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A nanoformulated weed with antioxidant and hypoglycemic activity: *Sonchus asper* (L.) Hill

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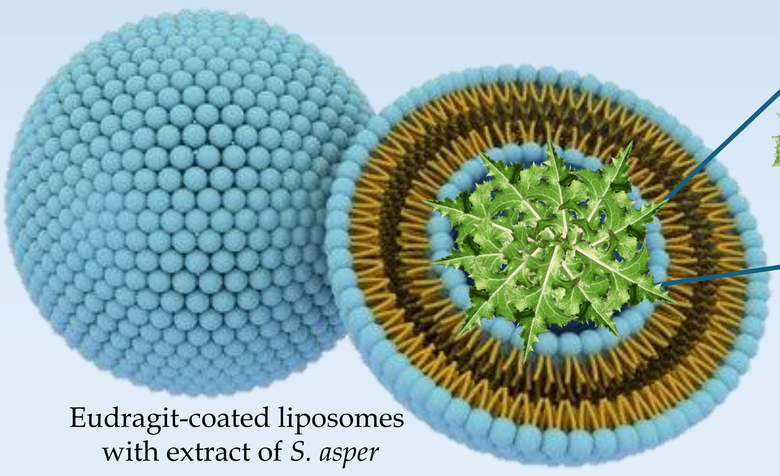
INTRODUCTION

Sonchus asper (L.) Hill is an annual or biennial wild edible herb belonging to the Asteraceae family. It is widely used in traditional Mediterranean cuisine typically eaten as a side dish or a soup in southern Italy. Moreover, different scientific studies report the numerous beneficial properties of *S. asper* attributable to the polyphenols, terpenes, and carotenoids present in fresh leaves and their extracts [1-4].



Eudragit-coated liposomes with extract of *S. asper*

Discarded non-edible leaves of *S. asper*

Sonchus asper (L.) Hill

AIM

- Evaluation the non-edible leaves of *S. asper* for recovery and valorization of food waste through the creation of a nanoformulation with potential antioxidant and hypoglycemic activities.
- Rediscovery of ancient dishes along with the preservation and sustainable use of biological diversity.

METHODS

EXTRACTION: Ultrasound assisted extraction was carried out for 15 minutes, using an hydroalcoholic mixture (EtOH:H_2O 7:3), with plant material to solvent ratio of 1:10 (*w/v*).

PHYTOCHEMICAL CHARACTERIZATION: The extract was analyzed with Q-Exactive™ Hybrid Quadrupole™Orbitrap™ Mass Spectrometer Q-trap (Thermo Fisher Scientific, Milan, Italy) coupled with an UltiMate 3000 UHPLC system (Thermo Fisher Scientific) [5].

NANOFORMULATION: For a potential application in health and nutritional fields, the extract from discarded non-edible leaves was incorporated in Eudragit-coated liposomes to provide protection during transit in the gastrointestinal tract, improving the bioavailability and efficacy of the extract's bioactive compounds [6].

BIOLOGICAL ACTIVITY: The content of phenolic compounds (TPC), and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl radixcal - DPPH, Ferric Reducing Antioxidant Power – FRAP) were evaluated. Then the potential hypoglycemic effect of the extract was carried out by *in vitro* *a*-amylase and *a*-glucosidase inhibition assays. The enteroendocrine cell line STC-1 was used to study the cytotoxicity by MTT assay, the effect of the extract on glucose absorption using the fluorescent deoxyglucose analog probe 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG) and of the secretion of the incretin hormone Glucagon like Peptide 1 (GLP-1) by ELISA assay [7-10].

RESULTS

The non-edible leaves extract of *S. asper* **(S)** showed the presence of 45 compounds as reported in **Table 1**.

Table 1. LC-HRESIMS profile of *S. asper* extract from discarded non-edible leaves

