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Research Article

The phenolic profile and biological activities of common *Scorzonera* species from Eastern Anatolia

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Abstract: The present study focused on chemical composition and base therapeutic potential of common three *Scorzonera* species (*Scorzonera mollis* M.Bieb. subsp. *mollis*, *Scorzonera papposa* DC. and *Scorzonera semicana* DC), which have been utilized as food and medicine by local people of Eastern Anatolia for a long time. Comparative analytical studies were performed on ethanol-based extracts and traditional preparations (infusions) through chromatographic (HPLC-MS/MS) and reagent-based antioxidant and enzyme inhibitory assays. Results revealed that leaf extracts were rich in phenolics, particularly hydroxycinnamic acids that were confirmed by HPLC-MS/MS, chlorogenic acids and luteolin were the major phytochemical compounds of extracts. With regards to biological activities findings, it was determined that ethanol-based extracts showed better antioxidant activities and effectively suppressed the activities of α-glucosidase. In addition, both of the extracts were found as strong suppressive agents of pancreatic lipase activity so *Scorzonera* species were rich sources of bioactive compounds that able to deactivate reactive oxygen species and free radicals and as well as suppress the activities of α-glucosidase and pancreatic lipase. Finally, obtained findings reveal base data of *Scorzonera* species for researches that focused on novel candidates of nutraceuticals and biotherapeutics.

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1. INTRODUCTION

Food and medicinal plant sources have been examined by mankind through the process of trial and error for a long time. It was documented that 5000 years ago Sumerian clay slab described twelve recipes from nearly two hundred fifty plants for food and drug preparation, which are still in use today (Petrosvka, 2012). Recently, the trend of natural alternatives usage as a source of new commercial products has become very popular. Although, the plant use in medicine is

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frequently undervalued, the active components of the plants, which allow them to perform the healing process, should be valued more (Reid *et al.*,, 2018).

Several researchers in Turkey have thoroughly investigated the use of wild medicinal plants over the last decade. Because of its varied weather and environmental zones, the eastern part of Anatolia has a plethora of the flora. This variety supplies an abounding source of medicinal plants (Özgökçe & Özçelik 2004; Ergün 2021). Medicinal plants are acknowledged to have antioxidant activities along with multiple bioactivities; for instance, antimicrobial, antiinflammatory, and antiallergic activities (Gülçin *et al.*, 2010).

Asteraceae is one of major flowering plants families globally, which chemically contained significant biologically active compounds (Hattori & Nakajima 2008; Funk *et al.*, 2009). *Scorzonera* genus is belong to Asteracea family among the most utilized species in food and medicinal plant culture in Turkey and there is a need to investigate their biologically active chemical compounds bioactivities to contribute the scientific literature of plant-based pharmaceuticals .

The main purposes of the present study were to (i) detect the biotherapeutic potential and (ii) chemical composition of leaf organs of three *Scorzonera* taxa (*Scorzonera mollis* M. Bieb. subsp. *mollis*, *Scorzonera papposa* DC. and *Scorzonera semicana* DC. Therefore, ethanol-based extracts and water-based infusion preparations obtained from plant leaf matrix were assayed for antioxidant and enzyme inhibitory activities. Major contributors of the activities were analysed by reagent-based spectrophotometric assays and HPLC-MS/MS.

2. MATERIAL and METHODS

2.1. Plant Materials

Leaf samples of *Scorzonera* species (*S. mollis*, *S. semicana* and *S. papposa*) were collected from the Van/Türkiye (Table 1). Plant samples were transferred to the laboratory at the appointed time (within 2 hr). The identification of the plant samples was done at Van Yuzuncu Yil University, Faculty of Pharmacy. Voucher specimens were placed at Van Pharmaceutical Herbarium. The plant materials were cleaned properly for dust and contaminants and air-dried in the dark at room temperature (RT) (22±2 °C) for 96 hr. Subsequently, they were ground into a fine powder using a grinding mill.

Table 1. Location and herbarium details of *Scorzonera* species.

