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Phytochemical and Biological Investigation of *Sonchus tenerrimus* Growing in Libya

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Abstract: *Sonchus tenerrimus* is known as slender sow thistle and belongs to the family Asteraceae. It is native to the Mediterranean regions. The seeds and leaves of the plant were subjected to phytochemical and biological investigations. Protein hydrolysate of the seeds of *S. tenerrimus* was analyzed using HPLC resulting the identification of 14 amino (29.15 mg/ 100 g seed). Glutamic acid was the major constituent in the plants. GC/MS analysis GLC/ MS analysis of unsaponifiable matters of *S. tenerrimus* revealed the identification of 36 compounds and phytolis the major constituent while ethyl acetate fraction contains hyperoside as a major product. All plant fractions showed significant antioxidant and anticancer activities except unsaponifiable fraction showed weak activities.

Keywords: *Sonchus tenerrimus,* Hyperoside, Amino acids, GC/MS, Cytotoxicity, Antioxidant.

I. INTRODUCTION

Asteraceae is the largest family of Angiosperms comprises of over 1535 genera and 23000 species, distributed in three sub-families and 17 tribes [1]. The genus *Sonchus* belongs to sub-tribe Crepidinea, tribe Lactuceae and includes more than 50 species [2]. *Sonchus* species are widely distributed throughout the world. Their aerial parts are considered the cheapest source of protein, vitamins, minerals and essential amino acids in the diet of many people [3]. *Sonchus* has the questionable distinction of being considered one of the world's worst weeds [4].

Sonchus tenerrimus is known as slender sow thistle. It is native to the Mediterranean regions of southern Europe, northern Africa, and the Middle East [5].

The genus *Sonchus* is characterized by the presence of sterols, triterpenes and their glycosides [6-7], sesquiterpene lactones of eudesmanolide type [8-9], Ionone glycosides [10], Flavonoids, Tannins and hydroxycoumarins [11].

Sonchus species were reported for many biological activities as cytotoxic and antioxidant activity [6,12], antidiabetic activity [13], antidepressant and anxiolytic activity [14], Anti-inflammatory; and wound healing activities [15].

This is the first report concerning phytochemical and biological investigation of *S. tenerrimus*. Here on, GC-MS of the unsaponifiable matters, amino acids of seeds, flavonoids and phenolics from the chloroform and ethyl acetate soluble fractions of the alcoholic extract, of the species under investigation was studied, in addition to isolation and structure elucidation of major compound from the ethyl acetate soluble fraction. Anticancer and antioxidant activities of fractionated alcoholic extract of the plant were also investigated.

Fig. 1 *Sonchus tenerrimus*

II. MATERIAL AND METHODS

2.1. Plant material

Fresh samples of *S. tenerrimus* were collected during the flowering stage (March. 2014) from Benghazi, Libya and they were identified by Dr. Hussein Abdel Baset .A voucher specimen has been deposited at the Department of Botany, Faculty of Science, Zagazig University, Egypt.

2.2. Extraction and isolation

Air dried aerial parts (1 Kg) of *S. tenerrimus* were separately extracted by 90 % ethyl alcohol till complete exhaustion to afford (25g) of dry extract. The dried extract were separately suspended in water, fractionated with light petroleum, followed by chloroform, and finally ethyl acetate to afford (7g), (3 g) and (5 g), respectively.

Ethyl acetate soluble fraction of *S. tenerrimus* was applied on silica gel column packed with light

petroleum, polarity increased by methylene chloride then methanol to afford one compound as a major constituent which further purified by Sephadex LH 20 using Methanol as eluent to afford 400 mg of 1.

2.3. General

Melting points were measured by melting point apparatus, Electrothermal LTD Digital (England) and were uncorrected; UV spectra were measured in methanol by Schimadzu UV-1700 Spectrophotometer (Japan);.1H and13C-NMR spectra were run in DMSO-d6; at 300 MHz and 75 MHz, respectively using Varian Mercury-VX-300 NMR Spectrometer; Chemical shifts are given in ppm with TMS as internal standard.

2.4. GC-MS analysis

GLC/MS analysis of the unsaponifiable matter were analyzed on Agilent 6890 gas chromatograph, USA with fused silica capillary column PAS-5 ms (30 m × 0.25 um film thickness), under the following operating conditions: Detector: FID; Temperature of detector: 280ºC; Temperature of injector: 250ºC; Recorder: Dual channel recorder; Column temperature: 55ºC-280ºC (8ºC/min); Carrier gas: Helium 1 ml/min, E.Imode;70ev. Identification of the components was based on matching the fragmentation pattern in the resulted mass spectra with those of library reference and available published data.

