence of chlorogenic acid, caffeic acid, ferulic acid, syringic acid, apigenin, apigenin-7-glucoside, and hesperidin; luteolin, naringenin, quercetin, and resveratrol in different *Scorzonera* species (Albayrak et al., 2010).

Many studies have been done to isolate and identify the phytochemicals that are responsible for the anti-diabetic activity of different medicinal plants. In a study done by Narita et al., on different *Helichrysum* species, they identified chlorogenic acid as an active metabolite against alpha amylase & alpha glucosidase enzymes (Narita et al., 2008). On the other hand, hydroalcoholic extracts from two *Juniperus* plants rich in coumarins, sterols, terpenes, and ligannas were shown to have antidiabetic activity mainly by inhibiting the alpha glucosidase enzyme (Lohani et al., 2013). Therefore the observed inhibitory effect of *Scorzonera phaeopappa* extracts could be related to the presence of different phytochemicals like chlorogenic acid, rutin, hyperoside, apigenin-7-glucosidase, sequestiterpenes that are known to be found in the genus *Scorzonera.*

Table 12: Results of alpha amylase and alpha Glucosidase inhibitory assays of 5 *S. Phaeopappa* leaves extracts using solvents with varying polarities

Extract	IC50 alpha amylase mg/ml	IC50 alpha glucosidase mg/ml
Acetone	0.21	6.3
DCMa	3.43	12.55

Dichloromethane	0.97	16.8
Ethanol	0.56	5.46
Methanol	2.06	9.01
Acarbose	0.42	0.277

Table 13: Correlation between total phenols, total flavonoids and total terpene content and anti-diabetic activity (alpha amylase & alpha glucosidase)

		Alpha amylase inhibitory activity	Alpha glucosidase inhibitory activity
Total Phenol content	Correlation coefficient	.800	.900*
	P value	.104	.037
	N	5	5
Total Flavonoid content	Correlation coefficient	-.400	-.500
	P value	.505	.391
	N	5	5
Total Terpene content	Correlation coefficient	.600	1.000**
	P value	.285	.
	N	5	5

*: correlation is significant at the 0.05 level (2-talied)

**: correlation is significant at the 0.01 level (2 tailed)

4. Conclusion

The screening of different extracts from the leaves of *Scorzonera Phaeopappa* for total phenols, total flavonoids, total terpenes, anti-diabetic and antioxidant activities was performed. Acetone was found to have the highest extraction capacity. Where methanol exhibited the highest antioxidant against DPPH radical scavenging and Fe^{2+} chelating activities. On the other hand, acetone and ethanol exhibited the highest anti-diabetic activities using alpha amylase and alpha glucosidase inhibitory assays, noting that acetone was more active than acarobse on the inhibition of alpha amylase. Moreover, total flavonoids were found to be strongly correlated with the antioxidant and anti-diabetic activities.

The present findings can serve as a building block for further researchers that should aim to validate the anti-diabetic activity of the total phenol and total terpene content using other methods, and to determine the bioactive molecules that are responsible for this activity.

5. References

Acikara, H. O. O. B., Yigitbasi, I. K. N. K. T., & Iscan, G. S. (2017). Preclinical Evaluation of Scorzonera sp. Root Extracts and Major Compounds Against Acute Hepatotoxicity Induced by Carbon Tetrachloride. *Indian Journal of Pharmaceutical Sciences*, *79*(5), 715-723.

Acikara, O. B., Çitoğlu, G. S., & Çoban, T. (2013). Phytochemical Screening and Antioxidant Activities of Selected Scorzonera Species. *Turk J Pharm Sci*, *10*, 453-462.

Acıkara, Ö. B., Çitoğlu, G. S., Dall'Acqua, S., Šmejkal, K., Cvačka, J., & Žemlička, M. (2012). A new triterpene from Scorzonera latifolia (Fisch. and Mey.) DC. *Natural product research*, *26*(20), 1892-1897.