Species	Location/GPS/Date	Herbarium Code
Scorzonera mollis	Van/Türkiye, Çatak, Konalga village, steppe/ 37° 50' 255"N 043° 09' 857"E; 2258 m/ 08.05. 2019,	Herbarium code: VPH553; Collector code: DM350.
Scorzonera semicana	Van/Türkiye, Çatak, Konalga village, steppe/ 37º50'191"N 043º08' 185"E, 2290m/ 09.05.2019	Herbarium code: VPH355; Collector code: DM301
Scorzonera papposa	Van/Türkiye, Çatak, Konalga village, steppe/ 37º 51' 555" N, 43º 08' 984" E, 1808m/ 12.05. 2019	Herbarium code: VPH356; Collector code: DM302

For extraction of plant samples two different extraction methods (aqueous extraction and herbal infusion) were preferred. The plant samples were prepared for in accordance to Dalar *et al.*, 2012 with slight adjustments. In brief, 1 g of pulverized plant materials were thoroughly mixed with 10 mL of ethanol (80%) then set approximately 5 minutes for shaking at RT. Subsequently, the solutions were sonicated at 37 °C for 40 minutes then incubated for 24 hr at 4 °C. Extracts were centrifuged at 15,300 x g for 20 minutes at 4 °C, aliquots of the supernatants were collected kept at -20 °C to be subsequently analyzed.

Herbal infusion extracts preparation was done according to Baytop 1999, from the pulverized plants. In brief, plant material was mixed with a 20-fold volume (g/mL) of pre-boiled

distilled water then left for incubation for 10 min followed by sonication for 10 minutes at 50 °C for homogenizing. The solutions were left for overnight incubation at 4 °C, Next the mixtures were filtered by cotton and vacuum filtering. Supernatants were stored (-20 °C) until analyses.

2.3. Determination of Antioxidant Capacities

In ABTS capacity the extracts were evaluated for their antioxidant activities by the decolorization of ABTS radical (Re *et al.*, 1999). The resulting color changes were measured using spectrophotometer and the results were expressed as percent inhibition of absorbance at 734 nm compared with the ascorbic acid regression curve.

Determination of total reducing capacity of the extracts were done using the FRAP assay (Benzie & Strain, 1996) with minor modifications as described by Dalar *et al.*, (2012) and the reducing capacity of the extracts were expressed as mmol of iron (Fe^{2+})/g dw according to iron sulphate standard (Fe_2SO_4) curve against a blank control. The analyses were performed in triplicate. Folin-Ciocalteu reducing (FCR) assay was performed as described formerly by Ainsworth and Gillespie (2007), with minor modifications as described by Dalar *et al.*, (2012). Shortly, plant extracts already prepared in the selected solvent for extraction were mixed with Folin-Ciocalteu reagent which had been previously diluted with MilliQ water in 96-multiwell microplates. The absorbance was measured at 600 nm for ascorbic acid correction. Next, the addition of Na_2CO_3 to the microplate was done. The absorbance was measured once more at 600 nm. The results were expressed as mg gallic acid (GA) E/g dw according to GA standard curve. The analyses were performed in triplicate

The assay of oxygen radical absorbance capacity (ORAC) was done using the ORAC assay according to Prior *et al*. (2005) with some modifications as described previously by Dalar *et al.*, (2012). The ORAC capacities of the samples was expressed as mmol Trolox equivalents (E)/g dw according to Trolox standard curve.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was performed according to Konczak *et al.*, (2003). In brief, the prepared sample solution in the solvent used for extraction, 4-morpholineethanesulfonic acid (MES) buffer, and DPPH solution were combined and set for 2 minutes at RT. The decline in absorbance of DPPH at 520 nm was measured employing the microplate reader. The measurements were assessed in triplicate. The relative DPPH inhibitions (%) were calculated applying the equation of; [(Abs Control-Abs Sample) /Abs Control]*100

2.4. Enzyme Inhibitory Activity

Pancreatic lipase inhibitory activity was performed as described by Dalar & Konczak (2013) 4-methylumbelliferyl oleate was used as a substrate, except for porcine pancreatic lipase (Sigma type II). Sample solutions were prepared employing citricphosphate buffer. 4-Muo and the enzyme solution were placed in a 96-multiwell microplate and incubated for 20 minutes at 37°C. The reaction was ended with the addition of hydrochloric acid and sodium citrate, respectively. The lipase inhibition activity was relatively calculated applying the formula: % Inhibition = (1- (FS-FSB) / (FC-FCB)) x100. While the FS and FC were the sample values as well as negative control measured fluorometrically at an emission wavelength of 460 nm and excitation of 320 nm, respectively.

