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CHEMICAL PROFILE OF SCORZONERA UNDULATA GROWN IN LIBYAN WESTERN COAST

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ABSTRACT
The chemical composition and antioxidation ability of *Scorzonera Undulata* plant was investigated. Phytochemical screening tests of ethanolic extract of aerial part of the plant revealed the presence of all known phytochemical compounds; alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins, terpenes and quinones. The plant showed moderate antioxidation ability with IC_{50} of 33.37 µg/ml. GC-MS technique was also used for chemical composition analysis, the results showed presence of fiftyone compounds with very diverse chemical classes including fatty acids and their esters, long chain hydrocarbons, long chain alcohols and heterocyclic compounds. The most abundant compounds were: 17-Octadecynoic acid, (*E*)-9-Tetradecen-1-ol, acetate, *E*-7-Tetradecenol, (*Z*,*Z*)-9,12-Octadecadien-1-ol, 4,9-Tetradecadien-1-ol, Deoxyspergualin, Undecane, 6,6-Methyl-2-Methylundecane-6,6, Trans-3-Cyclopentene-3,5-Diol.

KEYWORDS: *Scorzonera Undulata*, GC analysis, Fatty acid esters, Bioactive compounds.

INTRODUCTION
The genus *Scorzonera* L. belongs to the sunflower family (Asteraceae), Lactucoideae subfamily, Lactuceae tribe and Scorzonerinae subtribe.[1] *Scorzonera* genus belonging to Asteraceae family is widely distributed in Eurasia and northern Africa with about 160 species. Podospermum genus (*Asteraceae*), represented by several tens of species, and also grows mainly in Mediterranean and Western Asia. Members of the *Scorzonera* genus are used as vegetables and medicinal plants. Phenolic compounds such as dihydroisocoumarins, bibenzyl derivatives, flavonoids, lignans, stilbene derivatives, quinic and caffeic acid derivatives, sesquiterpenes and triterpenes have been isolated from *Scorzonera* species.[2] *Scorzonera undulata* subsp. *deliciosa* (Guss.) Maire (Figure 1) is a perennial plant growing to 0.3 m. The stems are simple possessing of linear lanceolate leaves. The flowerheads have a cylindrical involucre of green bracts with purpurins and herma phrodites flowers. The external bracts are ovals and applied to the interns ones. The roots are fleshy. The akenes are slightly inflated and corded in their lower part.[1]

Figure 1: *Scorzonera undulata* plant.

The plant is well known in Libya and distributed mostly in the northern part of the country, flowering in the spring time where it is especially abundant in the pastures and slopes, grows mostly in sandy or clay but well-drained soil. *Scorzonera* genus, which are a significant part of Turkish traditional medicine in the therapy of arteriosclerosis, kidney disorders, wounds, rheumatism, but also as antidiabetic, antihypertensive, and antinociceptive medications.[3] The *S. undulata* sub sp. *deliciosa* oil exhibited an interesting antibacterial activity against gram-positive and gram-negative bacteria but no antifungal activity was detected.[4] Geroushi and his co-workers performed a biological study on aerial and roots parts *of S. Undulata* from Libya, the

methanolic extract of showed promising antimicrobial, antibacterial and laxative properties.[5]

MATERIALS AND METHODS

Plant material

Scorzonera Undulata plant was collected from Gaser Khiar region (East of the capital Tripoli, 32°46'26"N 13°46'56"E) in flowering time, March of 2022. The plant was identified by plant taxonomist from Botany department, Science Faculty, Elmergib University. The plant material was cleaned and foreign materials were removed, dried in the shade then grinded to a fine powder using electrical blender, the powder was stored in airtight container and stored at room temperature until further use.

Extraction method for phytochemical screening

About 10 g of the plant powder and 200 ml of the solvent was put in 250 ml flask, then stirred for 24 hours using Jenway 1002 Stirrer machine at room temperature to keep most of the low boiling point components. Different polarity solvents were used to guarantee extraction of all types of chemical classes, the solvents were; ethyl acetate, ethanol, chloroform and hexane. Aqueous extract was prepared by heating 10 g of the powder in 200 ml of water at 70 °C for 20 min., after cooling the mixture was filtered and kept in fridge. The extracts were filtered then concentrated using water bath then kept until using in phytochemical screening process illustrated by Harborne.[9]

DPPH radical scavenging method

Free radical scavenging activity of different extracts of leaves plant were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 m*M* solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/ml). Here, only those extracts are used which are Solubilize in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS milton roy). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.[7] The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or inhibition Percent = $A_0 - A_1 / A_0 \times 100$.