Peak	RT	Compounds	[M-H]⁻	MS²
		Phenolic acids		
1	7.6	Vanillic acid hexoside	329.0882	167, 152
2	7.7	Protocatechuic acid hexoside	315.0722	153, 109
3	11.1	Syringic acid hexoside	359.0986	197, 153
4	11.9	Caffeoyl quinic acid hexoside	515.1407	191, 179, 161
5	12.8	Caffeoyl hexoside	341.0867	179, 135
6	12.9	Caftaric acid	311.0410	179, 149, 135
7	16.6	Chlorogenic acid	353.0880	191, 179
8	16.9	Caffeoyl hexoside isomer 1	341.0867	179, 135
9	17.0	Caffeoyl hexoside isomer 2	341.0867	179, 135
10	19.4	Caffeoyl quinic acid isomer	353.0880	191, 179
11	27.2	Dicaffeoyl quinic acid	515.1193	353, 191, 179
12	27.8	Chicoric acid	473.0718	179, 149, 133
		Flavonoids		
13	17.9	Quercetin-*O*-hexosyl uronide	639.1210	463, 301
14	19.3	Quercetin-*O*-hexosyl uronide isomer	639.1210	463, 301
15	21.3	Luteolin-*O*-dihexoside	609.1459	285
16	21.5	Luteolin-*O*-hexosyl uronide	623.1249	285
17	22.1	Apigenin-*O*-hexosyl uronide	607.1307	431, 269
18	22.8	Luteolin dihexoside isomer	609.1459	285
19	24.1	Luteolin-*O*-hexosyl uronide isomer	623.1249	285
20	24.9	Luteolin-*O*-hexosyl deoxyhexoside	593.1512	285
21	25.7	Luteolin-7-*O*-glucoside	447.0935	285
22	26.3	Luteolin-*O*-uronide	461.0970	285
23	28.2	Apigenin-7-*O*-glucoside	431.0724	269
24	29.2	Apigenin-*O*-uronide	445.0771	269
25	34.4	Luteolin	285.0407	193, 149
26	38.7	Apigenin	269.0454	191, 179
		Coumarins and glycoside		
27	13.7	Esculin	339.0721	177, 133, 105
28	17.1	Aesculetin	177.0185	133, 105
29	19.8	Roseoside	385.1866	205, 135
30	25.5	Dihydroroseoside	387.0756	207
		Fatty acids		
31	35.8	Trihydroxyoctadecadienoic acid	327.2180	229, 211
32	42.1	Trihydroxyoctadecenoic acid	329.2116	229, 211
33	58.5	Hydroxy C18:3	291.2000	185, 121
34	58.7	Hydroxy C18:3	291.2000	185, 121
35	59.0	Hydroxy C18:2	293.2115	275, 195
36	59.3	Hydroxy C18:2	293.2115	275, 195
37	63.6	Linolenic acid	277.2160	259, 231, 181
38	64.8	Linoleic acid	279.2322	261, 209, 187
		Essential aminoacids		
39	1.35	Lysine	147.1128	130, 79
40	1.66	Threonine	120.0655	79
41	3.58	Isoleucine	132.1019	86, 79
42	3.94	Leucine	132.1019	86, 79
44	8.35	Phenylalanine	166.0862	120, 79
45	12.7	Tryptophan	205.0971	188, 79

Empty uncoated liposomes displayed small size, good homogeneity, and positive charge due to stearylamine. The loading of the extract did not affect these values significantly. On the other hand, the Eudragit coating induced an increase in size and in homogeneity and a decrease in zeta potential due to anionic Eudragit. The entrapment efficiency of the Eudragit-coated liposomes was very high (~90%). The Eudragit coating protected the vesicles and increased their physical stability with an optimal resistance to the gastrointestinal environments (**Table 2**).

Table 2. Composition and main features of the liposome formulations

Formulations	P90G	SA	S	PBS	Eu in PBS (0.1% *w/v*)	pH	time	MD nm ± SD	P.I.	ZP mV ± SD	EE % ± SD
Empty liposomes	120 mg	6 mg		1 mL				80 ± 5.6	0.25 ± 0.02	+8 ± 0.8	--
S liposomes	120 mg	6 mg	4 mg	1mL				81 ± 4.2	0.24 ± 0.02	+7 ± 0.6	--
Empty Eu-liposomes	120 mg	6 mg		1mL	1 mL			**103 ± 13.9	**0.36 ± 0.06	**+6 ± 1.4	--
S Eu-liposomes	120 mg	6 mg	4 mg	1mL	1 mL			**108 ± 15.2	**0.39 ± 0.05	**+6 ± 0.9	Luteolin-glucuronide 93 ± 3.2 Apigenin-glucuronide 94 ± 2.3
Incubation with gastrointestinal media at 37°C											
S liposomes						1.2	t_0	91 ± 7.5	0.29 ± 0.08	+10 ± 0.8	
							t_{2h}	97 ± 2.9	0.35 ± 0.01	+11 ± 1.0	
						7.0	t_0	105 ± 9.0	0.31 ± 0.03	+4 ± 1.4	
							t_{6h}	103 ± 3.6	0.32 ± 0.05	+4 ± 0.3	
S Eu-liposomes						1.2	t_0	94 ± 2.6	0.31 ± 0.09	+11 ± 0.7	
							t_{2h}	94 ± 1.2	0.29 ± 0.06	+10 ± 0.7	
						7.0	t_0	90 ± 0.7	0.31 ± 0.05	+4 ± 1.0	
							t_{6h}	97 ± 4.3	0.34 ±0.07	+2 ± 0.1	