The inhibition of α-glucosidase enzyme, the assay was assessed with mild adjustment. Dalar & Konczak (2013). Sucrose was applied as a substrate. The enzymatic reaction was ended by increasing the temperature of the mixture to 100 °C by heating for 10 minutes and the absorbance was measured at 505 nm.

2.5. Antimicrobial Activity Test

The antimicrobial capacity of each of the extracts (ethanol and water) were examined against six different bacterial pathogen applying disc diffusion method. Pencilline was the positive control. This test was assessed as formerly described by Boussaada *et al.*, (2008) with mild adjustment.

2.6. Phytochemical Profile

Total phenolics levels of the extracts were done by high-performance liquid chromatography as described previously, (Dalar & Konczak 2013). The levels of total phenolics were expressed as mg chlorogenic acid/g extract. Total flavonoid content **(**TFC) was determined according to previous description by Dalar *et al.,* (2012) with minor changes. In brief, plant extracts prepared in the solvent were placed in 96-multiwell microtiter plates. Next, addition of dH_2O and $NaNO_2$ (1:20 w/v in water) took place, then incubation was followed further $AlCl_3$ was added. The samples were set to be incubated for 6 minutes at RT then NaOH was added and mixed thoroughly till samples turned pinkish into a pinkish color. The absorbance was measured at 510 nm. The extracts were tested in triplicates and the results were expressed as mg rutin (R) E/g dw, The Total hydroxycinnamic acids (THA) content THA contents were determined According to Dalar *et al.*, (2012). In brief, plant extracts were placed in 96-multiwell microplate and HCl in 95% ethanol was added, followed by addition of HCl. The solution was mixed thoroughly and left ~15 minutes at RT.

2.7. Identification and Quantification of Phenolic Compounds

Identification and quantification of phenolic compounds were done using high performance liquid chromatography-diode array detector (HPLC-DAD) and liquid chromatography-photo-diode array–mass spectrometry (LC-DAD–MS/MS) on a Quantum triple stage quadrupole mass spectrometer equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an auto sampler (Thermo Fisher Scientific, Waltham, MA, USA) as described previously (Dalar & Konczak, 2013). An aliquot (3 µl) of each sample solution prepared in the solvent used for extraction was chromatographed on a 150 x 2.1 mm, 5 µm Luna Synergy Hydro column (Phenomenex, Torrance, CA, USA) which was heated to 30 °C. Analytes were separated using 0.5% formic acid and acetonitrile with a flow rate of 200 µl/min. The photodiode array detector was used to acquire data from 190-520 nm.

The composition of phenolic compounds was characterized based on their UV spectrum, retention time, co-chromatography with commercial standards, when available, and MS/MS fragmentation patterns. Mass spectrometry data were obtained using an electrospray source in either the positive (quercetin 3-glucoside) or the negative (chlorogenic acid) modes. MS experiments in the full scan (parent and product-specific) and the selected reaction monitoring (SRM) mode were done. The quantification of phenolic compounds was done using the HPLC-DAD system, which consisted of two LC-10ADVP pumps, SPD-M10ADVP diode array detector, CTO-1-ADVP column oven, DGU-12A degasser, SIL-10ADVP auto injector, and SCL-10A system controller equipped with an Atlantis column (dC_{18}, 4.6 x 100 mm, 5 µm particle size) (Waters Associates, Milford, MA, USA). Analytical HPLC was run at 30 °C and monitored at 250, 280, 320, 370 and 520 nm. Injection volume was 10 µl. The levels of phenolic compounds were quantified as authentic standard E/g dw based on a calibration curve.

To assist the spectrophotometric analysis of phenolic composition, the extracts were hydrolysed according to Pinto et al. (2008). Briefly, lyophilized plant extracts (10 mg) were added to 2 mL of 2N trifluoroacetic acid (TFA) in pyrex glass tube. The tube was placed into a heating instrument and maintained at 120 °C for 2 h. Subsequently, the solution was transferred into 5 mL volumetric flask and 80% methanol was added to adjust the volume. The extract was then directly analysed by HPLC-MS/MS as described above.