2.5. HPLC analysis of amino acids in seeds

Amino acids content of *S. tenerrimus* was determined as described by Spakman et al. (1958). The analysis was performed in central service Unit, National Research Center, Egypt. HPLC analysis of protein was carried out using LC3000 amino acids analyzer (Eppendorf-Biotronik, Germany): flow rate, 0.2 ml/ min, Pressure of buffer 0 -50 bar Pressure of reagent, 0 - 150 bar, Reaction temp., 123°C; silica gel (60 to 120 mesh, Merck) was used for column chromatography. The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrinecolour reaction and photometric detection at 570 nm. Samples were hydrolyzed with 6 N HCL at 110° C in Teflon capped vials for 24h. After vacuum removal of HCL, the residues were dissolved in a lithium citrate buffer, pH2.2. Twenty microliter of the solution were loaded onto the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7, respectively, were successively applied to the column at flow rate 0.2 ml/min. The ninhydrine flow rate was 0.2ml/min and pressure of 0-150 bar and reaction temperature was 130° C. Qualitative and quantitative determination were carried out by comparing their retention times with those of.

III. RESULTS AND DISCUSSION

3.1 Identification of major compound of Ethyl acetate fraction

Column chromatography of ethyl acetate soluble fraction of *S. tenerrimus* afforded Compound 1; which showed chromatographic properties characteristic of flavonol mono glycosides and upon acid hydrolysis released quercetin and sugar

identified with D-galactose. The UV spectra analysis indicated the site of glycosylation at position 3. By the observed ^{1}H and ^{13}C NMR spectral data listed below, compound 1 was confirmed as quercetin 3-O-*β*- -D-galactopyranoside, hyperoside (Olszewska, 2005 and Sukito, 2014). Yellow crystals (methanol), m.p. 226-228 ^{o}C, R_f 0.6 (20% methanol in methylene chloride). ^{1}H-NMR (DMSO-d6) δ: 3.28-3.65 (sugar protons), 5.38(1H, d, J= 7.6 Hz, anomeric). 6.20(1H, d, J= 2Hz, H-6), 6.40(1H, d, J= 2Hz, H-8), 6.80 (1H, d, J = 8.8 Hz, H-5\`), 7.52 (1H, d, J = 2 Hz, H-2\`), 7.68 (1H, dd, J =2 and 8.8 Hz, H-6\`). The $^{13}$C-NMR (DMSO-d6) δ: 93.49 (C-8), 98.67 (C-6), 103.88 (C-10), 115,17(C-2\`), 115.92 (C-5\`), 121.08 (C-1\`), 121.99 (C-6\`), 133.47 (C-3), 144.81 (C-3\`), 148.44 (C-4\`), 156.29 (C-2 and C-9), 161.22 (C- 5),164.18 (C-7), 177.47 (C-4). Galactose signals: 60.12 (C-6"), 67.9 (C-4"), 71.18 (C-2"), 73.17(C-3"), 75.83(C- 5"), 101.78 (C-1").

3.2. GLC of unsaponifiable extract:

GLC/ MS analysis of unsaponifiable matters of *S. tenerrimus* (Table 1) revealed the identification of 36 compounds. phytol was the major constituent, followed by hydrocarbons; Benzene,4-ethyl-1,2-dimethyl, Benzene,(1-methylethyl), Benzene, 1-methyl-3-propyl and o-Cymene.

3.3. Protein hydrolysate of the seeds

Protein hydrolysate of the seeds of *S. tenerrimus* was analyzed using HPLC resulting the identification of 14 amino (29.15 mg/ 100 g seed). Glutamic acid was the major constituent in the plants (7.51 mg / 100 g seed), while the essential and semi essential amino acids contents were 12.84 mg/ 100 g seed.

IV. BIOLOGICAL EVALUATION

4.1. Antioxidant activity

The Chloroform fraction of *S. tenerrimus* possessed strong antioxidant scavenging activity as shown in (Table 3) against DPPH radicals as concluded from their low SC_{50} value. This activity is due to presence of conjugated and phenolic metabolites in different fractions.