Where A_0 the Absorbance of control reaction, A_1 the Absorbance in of test sample

GC-MS Analysis

About 10ml of methanol was added to 2.0g of a homogenized powder sample, the mixture was shaken vigorously for 60 min to transfer phytochemicals from the sample matrix into the organic layer. The extract was centrifuged and the supernatant was collected and filtered through 0.2µm syringe to remove particulate matter. The filtered extract was concentrated using rotary evaporation. The dried concentrated extract was dissolved in 5.0ml ethanol, then, 1.0µl of reconstituted sample was injected into the GC injection port using a microliter syringe.[8]

The chemical composition of roots powder ethanolic extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 °C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290 °C by 30 °C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260 °C respectively; Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min. and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical analysis of different solvent extracts of ***S. Undulata*** are shown in **Table 1.** The tests revealed the presence of all known phytochemical compounds; alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins, terpenes and quinones.

Table (1): Phytochemical screening of *Scorzonera Undulata* extracts.

Test \ Extract		EtAc	EtOH	$CHCl_3$	H_2O	Hexane
Alkaloids	Wagner	-	+	-	+	-
	Dragendrof	-	+	-	+	-
Carbohydrates	Molish	+	+	+	+	+
Reducing sugars	Fehling	+	+	+	+	+
Glycosides	Keller- Kelani	+	+	+	+	-
Saponines		-	+	-	+	-

Phytosterols	Salkowsky	+	+	-	+	-
	Liberman	+	+	+	+	-
Phenols		-	+	-	+	-
Tannins		+	+	-	-	-
Flavonoids	Basic test	+	+	+	+	+
	Lead acetate	+	+	+	+	-
Proteins	Xanthoprotein	+	+	+	+	-
	Nenhydrin	-	+	-	+	-
Terpenes		-	-	-	+	-
Diterpenes		-	+	-	+	-
Quinones		+	+	+	+	-
Anthraquinones		-	-	-	-	-

Ethanolic extract showed presence of all phytoconstituents except anthraquinones. Carbohydrates, reducing sugars and flavonoids were present in all extracts, while phytosterols, glycosides, proteins and quinones were present in all extracts except hexane extract, tannins were absent in aqueous, chloroform and hexane extracts, alkaloids, saponins, amino acids, phenols and terpenes were absent in the ethyl acetate, chloroform and hexane extracts.

Phytosterols act as an adjuvant in the prevention and treatment of cardiovascular diseases. Anthraquionoes derivatives have been used as laxatives and antimicrobial and anti-inflammatory agents, in addition to treatment of constipation, arthritis and cancer. Tannins are working as anticancer, virucides, antioxidant, antimicrobial and anti-inflammatory agents.[9] Phenolic compounds, flavonoids exhibited with antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities.[10]

Antioxidation analysis

The extract of *Scorzonera Undulata* plant showed low antioxidant potential when compared with the standard, ascorbic acid by DPPH scavenging assay method. The better antioxidant agents should have lower IC_{50} value, especially comparing with well-known super scavenging agent, ascorbic acid.[11] IC_{50} value of studied plant was 33.37 µg/ml, higher than ascorbic acid with 13.49 µg/ml, indicates mostly to a low content of phenols and flavonoids.

GC-MS analysis

GC-MS analysis was performed on ethanol extract of ariel part powder of *S. Undulata* plant as mentioned in the experimental part, affording the chromatogram shown in **Figure 2**. The identification was based on the comparison of the retention times and molecular weights to those found in NIST spectra database, the compounds were very diverse; unsaturated fatty acids and their esters, substituted aromatic hydrocarbon, long chain alkanes and alkenes, substituted heterocyclic aromatic compounds and wide range of phenolic compounds.