3. RESULTS

3.1. Antioxidant and Enzyme Inhibitory Activities

Antioxidant abilities of the extracts were presented in Table 2 through complementary methods including FCR, FRAP, ORAC, DPPH, and ABTS assays. Findings revealed a pattern of *S. papposa* ≥ *S. mollis* > *S. semicana* in ethanol extracts. A similar pattern was observed in infusion preparations with slight differences in ORAC and DPPH results, where *S. semicana* exhibited pronounced activities (Table 2). Ethanol-based extracts had higher antioxidant values which were approximately double that of the infusion preparations, and therefore it can be concluded that ethanol solvent had more effective than water solvent in terms of extracting of antioxidative compounds (Table 2).

Table 2. Antioxidant capacities of ethanol-based extractions and infusion preparations

Taxa	FCR [1]		FRAP [2]		ORAC [3]		DPPH (%) [4]		ABTS(%)[5]	
	Ethanol	Infusion	Ethanol	Infusion	Ethanol	Infusion	Ethanol	Infusion	Ethanol	Infusion
SP	15.6±1.1^{a}	8.3±0.7^{a}	796.3±26.6^{a}	318.9±25.8^{b}	1573.1±77.0^{a}	801.1±43.6^{b}	73.9±1.0^{a}	35.7±1.4c	98.92±6.3^{b}	90.15±8.9^{a}
SM	17.5±1.2^{a}	8.6±0.3^{a}	780.6±35.8^{a}	378.4±8.6^{a}	1483.1±68.9^{a}	768.9±22.9^{b}	73.1±1.1^{a}	58.8±1.7^{b}	93.54±8.2^{a}	83.53±4.5^{b}
SS	9.4±0.4^{b}	6.9±0.2^{b}	409.7±14.3^{b}	308.6±4.9c	1256.8±21.9^{b}	885.6±33.3^{a}	69.9±1.6^{b}	66.4±2.7^{a}	94.15±7.9^{a}	84.61±5.2^{b}

Means with different letters in the same column were significantly different at the level (p<0.05); n=9. [1] Folin-Ciocalteu reducing-total phenolics content–mg Gallic acid Eq. /g dw, [2] Ferric reducing antioxidant power – µmol Fe $^{2+}$ /g dw, [3] Oxygen radical absorbance capacity - µmol Trolox Equivalent/g dw, [4] DPPH radical scavenging activity and [5] ABTS % inhibition at 1 mg/mL concentration. (Taxa: SP: *S. papposa*, SM: *S. mollis;* SS: *S. semicana*)

With regards to enzyme inhibitory activities, suppressive abilities of the extracts were measured towards pancreatic lipase and alpha-glucosidase (Table 3). Similar to antioxidant findings, ethanol extracts had superior activities than infusion preparations. All species showed similar and pronounced antilipase activities. With regards to glucosidase enzyme, *S. papposa* in ethanol extracts and *S. semicana* in infusion preparations had better inhibitory activities (Table 3).

Table 3. Enzyme inhibitory activities of ethanol-based extractions and infusion preparations.

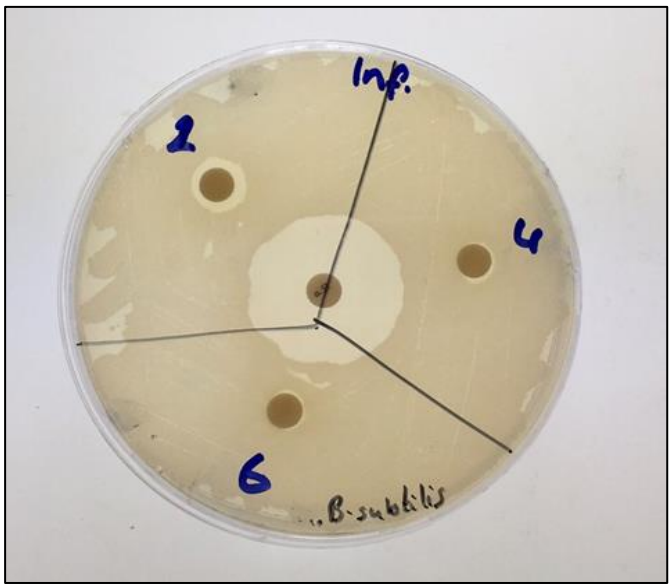
Taxa	Pancreatic Lipase (%)*		Alpha Glucosidase (%)*	
	Ethanol	Infusion	Ethanol	Infusion
S. papposa	96.5±0.9^{a}	74.2±6.0^{a}	82.1+1.0^{a}	62.1±1.1^{b}
S. mollis	96.3±0.6^{a}	72.9±2.3^{a}	78.2±0.8^{b}	60.2±1.0^{b}
S. semicana	96.4±0.8^{a}	71.5±3.0^{a}	79.5±1.3^{b}	71.3±0.9^{a}

Means with different letters in the same column were significantly different at the level (p<0.05); n=3.
*Inhibition of samples at 1 mg/mL concentration.

