Capsicum annuum L. cv Senise: from food to liposomes with high-value antioxidant potential

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INTRODUCTION

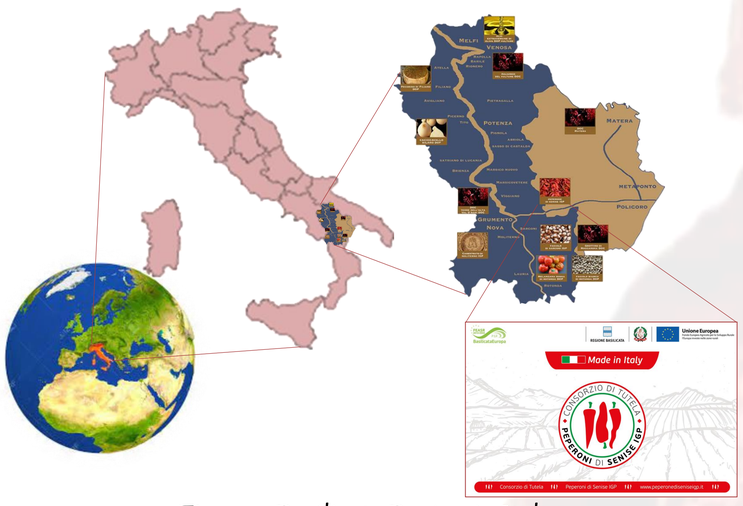


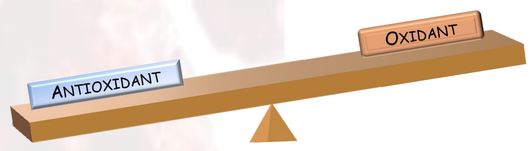
Figure 1. Basilicata Region in Italy

Different factors increase the production of free radicals in human body altering the homeostasis [1]. The increase in free radicals resulting in the oxidative stress is involved in the etiology of several major human diseases such as diabetes, cardiovascular diseases, cancer, and neuronal disorders. Specialized metabolites extracted from plant species are able to neutralize free radicals by carrying out numerous biological activities [2].

Among all, *Capsicum annuum* L. cultivar Senise, belonging to the Solanaceae family, also known as "*red gold of the Basilicata region*", is a precious source of health-promoting compounds. It is a sweet pepper traditionally sun-dried and eaten fried ("*cruschi peppers*") or powdered and used as a spice in the Basilicata region in Italy (**Fig. 1**).

Recent studies have investigated its health-promoting activities and chemical composition [3, 4]. As far as we are aware, this is the first study of the biological activity of *C. annuum* cv Senise incorporated in a vesicular carrier system. The purpose of this study is the evaluation of the antioxidant activity on HepG2 cell line used as cell model as well as the investigation of the phytochemical profile of *C. annuum*.

Furthermore, the molecular signalling pathways involved in the antioxidant activity of the extract are assessed [5].



METHODS

C. annum was collected in Senise, Basilicata, Italy during the autumn of 2016. Sun-dried red peppers without seeds and petiole were extracted by maceration with absolute ethanol for 48 h. Then, the dried extract was injected in positive and negative mode on LC-ESI/LTQOrbitrap/MS for the quali-quantitative determination analysis. The extract of *C. annum* was also incorporated in liposomes. For comparative purposes, empty liposomes were produced following the above procedure, but without including the extract. The extract and its liposomal formulation were evaluated for the antioxidant activity by *in vitro* cell free assay as Oxygen Radical Absorbance Capacity (ORAC), or measurement of cell viability by the MTT assay and intracellular Reactive Oxygen Species (ROS) in HepG2 cells. Quantitative RT-PCR was used to evaluate the expression of some genes involved in antioxidant defence [5].

RESULTS

From the phytochemical analysis of *C. annum* extract 24 compounds were identified based on the mass accuracy value, tandem mass experiments, literature data, and reference standards (**Fig. 2**).

The extract slowed fluorescein degradation by quenching the peroxyl radicals in a dose-dependent manner with an ORAC value of 38.14 µmol TE/100 g of dried extract (**Fig. 3**).

C. annuum extract had no cytotoxic effect on HepG2 cells after 24 and 48 h of treatment (**Fig. 4**).

Instead the pre-treatment with the extract for 24 h reduced ROS levels dramatically, restoring the basal level similar to that of cells treated with *N*-acetylcysteine (NAC), a known antioxidant (**Fig. 5**).

Moreover, the extract did not affect the expression of genes after 24 h, as compared to the control but it upregulated the expression of SOD-2 and GPx-1 after 48 h, as well as the nuclear factor erythroid 2-related factor 2 (Nrf2) and ATP-binding cassette transporter G2 (ABCG2) (**Fig. 6**).

Interestingly, after extract incorporation, ROS levels were significantly decreased even showing twice as potent activity in comparison with both the raw extract and NAC used as control (**Fig. 5**).