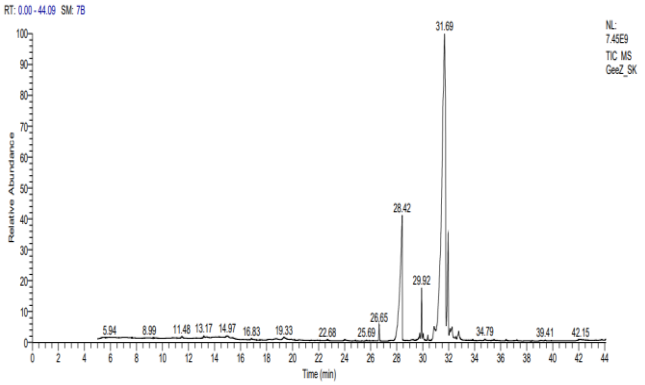


Figure 2: GC-MS chromatogram of ethanolic extract of *S. Undulata* plant.

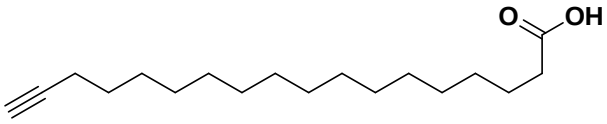
Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The retention times, names, peak areas, molecular weights and molecular formulas of all components are shown in Table 2.

Table 2: Compounds identified from GC-MS analysis of ethanolic extract of *S. Undulata.*

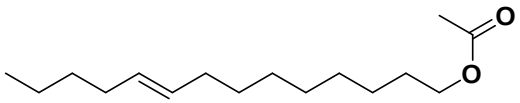
No.	RT	Compound Name	Peak Area	MW	MF
1	26.65	2-Aaminhexanoic acid	1.13	131	C6H13NO2
2	26.65	10-Undecnoic acid methyl ester	1.13	198	C12H22O2
3	26.65	DL-Norleucine	1.13	131	C6H13NO2
4	26.62	Cyclopentane undecanoic acid, methyl ester	1.13	268	C17H32O2
5	28.42	Deoxyspergualin	17.27	387	C17H37N7O3
6	28.42	Undecane	17.27	172	C12H26
7	28.42	Cyclopentene-3,5-Diol	17.27	101	C5H8O2
8	28.42	Dodecane	17.27	172	C12H26
9	29.78	*Cis*-3-Methyl-2-(2'-Pentenyl)- Cyclopent-2-en-1-one	0.49	168	C11H16O
10	29.78	(Z,Z,Z)-8,11,14-Eicosatrienoic acid	0.49	306	C20H34O2
11	29.78	*C*is-1,2-c*is*,*trans*-5,9-Cyclododecadienediol	0.49	196	C12H20O2
12	29.78	(Z,Z)-9,12-Octadecadienoyl chloride	0.49	298	C18H31ClO
13	29.92	Methyl 12,13-tetradecadienoate	3.68	238	C15H26O2
14	29.92	(*Z*,*Z*)-9,12-Octadecadienoyl chloride	3.68	298	C18H31ClO
15	29.92	13,16-Octadecadienoic acid, methyl ester	3.68	294	C19H34O2
16	30.04	10-Methyl-E-11-tridecen-1-ol propionate	0.48	268	C17H32O2
17	30.04	Methyl 12,13-tetradecadienoate	0.48	238	C15H26O2
18	30.04	1,12-Tridecadiene	0.48	180	C13H24
19	30.04	2-Hexyl Cyclopropaneacetic acid	0.48	184	C11H20O2
20	30.39	3-Nonenoic acid methyl ester	0.39	172	C10H18O2
21	30.39	7- Nonenoic acid methyl ester	0.39	172	C10H18O2
22	30.39	4- Nonenoic acid methyl ester	0.39	172	C10H18O2
23	30.39	2-Methyl malonic acid	0.39	118	C4H6O4
24	30.88	R-Limonene	1.22	184	C10H16O3
25	30.88	6-Heptenyl malonic acid	1.22	198	C10H14O4
26	30.88	(*Z*,*Z*)-9,12-Octadecadienoyl chloride	1.22	298	C18H31ClO
27	30.88	(9*E*,12*E*)-9,12-Octadecadienoyl Chloride	1.22	298	C18H31ClO
28	30.88	Hi Oleic Safflower oil	1.22	450	C21H22O11
29	31.39	(*Z*)6,(*Z*)9-Pentadecadien-1-ol	0.10	224	C15H28O
30	31.39	Methyl- 12,13-tetradecadienoate	0.10	238	C15H26O2
31	31.39	1-Oxacyclopropyl -3,4-Epoxy cyclohexane	0.10	140	C8H12O2
32	31.39	4-Chloro-3-n-butyltetrahydro pyran	0.10	176	C9H17ClO
33	31.69	17-Octadecynoic acid	62.51	280	C18H32O2
34	31.69	(*E*)-9-Tetradecen-1-ol, acetate	62.51	254	C16H30O2
35	31.69	E-7-Tetradecenol	62.51	212	C14H28O
36	31.69	(*Z*,*Z*)-9,12-Octadecadien-1-ol	62.51	266	C18H34O
37	31.69	4,9 -Tetradecadien-1-ol	62.51	210	C14H26O
38	31.96	2-Amioethanethiol Hydrogen sulfate	9.75	157	C2H7NO3S2
39	31.96	(2*E*)-1-(Methoxymethoxy)2-Octene	9.75	173	C10H20O2
40	31.96	4-Vinyl Cyclohexene	9.75	114	C8H12
41	31.96	1,2-Divinyl cyclobutene	9.75	114	C8H12
42	32.09	Retreversine	0.51	393	C21H27N7O
43	32.09	1,4-Dodecyl benzene	0.51	358	C26H46
44	32.25	*Z*,*Z*,*Z*-1,4,6,9-Nonadecatetraene	1.56	260	C19H32
45	32.25	*Cis*-5,8,11,14,17-Eicosapentaenoic acid	1.56	302	C20H30O2
46	32.25	*Z*,*Z*,*Z*-4,6,9-Nonadecatriene	1.56	262	C19H34
47	32.25	5,8,11,14-Eicosatetraenoic acid, methyl ester	1.56	318	C21H34O2
48	32.77	*Z*,*Z*,*Z*-9,12,15-Octadecatrienoic acid	0.90	278	C18H30O2
49	32.77	Octahydro -4,7-Methano-Idene	0.90	153	C10H16O
50	32.77	1-(2-Nitrobenzyl)-2(1H)-Pyrimidinimine	0.90	230	C11H10N4O2
51	32.77	Linoleic acid ethyl ester	0.90	308	C20H36O2