3.2. Antimicrobial Activity

The antimicrobial activity was tested for each ethanol extracts and infusion extracts against pathogenic bacteria. The obtained results from the ethanol extract of *S. papposa*, *S. mollis* and *S. semicana* showed no significant effect against *P. aeruginosa*, *S. aureus*, *B. cereus B. subtilis*, *E. faecalis* or *E. coli*. However, for the water extracts *S. papposa* displayed a converse effect as opposed to *S. mollis* and *S. semicana.* Unpredictably the zone of inhibition of approximately 5mm was obtained against *Bacillus subtilis* (Figure 1). Evaluation of antibacterial activity was extended further when the extracted were lyophilized and the antimicrobial test was repeated after the lyophilizing, yet no significant difference in the results were obtained.

Figure 1. Antimicrobial results of ethanol-based extractions and infusions



3.3. Phytochemical Profile

Preliminary studies of phytochemical profile were conducted through reagent-based spectrophotometric assays following mass data based chromatographic studies by LC-MS/MS. The preliminary studies showed that the extracts were rich in total phenolics and phenolics subgroups including flavonoids and particularly hydroxycinnamic acids. The similar pattern of *S. papposa* ≥ *S. mollis* > *S. semicana* observed in total phytochemical content determination (Table 4).

Table 4. Phytochemical profile of ethanol-based extractions and infusion preparations

Taxa	Total phenolics[1]		Total flavonoids[2]		Total hydroxycinnamic[3]	
	Ethanol	Infusion	Ethanol	Infusion	Ethanol	Infusion
S. papposa	30.1±1.0^{a}	14.1±0.8^{a}	6.7±0.2^{b}	2.4±0.2b	22.8±1.5^{a}	9.6±0.8^{a}
S. mollis	32.4±1.2^{a}	14.7±1.0^{a}	8.9±0.4^{a}	2.9±0.4^{a}	24.2±1.1^{a}	9.2±1.0^{a}
S. semicana	16.5±0.9^{b}	11.9±0.5^{b}	3.4±0.3^{c}	3.0±0.2^{a}	11.6±0.9^{b}	7.3±0.2^{b}

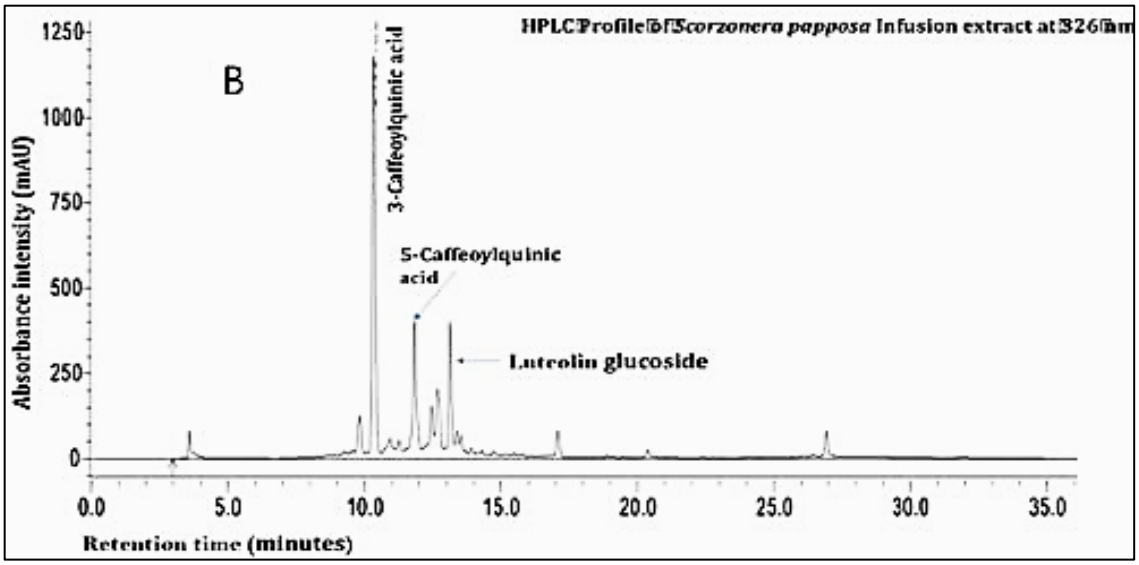
Means with different letters in the same column were significantly different at the level ($p< 0.05$); n=3. [1] mg Chlorogenic acid Eq./g dry weight at 326 nm by HPLC, [2] mg Rutin Eq./g dry weight, [3] mg Chlorogenic acid Eq./g dry weight.