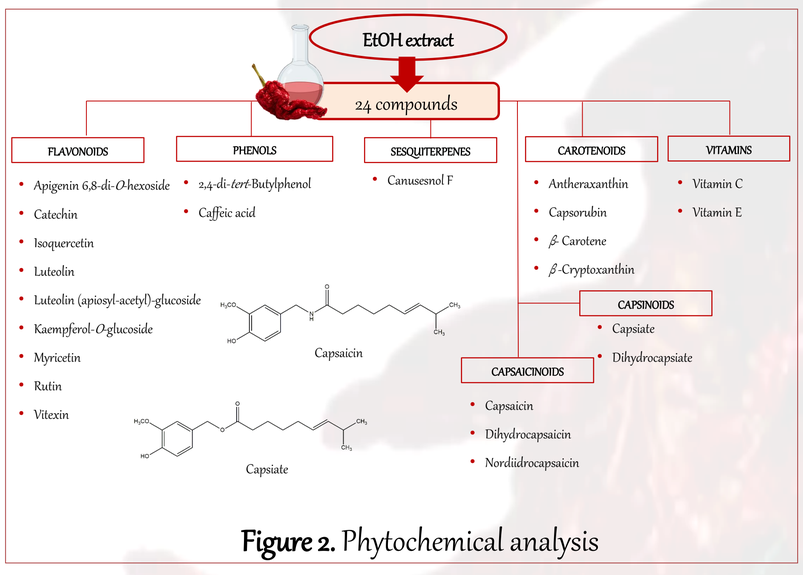


Figure 2. Phytochemical analysis

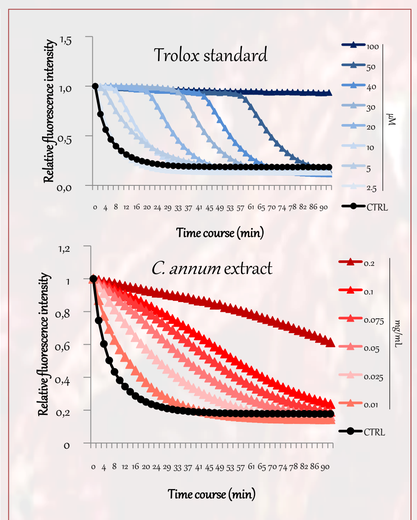


Figure 3. ORAC assay of *C. annum*

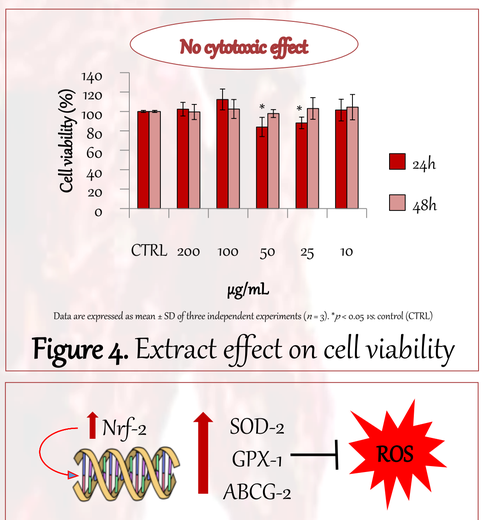


Figure 4. Extract effect on cell viability

Figure 6. *C. annum* molecular mechanism

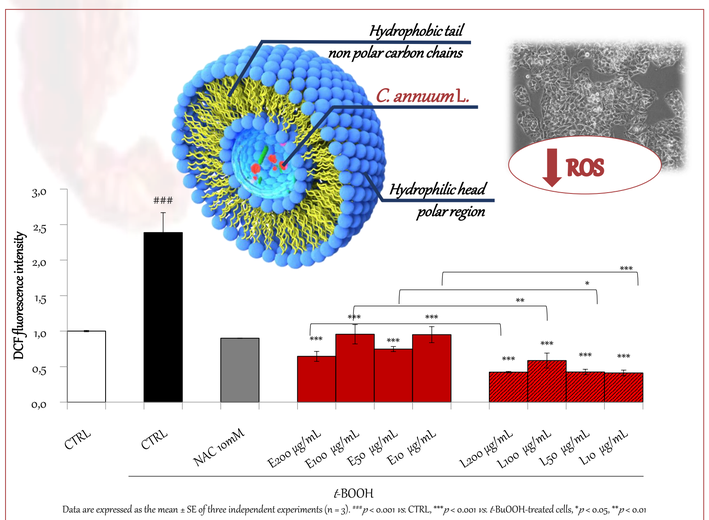


Figure 5. Extract and liposome effects on ROS levels

CONCLUSIONS

For the first time, this study, has investigated the protective effect of *C. annuum* L. cv Senise raw extract and its liposomal formulation against oxidative stress in cells.

Twenty-four compounds have been identified by LC-MS and the results obtained demonstrated the best antioxidant activity of the *"red gold"* when introduced into liposomes. Furthermore, it has been demonstrated that several genes involved in the redox cell system are upregulated during treatment with the extract, with an evident impact on SOD- 2 and GPX-1 as well as Nrf2 and ABCG2.

Overall, this study suggests that the incorporation in liposomes of a typical food of the Basilicata region could represent a new strategy in nutraceutical and pharmaceutical fields.

SCAN ME

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