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Insight into the biochemical, kinetic and spectroscopic characterization of garlic (*Allium sativum*) phytocystatin: Implication for cardiovascular disease

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Phytocystatins are cysteine proteinase inhibitors present in plants. They play crucial role in maintaining protease-anti protease balance and are involved in various endogenous processes. Thus, they are suitable and convenient targets for genetic engineering which makes their isolation and characterization from different sources the need of the hour. In the present study a phytocystatin has been isolated from garlic (Allium sativum) by a simple two-step process using ammonium sulfate fractionation and gel filtration chromatography on Sephacryl S-100HR with a fold purification of 152.6 and yield 48.9%. A single band on native gel electrophoresis confirms the homogeneity of the purified inhibitor. The molecular weight of the purified inhibitor was found to be 12.5 kDa as determined by SDS-PAGE and gel filtration chromatography. The garlic phytocystatin was found to be stable under broad range of pH (6-8) and temperature (30-60 °C). Kinetic studies suggest that garlic phytocystatins are reversible and non-competitive inhibitors having highest affinity for papain followed by ficin and bromelain. UV and fluorescence spectroscopy revealed significant conformational change upon garlic phytocystatin-papain complex formation. Secondary structure analysis was performed using CD and FTIR. Garlic phytocystatin possesses 33.9% alpha-helical content as assessed by CD spectroscopy.

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