Mean diameter (MD), polydispersity index (P.I.), zeta potential (ZP), and entrapment efficiency (EE). Mean values ± SDs are reported (n=10). **values statistically different (p<0.01) from uncoated liposomes. MD, P.I. and ZP of *S. asper* (**S**) liposomes and Eudragit-coated liposomes are reported upon dilution (t_0) with gastrointestinal media (pH 1.2 or 7.0) and incubation at 37°C for 2 h (t_{2h}) or 6 h (t_{6h}). Mean values ± SDs are reported (n=4). Phospholipid (**P90G**), stearylamine (**SA**), *S. asper* extract from discarded non-edible leaves (**S**), phosphate buffered saline (**PBS**), Eudragit® L100 (**Eu**).

The nanoformulation process did not alter the extract's phenolic content and antioxidant activity.

The *a*-glucosidase enzyme was not inhibited by the extract and the liposomes (**Table 3**).

Table 3. Total phenolic content (TPC), antioxidant and hypoglycemic activities of *S. asper*, in solution or in Eudragit-coated liposomes and empty eudragit-coated liposomes

	TPC mg GAE/g (mg GAE/mL)	DPPH mg TE/g (mg TE/mL)	FRAP mg TE/g (mg TE/mL)	*a*-amylase inhibition IC_{50} µg/mL
Extract (S)	63.36±3.73^a (1.69±0.10^b)	209.56±19.14^a (9.00±0.94^a)	1540.77± 110.28^b (12.33±0.88^b)	114.60±1.08
Eudragit-liposomes (SL)	79.17±6.03^a (2.02±0.02^a)	266.58±23.53^a (11.46±1.10^a)	2526.34± 151.25^a (20.35±2.85^a)	nd
Empty Eu-liposomes (EL)	(0.26±0.04^b)	(4.15±1.06^b)	(1.52±0.22^c)	nd
Acarbose				3.76±0.20

mg GAE/g (mL), mg gallic acid equivalents per gram (mL) of dried extract; mg TE/g, mg of trolox equivalents per gram of dried extract; IC_{50}, half maximal inhibitory concentration. Different letters (a-c) indicate significant differences among extracts (p≤0.05; Tukey's test). nd= not determinable at tested concentrations.

The inhibition of intestinal glucose absorption represents a key strategy for the treatment of diabetes, which was significantly inhibited by *S. asper* extract and liposomal formulation (**Figure 4**).

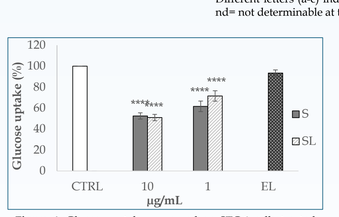


Figure 4. Glucose uptake measured on STC-1 cells treated for 2 h with different concentrations of *S. asper* (**S**) extract, Eudragit-coated liposomes (**SL**) and empty liposomes (**EL**) by using the 2-NBDG fluorescent glucose analog. ****p<0.0001 *vs* CTRL.

The levels of GLP-1, an incretin hormone involved in insulin secretion and suppression of glucagon production, are increased at the tested concentrations of *S. asper* extract compared to the control and further increase when the extract was nanoformulated (**Figure 5**).

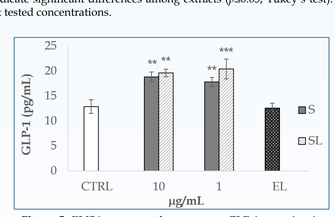


Figure 5. ELISA assay used to measure GLP-1 secretion in STC-1 cells treated for 2 h with different concentrations of *S. asper* (**S**) extract, Eudragit-coated liposomes (**SL**) and empty liposomes (**EL**). ***p<0.001, **p<0.01 vs CTRL.

CONCLUSIONS

This study valorizes ***S. asper*** **non-edible leaves**, ensuring that they do not become waste but become a source of metabolites with an interesting **antioxidant** and **hypoglycemic** potential activity. Furthermore, the development of a **nanoformulation**, stable under mimicked gastrointestinal conditions, rich in bioactive compounds, maximized its health-promoting properties that could contribute to an economic return.

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