ACIKARA, Ö., CITOGLU, G. S., & GENCLEROZKAN, A. (2013). Qualitative and quantitative analysis of phenolic acids in Scorzonera tomentosa L. *Turkish Journal of Pharmaceutical Sciences*, *10*(1), 1-8.

Adams, C. D., 1963, Compositae. In Flora of West Tropical Africa, 2nd ed., F.N. Hepper (ed.), Crown Agents, London.

Addai, Z. R., Abdullah, A., Mutalib, S. A. (2013). Effect of extraction solvents on the phenolic content and antioxidant properties of two papaya cultivars. J. Med. Plants Res., 7, 3354–3359.

Ademiluyi, A. O., & Oboh, G. (2013). Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Experimental and Toxicologic Pathology*, *65*(3), 305-309.

Akkol, E. K., Acıkara, O. B., Süntar, I., Citoğlu, G. S., Keleş, H., & Ergene, B. (2011). Enhancement of wound healing by topical application of Scorzonera species: Determination of the constituents by HPLC with new validated reverse phase method. *Journal of ethnopharmacology*, *137*(2), 1018-1027.

Akkol, E. K., Acıkara, Ö. B., Süntar, I., Ergene, B., & Çitoğlu, G. S. (2012). Ethnopharmacological evaluation of some Scorzonera species: in vivo anti-inflammatory and antinociceptive effects. *Journal of ethnopharmacology*, *140*(2), 261-270.

Albayrak, S., Aksoy, A., Sagdic, O., & Hamzaoglu, E. (2010). Compositions, antioxidant and antimicrobial activities of helichrysum (asteraceae) species collected from turkey. Food Chemistry, 119(1), 114-122. doi:10.1016/j.foodchem.2009.06.003

Anam, K.; Widharna, R.M.; Kusrini, D. α-Glucosidase inhibitor of Terminalia species. Int. J. Pharmacol. 2009, 5, 277–280

Athmouni, K., Belghith, T., Bellassouad, K., El Feki, A., Ayadi, H. (2015). Eff ect of extraction solvents on the biomolecules and antioxidant properties of *Scorzonera undulata* (Asteraceae): Application of factorial design optimization phenolic extraction. Acta Sci.Pol. Technol. Aliment., 14(4), 313–330. DOI: 10.17306/J.AFS.2015.4.32

Auezova, L., Najjar, F., Selivanova, O., Hajj Moussa, E., & Diab Assaf, M. (2013). Antioxidant activity of brown alga saccharina bongardiana from kamchatka (pacific coast of russia). A methodological approach. Journal of Applied Phycology, 25(4), 1189-1196. doi:10.1007/s10811-012-9932-z

Bader, A., De Tommasi, N., Cotugno, R., & Braca, A. (2011). Phenolic compounds from the roots of the Jordanian Viper's Grass Scorzonera judaica. Journal of Natural Products, 74, 1421–1427

Bahadır Acikara, Ö., Hošek, J., Babula, P., Cvačka, J., Budešínský, M., Dračinský, M., ... & Šmejkal, K. (2015). Turkish Scorzonera species extracts attenuate cytokine secretion via inhibition of NF-κB activation, showing anti-inflammatory effect in vitro. *Molecules*, *21*(1), 43.

Bahadır, Ö., Çitoğlu, G. S., Šmejkal, K., Dall'Acqua, S., Özbek, H., Cvacka, J., & Zemlicka, M. (2010). Analgesic compounds from Scorzonera latifolia (Fisch. and Mey.) DC. *Journal of ethnopharmacology*, *131*(1), 83-87.

Bahmani, M., Zargaran, A., & Rafieian-Kopaei, M. (2014). Identification of medicinal plants of Urmia for treatment of gastrointestinal disorders. *Revista Brasileira de Farmacognosia*, *24*(4), 468-480.