Subsequently, HPLC-MS/MS studies were performed to confirm preliminary phytochemical studies. According to MS/MS data three major phenolics were found in the extracts (Figure 2). Caffeoylquinic acids were the dominant phenolics followed by luteolin glucoside, which confirms preliminary chemical content results (Table 5).

Table 5. Phenolic composition of *Scorzonera* species from Eastern Anatolia by HPLC-MS/MS profile.

MS/MS	3-Caffeoylquinic acid		5-Caffeoylquinic acid		Luteolin glucoside	
-/[M-1]$^-$	-/353		-/353		449/447	
Fragments (m/z) (+/-)	-/191, 179		-/191, 179, 173		287/285	
Concentration (mg/g)	Ethanol	Infusion	Ethanol	Infusion	Ethanol	Infusion
S. papposa	16.6±0.9^a	7.5±0.4^a	3.5±0.2^a	2.2±0.1^a	5.2±0.3^b	2.1±0.1^b
S. mollis	17.3±0.8^a	7.7±0.1^a	3.8±0.1^a	1.7±0.1^b	6.9±0.1a	3.3±0.1^a
S. semicana	8.4±0.2^b	5.1±0.2^b	2.7±0.1^b	1.7±0.0^b	2.9±0.0^c	3.1±0.0^a

Figure 2. Representative HPLC profile of *Scorzonera* species



4. DISCUSSION and CONCLUSION

Investigating various food sources and medicinal plants in order to obtain effective antioxidants and enzyme inhibitors are extremely accredited by researchers for the effective controlling and (optionally management) avoidance of many illnesses (Ak *et al.*, 2020). The recovery rate of phytochemicals varies depending on the type of extraction and solvent applied, solubility and chemical nature of phenolic compounds targeted and/or present in the matrix. A major factor which is critical for the compound's solubility in the plant samples is the polarity rate of used solvent (Luthria & Biswas 2007; Ergün 2022). Extraction of *Scorzonera veratrifolia* by different solvent and method exhibit variable results comparing to our study (Taskın *et al.*, 2021)

The limited scientific record related to the antioxidant capacity of *Scorzonera* species in the literature provoked the need to perform this study in order to provide up to dated data about the selected species. In order to accomplish the successive evaluation of the antioxidant capacity of *S. papposa*, *S. mollis* and *S. semicana* extracts, four bioassays were conducted. Since different methodologies are required to comprehend the biological activity of multipart mixture of secondary metabolites (Gironés *et al.*, 2012).

The antioxidant capacities of extracts from three different species of plants from the Asteraceae family were detected by applying DPPH, FCR, FRAP, ABTS, ORAC assays to

confirm the consistency of the results. Generally, the results from all of the following assays, DPPH, FRAP, ABTS and ORAC displayed a high antioxidant capacity. Interpreting the results of FRAP its indicated that the ethanol extract of *S. papposa* and *S. mollis* had the highest scavenging activity. This suggests the suitability of ethanol in extraction procedure of *S. papposa* and *S. mollis*. This result is in line with Kenny *et al.,* (2014) and Moe *et al.,* (2018) who recorded similar concept about the effectiveness of ethanol use as a solvent. While for infusion extracts there was variability in the results which by *S. mollis* exhibited the highest antioxidant activity following *S. papposa* and lastly *S. semicana*, the variation could be because of each method of antioxidant capacity detection has a different mechanism for scavenging the radicals.

Moreover, the obtained antioxidant activity from FCR, DPPH, FRAP, ABTS and ORAC displayed variability in the antioxidant potential. The variability might be resulted from diverse mechanism of action of different assays and to the variety of chemical constituents. On the other hand, the aerial parts of plants which are rich in antioxidant compounds infusion extracts seems to be verified to be effectual for recovering effective compounds.