The Components recorded the highest concentration in the spectrum were came out at 31.69% min. with peak area of 62.51% (shown in Figure 3) were; 17-Octadecynoic acid, a fatty acid with triple bond, improves contractile response to angiotensin II by releasing vasoconstrictor prostaglandins,[12] also inhibits

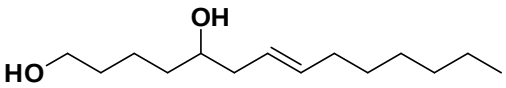
the metabolism of arachidonic acid by cytochrome P450 in renal cortical microsomes of rats.[13] (E)-9-Tetradecen-1-ol acetate, fatty alcohol, its isomer (*Z*) is a secondary sex pheromone of the fall armyworm,[14] E-7-Tetradecenol alcohol act as an anti-microbial agent.[15] (*Z*,*Z*)-9,12-Octadecadien-1-ol, which also known as linolenyl alcohol, showed considerable antibacterial effect and was particular effective against dental caries and periodontal disease.[16]



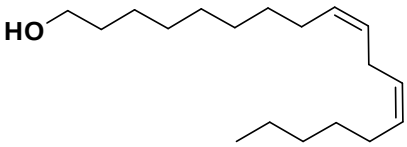
17-octadecynoic acid



(E)-9-Tetradecen-1-ol acetate



E-7-Tetradecenol alcohol



(Z,Z)-9,12-Octadecadien-1-ol

Figure 3: Dominant Fatty acids with 62.51% peak area.

The following highest concentration band of components came out at 28.42 min. with 17.27% peak area, the band afforded very interesting and biologically active phytochemical, Deoxyspergualin; also known as Gusperimus (Figure 4), this compound belongs to the class of organic compounds known as n-acyl-alpha amino acids, a derivative of the antitumor and antibiotic spergualin, Deoxyspergualin works as an immunosuppressive drug and exhibits cytoprotection, Immunomodulation properties.[17]

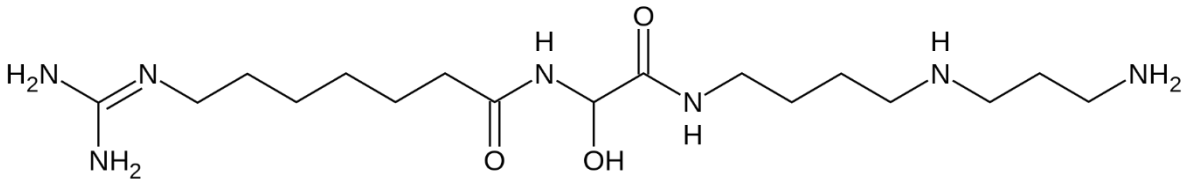


Figure 4: Deoxyspergualin.