Barros, L., Ferreira, M., Queirós, B., Ferreira, Isabel C. F. R., & Baptista, P. (2007). *Total phenols, ascorbic acid, β-carotene and lycopene in portuguese wild edible mushrooms and their antioxidant activities* doi:9443/10.1016/j.foodchem.2006.07.038

Baydoun, S., Chalak, L., Dalleh, H., & Arnold, N. (2015). Ethnopharmacological survey of medicinal plants used in traditional medicine by the communities of Mount Hermon, Lebanon. *Journal of ethnopharmacology*, *173*, 139-156.

Baytop, T., 1999. Turkiye'de Bitkiler ile Tedavi. Nobel, Ankara, pp. 236–237.

Buenger, J., Ackermann, H., Jentzsch, A., Mehling, A., Pfitzner, I., Reiffen, K. -., . . . Wollenweber, U. (2006). An interlaboratory comparison of methods used to assess antioxidant potentials1. International Journal of Cosmetic Science, 28(2), 135-146. doi:10.1111/j.1467-2494.2006.00311.x

Citoglu, G.S., Bahadır, O., Ozbek, H., 2008. Antinociceptive activity of some Scorzonera species. In: The 12th International Congress Phytopharm, 2–4 July, Saint Petersburg, Russia.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, andantioxidant activity of *limnophila aromatica* doi:https://doi.org/10.1016/j.jfda.2013.11.001

Donia, A. E. R. M. (2016). Phytochemical and pharmacological studies on Scorzonera alexandrina Boiss. *Journal of Saudi Chemical Society*, *20*, S433-S439.

Douglas, J., 2001. Scorzonera hispanica—a European vegetable. Crop and Food Research 28.
Availablefrom:http://www.crop.cri.nz/home/productsservices/publications/broadsheets/028scorzonera.pdf.

Durmuşkahya, C., & Öztürk, M. (2013). Ethnobotanical survey of medicinal plants used for the treatment of diabetes in Manisa, Turkey. *Sains Malaysiana*, *42*(10), 1431-1438.

Erden, Y., & Kırbağ, S. (2015). Chemical and Biological Activities of Some Scorzonera Species: An In Vitro Study. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*(1), 319-326.

Erden, Y., Kırbağ, S., & Yılmaz, Ö. (2013). Phytochemical composition and antioxidant activity of some Scorzonera species. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *83*(2), 271-276.

Formica JV, Regelson W (1995) Review of the biology of Quercetin and related bioflavonoids. Food Chem Toxicol 33(12):1061 1080

Gairola, S., Sharma, J., & Bedi, Y. S. (2014). *A cross-cultural analysis of jammu, kashmir and ladakh (india) medicinal plant use*9443/10.1016/j.jep.2014.06.029 Retrieved fromhttp://www.sciencedirect.com.neptune.ndu.edu.lb:2048/science/article/pii/S0378874114004772

Granica, S., & Zidorn, C. (2015). Phenolic compounds from aerial parts as chemosystematic markers in the Scorzonerinae (Asteraceae). *Biochemical Systematics and Ecology*, *58*, 102-113.

Gülçin, İ. (2012). Antioxidant activity of food constituents: An overview. Archives of Toxicology, 86(3), 345-391. doi:10.1007/s00204-011-0774-2

Gupta, R.; Gigras, P.; Mohapatra, H.; Goswami, V.K.; Chauhan, B. Microbial α-amylases: A biotechnological perspective. Process Biochem. 2003, 38, 1599–1616

Geraci, A., Amato, F., Di Noto, G., Bazan, G., & Schicchi, R. (2018). The wild taxa utilized as vegetables in Sicily (Italy): a traditional component of the Mediterranean diet. *Journal of ethnobiology and ethnomedicine*, *14*(1), 14.

Harkati, B., Akkal, S., Bayat, C., Laouer, H., & Franca, M. D. (2010). Secondary metabolites from Scorzonera undulata ssp. deliciosa (Guss.) Maire (Asteracae) and their antioxidant activities. *Records of Natural Products*, *4*(3), 171.