A linear relation between the antioxidant potency and TPC reveal the outcome of higher phenolic content provides the higher antioxidant potency. Due to the significantly high levels of phenolic compounds in the particular species of *Scorzonera* in our study, the species might be counted as a supply for natural antioxidants. There are multiple previous studies assessed assays about antioxidant abilities of some other species of *Scorzonera* L. which in accordance with our findings. Milella *et al.*, (2014) reported a high antioxidant potential of both *S. papposa* and *S. judaica*, which then lead to find a base for additional investigation on dihydroisocoumarins and phthalates. In addition, it can be argued that ethanol has better extraction capability than water due to its effectiveness capability of isolation of phenolic compounds from plant matrix and polarity index, which provides to extract utmost of the polar and relatively polar constituents (Mükemre *et al.,* 2020).

A major enzyme for absorbing lipid by hydrolysis of total dietary fats is pancreatic lipase enzyme. Hence, obesity which is one of the common diseases suggested to be controlled by regulating the pancreatic lipase activity inhibition. Pancreatic lipase (triacylglycerol acylhydrolase) is critical enzyme for absorbing triglycerides gained from nutrition (Slanc *et al.,* 2009). The results of both (ethanol and infusion) extracts revealed a high activity of pancreatic lipase, which implies that these herbs appear to be a powerful source for the inhibition of pancreatic lipase enzyme and therefore could be used as a safe natural product for the management of obesity.

Searching through literature, several species belonging to the same family (Asteraceae) have been investigated previously however, it is noticed that the *Scorzonera* species investigated in this study haven't been examined before for their anti-lipase activity. For comparison, both of the *Cnicus benedictus* L. and *Bellis perennis* L. belong to Asteraceae showed 40–70% pancreatic lipase inhibitory activity. However, *Arctium lappa* L extract from the same family showed pancreatic lipase inhibitory activity under 40% reported by Slanc *et al.,* (2009). While our results of the pancreatic lipase enzyme inhibition ranged (71.5-96.5%). Furthermore, from the same study the inhibition activity of glycosidases (α-amylase, α and β-glycosidases) which are major enzymes involved in the pathology of diabetes were detected from the lyophilized methanol extracts. Detected results revealed inhibition of α and β-glycosidases unlike amylase which showed no significant inhibition (Souza *et al.,* 2011). In our study, we also detected the inhibition of α-glucosidase activity. Since α-glucosidase is a major enzyme involved with declining the blood glucose in diabetics (Kwon *et al.,* 2008; Agarwal & Gupta 2016). Overall, comparing our results for the α-glucosidases inhibition activity, the found results showed a significantly high α-glycosidases inhibition activity. The results ranged between 60.2-82.1%

with ethanol extract of *S. papposa* being the highest 82.1% and infusion extract of *S. mollis* being the lowest 60.2%. While Ak *et al.,* (2020) reported their study on *S. hispanica* showed a poor inhibitory activity for both (a-amylase and a-glucosidase. Similarly, a study report by Dall'Acqua *et al.,* (2020) detected a modest α -amylase 0.08 mmol (ACAE)/g) and α glucosidase 0.09 mmol (ACAE/g) inhibitory activity of *Scorzonera tomentosa* L. (Dall'Acqua *et al.,* 2020).

Thus, the revealed results from our study gives credit to the popular use of the studied *Scorzonera* species for treating diabetes. The α-glucosidase inhibition rate was positively correlated with TPC, the higher the phenolic content the higher α-glucosidase inhibitory activity. Our results are in line with Mai *et al.* (2007); Wongsa *et al.*,, (2012) detected a strong linear relation between caffeic acid content and α amylase inhibitory activity ($r = 0.68$, $p < 0.05$), whereas, the correspondence relation between caffeic acid content and p-coumaric acid content with inhibition against α -glucosidase was 0.28 and 0.33 respectively.

