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].

The present study used a dual strategy: to contribute to the rediscovery of ancient dishes along with the preservation and sustainable use of biological diversity, and evaluate the non-edible leaves of *S. asper* for recovery and valorization of food waste through the creation of a nanoformulation with potential biological activities.

MATERIALS AND METHODS

The first step of the study was the extraction of specialized metabolites from the external leaves of *S. asper*, which are usually discarded after harvesting. A green extraction was carried out in an ultrasound bath for 15 minutes, using an hydroalcoholic mixture (EtOH:H_2O 7:3), with plant material to solvent ratio of 1:10 (*w/v*).

Subsequently, a phytochemical characterization of the extract was carried out by 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].

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].

Then, the content of phenolic compounds (TPC), antioxidant and hypoglycemic activities of the extract, Eudragit-coated liposomes, with or without extract, were evaluated by different *in vitro* cell-free or cell-based tests [7-10].

RESULTS AND DISCUSSIONS

The liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS) was used to analyze the non-edible leaf extract of *S. asper* that showed the presence of 45 compounds including phenolic acids, flavonoids and unsaturated fatty acids, as well as C13-norisoprenoid glycosides, coumarins, and essential amino acids.

Given the interesting phytochemical profile of the discarded leaves, the study proceeded by developing a nanoformulation of *S. asper* extract. Eudragit-coated liposomes containing *S. asper* extract were produced, characterized, and compared with empty Eudragit-coated liposomes, *S. asper* extract uncoated liposomes and empty uncoated liposomes. Empty uncoated liposomes displayed small size (80 nm), good homogeneity (P.I. 0.25), and positive charge (+8 mV) due to stearylamine. The loading of the extract did not affect these values significantly ($p > 0.05$). 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 behaviour of the Eudragit-coated liposomes containing extract and uncoated liposomes under pH and ionic strength conditions that simulate the gastrointestinal environment was studied. The results demonstrate that the Eudragit coating protected the vesicles and increased their physical stability with an optimal resistance to the gastrointestinal environments.

The total phenolic content and the antioxidant activity evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical test and Ferric Reducing Antioxidant Power (FRAP) assay of raw extract and of extract in Eudragit-coated liposomes were comparable demonstrating that the nanoformulation process did not alter the extract's phenolic content and antioxidant activity.

The evaluation of the potential hypoglycemic effect of the *S. asper* extract was carried out by *in vitro* α-amylase and α-glucosidase inhibition assays and in intestinal STC-1 cell line, which possesses many features of enteroendocrine cells

and is commonly used for studies on glucose uptake and gut hormone secretion. The extract showed no cytotoxic effect against STC-1. Empty Eudragit-coated liposomes showed reduced cell viability after 24 h of treatment, likely due to the presence of stearylamine. Nevertheless, the effect was mitigated when the *S. asper* extract was incorporated into the vesicles. Moreover, this effect was far less marked after 2 h of exposure, thus suggesting a safe use of the nanoformulation for hypoglycemic studies. Two hours is the ideal exposure time period for the following glucose-related experiments, and thanks to the viability assessment, the proper *S. asper* concentration range (1-10 µg/mL) was identified.

Since the inhibition of glucose absorption in the intestinal tract represents a key strategy for the treatment of diabetes, the fluorescent deoxyglucose analog probe 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose) (2-NBDG) was used to measure intestinal glucose absorption, which was significantly inhibited by. *S. asper* extract and liposomal formulation.

To verify the hypoglycemic effect of *S. asper*, its activity on Glucagon like Peptide 1 (GLP-1) secretion was also evaluated. GLP-1 is an incretin hormone produced by enteroendocrine cells, which induces insulin secretion, suppresses glucagon secretion, slows down gastric emptying, and decreases appetite. The results obtained indicate a marked increase of the hormone levels at the tested concentrations of *S. asper* extract compared to the control and further increase when the extract was nanoformulated.

CONCLUSIONS

This study for the first time enhances ancient culinary traditions and supports the consumption of *S. asper* for its beneficial effects on health. Above all, it valorizes *S. asper* plant materials leftover, ensuring that they do not become waste but become a new material from which to extract metabolites with an interesting potential biological activity useful for health.

Furthermore, the development of a nanoformulation stable under mimicked gastrointestinal conditions and containing *S. asper* extract from discarded leaves, rich in bioactive compounds, maximized its health-promoting properties that could contribute to an economic return.

In conclusion, the results obtained demonstrate that Eudragit-coated liposomes can represent a formulation that protects *S. asper* extract and enhances its bioactivity with site-specific delivery into the intestinal tract, even at lower concentrations than the free extract. This allows us to enhance the intestinal absorption of some natural compounds such as polyphenols, which have low bioavailability and stability in the harsh conditions of the digestive tract.

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