Harumi Iyda, J., Fernandes, Â., Calhelha, R. C., Alves, M. J., Ferreira, F. D., Barros, L., . . . Ferreira, I. C. F. R. (2019). Nutritional composition and bioactivity of umbilicus rupestris (salisb.) dandy: An underexploited edible wild plant. *Food Chemistry*, *295*, 341-349. doi:10.1016/j.foodchem.2019.05.139

Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L. *et al.* Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron*

californicum . *BMC Res Notes* 8, 396 (2015). https://doi.org/10.1186/s13104-015-1388-1

Johnson, L., & Lusas, E. W. (1983). Comparison of alternative solvents for oils extraction. Journal of the American Oil Chemists' Society, 60(2Part1), 229-242.

Justesen U (2000) Negative atmospheric pressure chemical ionisation low-energy collision activation mass spectrometry for the characterisation of flavonoids in extracts of fresh herbs. J Chromatogr A 902(2):369–379

Komal, S., Ranjan, B., Neelam, C., Birendra, S., Neeraj Kumar, S., 2011. *Berberis aristata*: a review. International Journal of Research in Ayurveda and Pharmacy 2(2), 383-388.

Koşar, M., 1999. Türkiye'de yetişen *Berberis* L. türlerinin alkaloitleri (Alkaloids of *Berberis* species growing in Turkey). Ph.D. Thesis, Anadolu University, Institute of Health Sciences, Eskişehir (Turkey).

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., ... & Chen, H. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules*, *21*(10), 1374.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition*, *134*(12), 3479S-3485S.

Lohani H, Haider SZ, Chauhan NK, Sah S, Andola HC. Aroma profile of two Juniperus species from Alpine region in Uttarakhand. J Nat Prod 2013; 6:38-43.

Loizzo, M. R., Saab, A. M., Tundis, R., Menichini, F., Bonesi, M., Piccolo, V.Menichini, F. (2008). In vitro inhibitory activities of plants used in lebanon traditional medicine against angiotensin converting enzyme (ACE) and digestive enzymes related to diabetes. Journal of Ethnopharmacology, 119(1), 109-116. doi:10.1016/j.jep.2008.06.003

Marc, E. B., Nelly, A., Annick, D., & Frederic, D. (2008). *Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of lebanon*9443/10.1016/j.jep.2008.08.

Milella, L., Bader, A., De Tommasi, N., Russo, D., & Braca, A. (2014). Antioxidant and free radical-scavenging activity of constituents from two Scorzonera species. *Food Chemistry*, *160*, 298-304.

Naczka, M., Shahidi, F., 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A 1054, 95-111.

Narita Y, Kimura R, Nakagiri O, Inouye K. Kinetic analysis and mechanism on the inhibition of chlorogenic acids against porcine pancreas αamylase. Proceedings of the 22nd International Conference on Coffee Science (ASIC), 2008, Campinas, SP-Brazil p. 171-175.

Nasseri, M. A., Bigy, S. S., Allahresani, A., & Malekaneh, M. (2015a). Assessment of antioxidant activity, chemical characterization and evaluation of fatty acid compositions of scorzonera paradoxa fisch and CA mey. Jundishapur Journal of Natural Pharmaceutical Products,10(4)

Nawaz, H., Shad, M. A., & Rauf, A. (2018). Optimization of extraction yield and antioxidant properties of brassica oleracea convar capitata var L. leaf extracts. Food Chemistry, 242, 182-187. doi:10.1016/j.foodchem.2017.09.041

Nieuwe vaderlandsche kookkunst, Bevattende een volledig en grondig onderricht, om, naar den hedendaagschen smaak, toe te bereiden allerleie soorten van spyzen […], door twee in dit vak zeer ervarene huishoudsters. ('New Dutch culinary arts […]') Johannes Allart, Amsterdam, 1797. Facsimile editie uitg. C. de Vries-Brouwers, Amsterdam/Antwerpen, 1976.

