

Optimization of Ellagic Acid Extraction from Pomegranate Rind Powder : Application in *In-vitro* cytotoxicity studies

Prajakta Dandekar, Parth Joshi, Meenal Ghune and Vandana Patravale

Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai

Ellagic acid (EA) (4,4',5,5',6,6'-hexahydroxydiphenic acid, 2,6,2,6'-dilactone) is a plant polyphenol which occurs in substantial amounts in fruits, nuts and berries. It is usually present in Ellagitannins (ET's) as an ester of hexa-hydroxy-diphenic acid (HHDP) with glucose. When exposed to acids or bases, ester bonds are hydrolyzed and HHDP spontaneously rearranges into EA. It has been reported to exert several pharmacological actions like anti-cancer, anti-inflammatory, anti-hypertensive, anti-atherosclerotic and hepato-protective activity.

The objective of the present study was to extract EA from pomegranate rind powder and to optimize different reaction parameters to maximize the yield. The purity of the extract was determined using high pressure liquid chromatography (HPLC). Furthermore, synergistic action of EA with curcumin was studied through *In-vitro* cytotoxicity assay in HT-29 human colon adenocarcinoma cell line.

ET was extracted by solvent extraction method and the extract was subjected to hydrolysis to yield EA. The extraction of ET's was studied with respect to various parameters like effect of temperature, period of extraction, solvents (water, methanol, chloroform, ethyl acetate) and ratio of raw material to solvent. The resulting ET containing extract was then hydrolyzed with mineral acids (HCl, HNO₃, H₂SO₄) to yield the final product. The isolated compound was further washed and dried to determine the product yield. The isolated compound was confirmed by thin layer chromatography (TLC) and the purity was determined by HPLC using standard EA (Hi-Media Laboratories Pvt. Ltd., Mumbai, India). Further, investigations of the isolated compound for the possible synergistic interactions with curcumin (Konark Herbals and Healthcare Pvt. Ltd., Mumbai, India) was studied through MTT-assay using HT-29 human colon adenocarcinoma cell line. EA was used in different concentrations (1, 5 and 10 μM) with curcumin to study the synergistic effect.

Maximum yield of ET's was obtained using water to methanol (2:1) (Fig.1) solvent mixture than the individual solvents. Maximum conversion of ET's to EA was obtained by hydrolyzing the extract with Conc. H₂SO₄ (2.5 %v/v). After purifying with di-chloro-methane, the yield of purified product was 1233.33 ± 30.81 mg, while the purity of the product was found to be 87.71 ± 0.09%, as determined by HPLC. MTT results revealed that curcumin exhibited better cytotoxicity when used with EA (10 μM) (Fig.2)

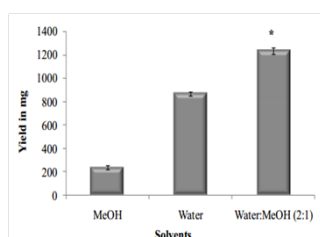


Fig.1 Effect of combination of methanol and water on the yield of ellagic acid

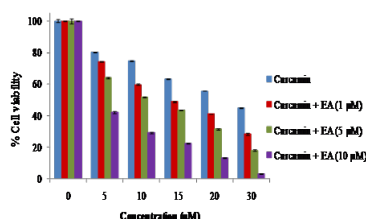


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Biography

Parth Joshi pursued his Bachelor of Pharmacy from B.N. College of Pharmacy, Udaipur and right now he is pursuing his M. Pharm (Pharmaceutics) at the Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Mumbai. He is working on the project titled as "Enhancement of solubility of a BCS Class-IV drug" under the guidance of Prof. Vandana B. Patravale. He has been active participant in the international symposium on "Drug Discovery for Infectious Diseases and Cancer" (DDIDC) and in a national symposium on "Regulatory Roadmap for Pharmaceuticals in Global Market" held at ICT Mumbai.