Undecane, (Also known as hendecane) is a liquid alkane hydrocarbon with the chemical formula $CH_3(CH_2)_9CH_3$. It is used as a mild sex attractant for various types of moths and cockroaches, and an alert signal for a variety of ants.[18] Undecane also possess Anti-Allergic, Anti-Inflammatory properties.[19] Another interesting compound observed in the chromatogram is Reversine (Figure 5), with peak area of 0.51%, Reversine, or 2-(4-morpholinoanilino)-6-cyclohexylaminopurine, used for stem cell dedifferentiation, selectively induction cell death in cancer cells.[20]

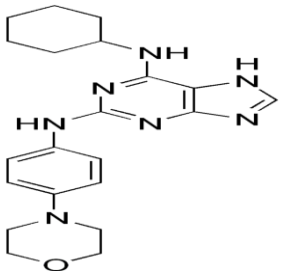


Figure 5: Retreversine.

Retreversine also is known to act as an antagonist of the adenosine A_3 receptor.[21] An important oil for human health was present in the chromatogram; Hi oleic Safflower oil with peak area of 1.22%; its considered the best oil choice since it contains higher amount of oleic and linoleic acids than any other oil

seed crops. Safflower oil has numerous applications in food, cosmetics, pharmaceutical industries and increase, and increase human health measures.[22]

From the table above, the domination of fatty acids is very clear, one of the important acids is *cis*-5,8,11,14,17-Eicosapentaenoic acid (Figure 6), polyunsaturated fatty acid found in fish oils. It serves as the precursor for the prostaglandin-3 and thromboxane-3 families.

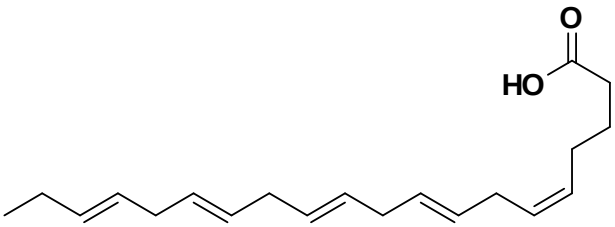


Figure 6: *Cis*-5,8,11,14,17-Eicosapentaenoic acid.

A diet rich in Eicosapentaenoic acid lowers serum lipid concentration, reduces incidence of cardiovascular disorders, prevents platelet aggregation, and inhibits arachidonic acid conversion into the thromboxane-2 and prostaglandin-2 families.[23]

In a previous study, the chemical composition of *S. Undulata* (tubers, leaves, and flowers) extracts collected from the southwest of Tunisia were determined by GC-MS using two solvents; water and ethanol. Twentyfive compounds were identified, volatile profile from the various extracts showed the presence of several phytochemical classes such as phenols, fatty acids, alcohol, organic acids, alkane and alkane derivatives.[24]

The volatile constituents of the aerial parts of *S. Undulata* obtained by steam distillation were analyzed by GC/MS. Thirtysix constituents were identified in the oil and the main components of which were methyl hexadecanoate (30.4%), methyl linolenate (23.9%) and heneicosane (12.2%).[3]

CONCLUSION

Ethanolic extract of aerial part of *S. undulata* showed very useful results in terms of chemical content of beneficial organic classes, using both qualitative phytochemical screening and GC-MS analysis. Elemental analysis presented reasonable concentrations of valuable dietary metals as the studied plant was used a food in the past. Extensive GC-MS analysis illustrated to the presence of valuable biologically active compounds. The documented activity such as antibacterial, antioxidation, anticancer, anti-inflammatory and cardioprotective effects, in addition to providing suitable supplements of fatty acids. The discoveries are very encouraging for more chemical studies especially isolation of active compounds.

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