Rees S, Harborne J (1984) Flavonoids and other phenolics of Cichorium and related members of the Lactuceae (compositae). Bot J Linn Soc 89(4):313–319

Rosa, A., Deiana, M., Atzeri, A., Corona, G., Incani, A., Melis, M. P., . . . Dessì, M. A. (2007). Evaluation of the antioxidant and cytotoxic activity of arzanol, a prenylated α-pyrone–phloroglucinol etherodimer from helichrysum italicum subsp . microphyllum. Chemico-Biological Interactions, 165(2), 117-126. doi:10.1016/j.cbi.2006.11.006.

Russo, D., Valentão, P., Andrade, P. B., Fernandez, E. C., & Milella, L. (2015). Evaluation of antioxidant, antidiabetic and anticholinesterase activities of Smallanthus sonchifolius landraces and correlation with their phytochemical profiles. International journal of molecular sciences, 16(8), 17696-17718.

Saeed, A. (2006). Synthesis of 6-O-methyl ether of scorzocreticin and scorzocreticoside I, metabolites from Sorzonera cretica. *Journal of Asian natural products research*, *8*(5), 417-423.

Sanchez-Moreno, C. (2002). Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Science and Technology International, 8(3), 121-137. doi:10.1177/1082013202008003770

Sarı, A. (2010). Two new 3-benzylphthalides from Scorzonera veratrifolia Fenzl. *Natural product research*, *24*(1), 56-62.

Sezer Senol, F., Acikara, O. B., Citoglu, G. S., Orhan, I. E., Acqua, S. D., & Özgökce, F. (2014). Prospective neurobiological effects of the aerial and root extracts and some pure compounds of randomly selected Scorzonera species. *Pharmaceutical biology*, *52*(7), 873-882.

Shai, L. J., Magano, S. R., Lebelo, S. L., & Mogale, A. M. (2011). Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. *Journal of Medicinal Plants Research*, *5*(13), 2863-2867.

Sokol-Letowska A, Oszmianski J, Wojdylo A (2007) Antioxidant activity of the phenolic compounds of hawthorn, pine and skullcap. Food Chem 103(3):853–859. doi:10.1016/j.foodchem. 2006.09.036

Soler-Rivas, C., Espin, J. C., & Wichers, H. J. (2000). An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochemical Analysis, 11(5), 330-338. doi:10.1002/1099-1565(200009/10)11:5<330::AID-PCA534>3.0.CO;2-G

Sun, Y., Liu, D., Chen, J., Ye, X., Yu, D. (2011). Effects of different factors of ultrasound treatment on the extraction yield of the all-trans-ß-carotene from citrus peels.Ultrason. Sonochem., 18, 243–249.

Syad AN, Shunmugiah KP, Kasi PD. Assessement of anticholinesterase activity of Gelidiella acerosa: implications for its therapeutic potential against Alzheimer's disease. Evid Based Complement Alternat Med. 2012;2012:497242

Tai, Z., Cai, L., Dai, L., Dong, L., Wang, M., Yang, Y., Cao, Q., Ding, Z. (2011). Antioxidantactivity and chemical constituents of edible fl ower of *Sophora viciifolia*. Food Chem., 126, 1648–1654.

Tenkerian, C., El-Sibai, M., Daher, C. F., & Mroueh, M. (2015). Hepatoprotective, antioxidant, and anticancer effects of the tragopogon porrifolius methanolic extract. *Evidence-Based Complementary and Alternative Medicine, 2015*.

Tiwari, B. K., Brennan, C., & Brunton, N. P. (2012;2013;). Handbook of plant food phytochemicals: Sources, stability and extraction (1. Aufl. ed.). GB: Wiley-Blackwell.