Current research has examined the antimicrobial activity against Gram-negative bacteria *P. aeruginosa* and *E. coli* as well as Gram-positive bacteria *E. faecalis B. cereus S. aureus,* and *B. subtilis* using disc diffusion method and Penicillin was applied as positive control. The results from the ethanol extracts had not shown a substantial antimicrobial activity on the gram negative or positive bacteria. Results from our study are in accordance with (Vergun *et al.*,, 2018; Sarı *et al.*,, 2019; Şahin *et al.*,, 2020). Sarı *et al.,* (2019) stated that no antimicrobial activity was detected against *S. aureus, E. coli and P. aeruginosa* apart from a weak activity against *E. faecalis*. Moreover, Şahin *et al.,* reported that fractions from *S. pygmaea* displayed no particular antimicrobial activity against tested bacteria or fungi. Boussaada *et al.,* 2008 investigated *Scorzonera undulata* subsp. *deliciosa* oil and detected that the antimicrobial capacity of the oil. This indicates that environmental factors such as soil composition, region, climate, humidity, can alter the structure of the bioactive compounds that are in control of the antimicrobial activity.

Many different compounds such as dihydro-isocoumarins, flavonoids, lignans, phenolic acid have been previously identified through chemical composition analysis of the *Scorzonera* genus (Abd el Raheim, 2016). Considering the results from the HPLC-MS/MS profile, three major phenolic compounds 3-Caffeoylquinic acid, 5-Caffeoylquinic acid and Luteolin glucoside were isolated from both infusion and ethanol extracts. Similar results were observed regarding 3-Caffeoylquinic acid among all of the investigated *Scorzonera* species in this study meaning that both ethanol and infusion are effective for extracting these phenolic compounds. This result is contrary to several studies in the literature which mostly indicates that a solvent with a higher polarity such as ethanol or methanol are better solvent for extracting phenolic content rather than water (Dall'Acqua *et al.,* 2020).

The results from our study are compatible with previous studies about various *Scorzonera* species that have found similar compounds (Granica *et al.,* 2015) has detected several chlorogenic acid isomers including 4-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 5-O-feroylquinic acid, 5-O-caffeoylquinic acid. It has been reported that luteolin (Rees and Harborne, 1984), luteolin 3'-(6-E-p-coumaroyl-beta-d-glucopyranoside) (Jiang *et al.,* 2007) has been detected in members of the *Scorzonera* genus. Additionally, number of mono and di-caffeoylquinic acids, hydroxycinnamates, and luteolin glycosides have been found as well as two luteolin-7-O-glycosides (Schütz *et al.,* 2005). Furthermore, our results were also in line with Şahin *et al.,* (2020), who used a spectroscopic technique in order to identify the presence of the derivatives of 3 phenolic acid (chlorogenic acid methyl ester, 3,5-di-O-caffeoylquinic acid, and chlorogenic acid). Comparing the species, ethanol and infusion extracts of *S. papposa, S. mollis* contained the highest 3-Caffeoylquinic and 5-Caffeoylquinic acid compound compared to *S. semicana*. This could be related with the fact that those methods of extraction

are most appropriate for isolating most of the phenolic compounds in those species. However, both of the ethanol and infusion extracts of *S. mollis* seemed to have the highest luteolin glucoside content interestingly same result applied to *S. semicana*.

While contrary ethanol extracts of *S. semicana* exhibited the lowest luteolin glucoside content. This could be due to genetic variation among the species and their correspondence to the polarity of the different kind of solvents that is applied for extracting them. There is a link between phenolic compounds and suppression of several enzyme activities which involved in metabolic disorders as previously reported by (Mai *et al.*, 2007; Gonçalves & Romano, 2017). There are several amino acids of various enzyme's protein which some phenolic compounds attach to (Oboh *et al.*, 2015). For instance, CA attaches to Phe78, Pro181, His264, Tyr115, and Ser153A.A in pancreatic lipase (Martinez *et al.*, 2017).

This is the first report of antioxidant testing and phytochemical composition of native botanicals including *S. papposa, S. mollis, and S. semicana.* In conclusion, the obtained results highlighted the potential benefits of the three species of *Scorzonera* as sources of bioactive constituents; it is thought that they play important role in managing metabolic disorders. Thereby, the use of these plants in pharmaceutical industry is strongly recommended due to the effectivity of their antioxidant, key enzyme inhibition potential and rich phenolic compound constituent. Among native botanicals investigated within this study, *Scorzonera mollis* might serve bioactive antioxidant both for industrial and pharmaceutical use. Nevertheless, more detailed research work must be perfomred to evaluate and optimize the extraction methods and detection of specific mechanism of action.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

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