4.2. Cytotoxic activity

As shown in (Table 4.), the chloroform and ethyl acetate extracts showed moderate cytotoxic activity against all tested cell lines while unsaponifiable extract showed a weak cytotoxic activity.

Table 1. Results of GLC/ MS analysis of the unsaponifiable matter of the aerial parts of *S. tenerrimus*

No	R_t	Name	Area	m+	No	R_t	Name	Area	m+
1	5.66	Isocomene	0.32	103	20	32.08	Heneicosane	1.4	158
2	7.00	Benzene, (1-methylpropyl)	0.94	147	21	32.62	Phytol	26.90	184
3	8.11	Benzene, 1-methyl-3-propyl	4.08		22	33.13	Phytol acetate	1.6	219
4	8.29	Benzene,(1-methylethyl)	4.32	120	23	33.83	Docosane	0.4	146
6	8.48	Benzene, 1-methyl-3-propyl	1.48	120	24	37.15	Tetracosane	0.46	338
7	8.79	Benzene,4-ethyl-1,2-dimethyl	6.38	120	25	38.72	Pentacosane	0.84	352
8	8.97	o-Cymene	4.04	134	26	40.22	Hexacosane	0.6	366
9	9.48	Benzene, 2-ethyl-1,4-dimethyl	0.46	134	27	42.42	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	0.44	291
10	9.73	Durol"Benzene,1,2,4,5-tetramethyl"	0.56	134	28	43.06	Octacosane	0.4	394
11	9.85	Isodurene "Benzene,1,2,3,5-tetramethyl"	0.58	120	29	44.50	9-Tricosene	1.28	322
12	14.27	Heptanoic acid, ethyl ester	0.44	112	30	45.33	stigmastan-3,5-diene	0.2	396
13	14.57	Tridecane	0.2	134	31	48.76	Campesterol	0.4	400
14	8.11	Benzene, 1-methyl-3-propyl	4.08	134	32	49.18	stigmasterol	0.82	412
15	27.14	Neophytadiene	3.14	134	33	49.89	beta-Sitosterol	0.86	414
16	27.26	2-Pentadecanone,	0.42	134	34	50.30	beta-Amyrin	0.54	426

		6,10,14-trimethyl							
17	27.98	1,3-Phytadiene	2.54	134	35	50.90	alpha-Amyrin	2	426
18	28.29	Nonadecane	0.2	134	36	52.09	Moretenol	0.4	426
19	30.20	Ecosane	0.26	134					

Table 2. HPLC analysis of amino acids of the seeds` proteins of *S. tenerrimus*

NO.	Amino acids	Rt (min.)	Conc. (mg/ 100g) seed)
			S. tenerrimus
Channel A			
1	Aspartic	11.57	2.11
2	Threonine **	14.93	0.57
3	Serine	16.18	0.95
4	Glutamic acid	18.25	7.51
5	Glycine	25.32	0.61
6	Alanine	26.55	1.28
7	Valine**	32.50	1.02
8	Methionine **	35.13	50
9	Isoleucine **	37.35	1.45
10	Leucine **	38.40	1.05
11	Phenylalanine **	42.95	1.61
12	Histidine **	49.95	0.81
13	Lysine **	54.08	2.50
14	Arginine *	61.97	2.39
Channel B			
1	Proline *	21.35	0.94
Total			29.15

* Occasionally essential amino acids; ** Essential amino acids

Table 3. Half maximum scavenging concentration of all samples in DPPH radicals. The data are presented as µg/ml. chloroform: chloroform soluble fraction; EtOAc: ethyl acetate soluble fraction of S. tenerrimus; unsap: unsaponifiable matter; Vit c; vitamin C standard.

Extract	Antioxidant activity (SC_{50})

Unsap.	39.17
Chloroform	15.78
EtOAc	24.14
Vit. C	1.84

Table 4. Half maximum inhibitory concentration of all samples in cell viability of HepG2, HCT-116 and MCF-7cells after the treatment for 48 hours, as measured by MTT assay. The data are presented as µg/ml.

Plant extracts	*S. tenerrimus*		
	HepG2	HCT-116	MCF-7
Unsaponifiable matters	162.42	169.28	159.80
Chloroform fraction	71.06	48.15	82.26
Ethyl acetate fraction	75.01	54.47	55.71

IV. CONCLUSION

S. tenerrimus seeds are good source for amino acids while leaves are good source for antioxidant and cytotoxic agents.

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