Encapsulation of pomegranate peel extracts in egg phosphatidylcholine liposomes

Sara Borgia, Francesco Cairone, Laura Di Muzio, Chiara Brandelli, Patrizia Paolicelli, Maria Antonietta Casadei, Stefania Cesa, Stefania Petralito and Jordan Trilli

Department of Drug Chemistry and Technology, University "La Sapienza", Piazzale Aldo Moro 5, 00185 Rome, Italy

INTRODUCTION

Pomegranate fruit is known as a good source of hydrolysable tannins, such as punicalagin (PU) and punicalins. In our recent papers, the extraction of bioactive polyphenols from pomegranate peels, provided high yields, especially in punicalagin (150-500 mg/100 g fresh weight)[1]. The so obtained extracts were also evaluated for enzyme inhibition activity on PDIA3 disulfide isomerase, an enzyme associated with different diseases such as cancer, Alzheimer's and Parkinson's diseases. The results demonstrated that the different ratios between PU and ellagic acid modulate the enzyme activity and other ellagitannins could interfere with this activity[2,3]. So, pomegranate extracts could be also useful for drug application and their relative development. Recent paper showed that the incorporation of plant-derived compounds into liposomes could overcome this limitation[4]. It has been reported that encapsulation of PU in liposomes can increase their stability and result in significant inhibition of cancer cell growth. Several hypotheses, including increased penetration of PU into cells and stability of encapsulated materials, may explain the mechanism of enhanced anticancer efficacy of these formulations. Moreover, liposomes represent an optimal delivery system due to the ability to entrap both lipophilic and hydrophilic compounds.

AIM OF THE WORK

The present work aimed to encapsulate pomegranate peels extracts in liposomal formulations and to evaluate the influence of the ellagitannic phytocomplex on the modulation of PDIA3 activity.

MATERIALS AND METHODS

Pomegranate fruits belonging to different cultivars (Dente di Cavallo, Black, Mollar, Wonderful and Provenza) were submitted to the same work-up and extraction technique. Manually separated peels were blended in a mixer for 30 sec and extracted by a hydroalcoholic mixture (70/30, v/v EtOH/H_2O 5% CH_3COOH). The so obtained hydroalcoholic dried extracts were partitioned with water and ethyl acetate. The resulting aqueous extracts (AE) were analyzed by HPLC-DAD. Wonderful showed the higher PU concentration and the most rich phytocomplex profile. On this bases, Wonderful aqueous extract was selected for further purification and submitted to solid phase extraction (SPE) (Discovery® DSC-18 SPE Tube). Two fractions (F1 and F2) were obtained. F1, eluted with acidified water was the most representative in weight (97%) whereas F2, eluted with MeOH and representing the only remainder of 3%, was enriched in ellagitannins, and especially punicalagins. AE of the different cultivars and F2 were selected for encapsulation in liposomal formulation (**Figure 1**). Liposomes were prepared by reverse-phase evaporation (REV) technique. The average diameter, polydispersity index (PdI) and zeta potential of liposomes were determined by dynamic and electrophoretic light scattering.

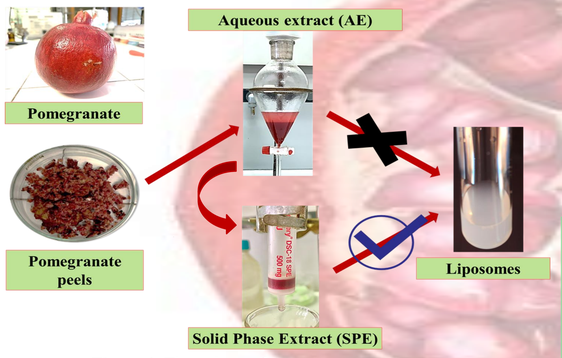

Figure 1. Flow chart of the adopted work-up

HPLC-DAD ANALYSES

The resulting aqueous extracts were analyzed by HPLC-DAD on reverse phase (RP18) column at 360 nm for the identification of ellagitannins phytocomplex. Ellagic acid and and α and β punicalagin anomers were identified and quantified. The different AE extracts showed different PU concentrations and phytocomplex profiles depending on cultivars. The two dried F1 and F2 fractions were also analyzed by HPLC-DAD. Chromatograms (**Figure 2)** showed that F1 (blue line) was characterized by a simpler profile and lower PU concentration than F2 (black line). F2 showed a more complex chromatographic profile, in which punicalagin is the most representative.

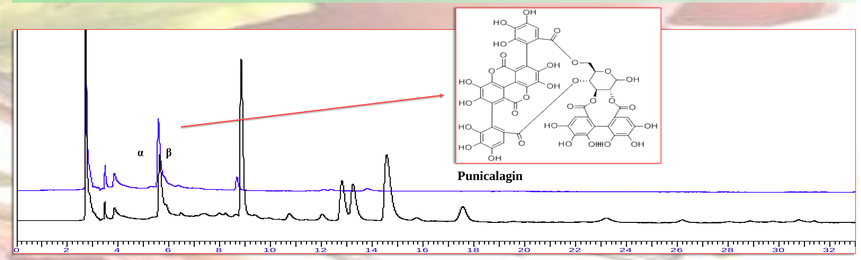

Figure 2. Chromatographic profile of the two fractions obtained after purification by SPE.

DLS ANALYSES

	Size (nm)	PdI	Zeta Potential (mV)
AE Liposomes	ND	ND	ND
SPE Liposomes	98.4 ± 3.4	0.102 ± 0.005	-27.3 ± 1.7
Control Liposomes	111.7 ± 3.8	0.195 ± 0.03	-15.3 ± 0.4

RESULTS AND CONCLUSIONS

The purification step by solid phase extraction (SPE) allows to obtain a small fraction (F2) highly enriched in punicalagins (**Figure 2**), probably due to the sugars' removal, making this step crucial for the next encapsulation in liposomes. In fact, preliminary results showed liposomes formation only when aqueous extracts were further purified. The obtained liposomes showed optimal size and PdI, combined with a high negative zeta potential, which together account for a very stable system. In light of these results, other AE from different cultivars will be purified and formulated in liposomes and entrapment efficiency (EE %) will be evaluated in order to quantify the entrapped PU. Afterwards, the obtained systems will be tested in vitro on PDIA3-overexpressing cells and their bioactivity will be compared with no-formulated aqueous extract.

References

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