Tsevegsuren, N., Edrada, R.A., Lin, W., Ebel, R., Torre, C., Ortlepp, S., Wray, V., Proksch, P., 2007. Four new natural products from Mongolian medicinal plants Scorzonera divaricata and Scorzonera pseudodivaricata (Asteraceae). Planta Medica 72,962–967

Ugur, A., Sarac, N., Ceylan, O., Duru, M. E., & Beyatli, Y. (2010). Chemical composition of endemic Scorzonera sandrasica and studies on the antimicrobial activity against multiresistant bacteria. *Journal of medicinal food*, *13*(3), 635-639.

Wang, Bin & Li, Guo-Qiang & Guan, Hua-shi & Yang, Li-ye & Tong, Guo-zhong. (2009). A new erythrodiol triterpene fatty ester from Scorzonera mongolica. Yao xue xue bao Acta pharmaceutica Sinica. 44. 1258-61.

Wang, Y., Edrada-Ebel, R.A., Tsevegsuren, N., Sendker, J., Braun, M., Wray, V., Lin, W., Proksch, P., 2009. Dihydrostilbene derivatives from the Mongolian medicinal plant Scorzonera radiata. Journal of Natural Products 72, 671–675.

Wu, Q. X., He, X. F., Jiang, C. X., Zhang, W., Shi, Z. N., Li, H. F., & Zhu, Y. (2018). Two novel bioactive sulfated guaiane sesquiterpenoid salt alkaloids from the aerial parts of Scorzonera divaricata. *Fitoterapia*, *124*, 113-119.

Wu, Q. X., Su, Y. B., & Zhu, Y. (2011). Triterpenes and steroids from the roots of Scorzonera austriaca. *Fitoterapia*, *82*(3), 493-496.

Yang CS, Landau JM, Huang MT, Newmark HL (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. Ann Rev Nutr 21:381–406. doi:10.1146/annurev.nutr.21.1.381

Yang, Y. J., Liu, X., Wu, H. R., He, X. F., Bi, Y. R., Zhu, Y., & Liu, Z. L. (2013). Radical scavenging activity and cytotoxicity of active quinic acid derivatives from Scorzonera divaricata roots. *Food chemistry*, *138*(2-3), 2057-2063.

Yang, Y. J., Yao, J., Jin, X. J., Shi, Z. N., Shen, T. F., Fang, J. G., ... & Zhu, Y. (2016). Sesquiterpenoids and tirucallane triterpenoids from the roots of Scorzonera divaricata. *Phytochemistry*, *124*, 86-98.

Yuan, Y. V., Bone, D. E., & Carrington, M. F. (2005). Antioxidant activity of dulse (palmaria palmata) extract evaluated in vitro. Food Chemistry, 91(3), 485-494. doi:10.1016/j.foodchem.2004.04.039

Zhu, Y., Wu, Q. X., Hu, P. Z., & Wu, W. S. (2009). Biguaiascorzolides A and B: Two novel dimeric guaianolides with a rare skeleton, from Scorzonera austriaca. *Food chemistry*, *114*(4), 1316-1320.

Zidorn, C. (2008). Sesquiterpene lactones and their precursors as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Phytochemistry*, *69*(12), 2270-2296.

Zidorn, C., Ellmerer, E. P., Sturm, S., & Stuppner, H. (2003). Tyrolobibenzyls E and F from Scorzonera humilis and distribution of caffeic acid derivatives, lignans and tyrolobibenzyls in European taxa of the subtribe Scorzonerinae (Lactuceae, Asteraceae). *Phytochemistry*, *63*(1), 61-67.

Zidorn, C., Petersen, B. O., Udovičić, V., Larsen, T. O., Duus, J. Ø., Rollinger, J. M., ... & Stuppner, H. (2005). Podospermic acid, 1, 3, 5-tri-O-(7, 8-dihydrocaffeoyl) quinic acid from Podospermum laciniatum (Asteraceae). *Tetrahedron letters*, *46*(8), 1291-1294.