

The discovery and development of new anticancer drugs derived from natural sources have recently gained significant attention. Among these sources, higher plants have emerged as the primary reservoir for drugs used in clinical practice. It is worth noting that the majority of recently approved anticancer drugs are derived from natural products or their derivatives, further reinforcing the pharmaceutical and medicinal value of plants. This study focuses on *Beta vulgaris* L., which is a great choice due to its affordability, ease of cultivation, and rich content of bioactive saponins. These saponins belong to the triterpene group and include aglycons such as oleanolic acid, hederagenin, gypsogenin, and akebonic acid conjugated with sugar chain units.

Saponins, with their remarkable structural and bioactive diversity, are emerging as a crucial class of natural compounds in the field of cancer treatment. In today's world, cancer poses a significant global health challenge and stands as one of the leading causes of death worldwide. According to an epidemiological study conducted by the World Health Organization, cancer claimed the lives of 7.6 million individuals in 2018, with a projected doubling of this number by 2030.

Conventional anticancer agents not only target cancer cells but also harm normal cells due to their high toxicity. Hence, the search for new natural anticancer compounds, such as saponins, has gained substantial interest, as they offer a promising alternative to synthetic compounds, demonstrating favourable efficacy and safety profiles. Scientific reports indicate that saponins exhibit potential anticancer properties, including cytotoxic, cytostatic, pro-apoptotic, anti-invasive, and high chemopreventive potentials. It is worth highlighting that previous research on the anticancer activity of triterpene saponins has predominantly focused on their extracts. Thus, our study aims to expand the investigation by exploring the bioactivity of pure saponins. Notably, triterpene saponins derived from *B. vulgaris* have never been tested for their anticancer activity, adding novelty to our research endeavor.

The proposed project aims to investigate the cytotoxicity and anticancer selectivity of triterpene saponins present in *B. vulgaris*, with the objective of evaluating their potential as anticancer drugs. Additionally, the project seeks to develop an effective procedure for isolating and separating saponins obtained from different varieties of *B. vulgaris* using countercurrent chromatography (CCC) and preparative high-performance liquid chromatography (prep-HPLC), with monitoring their stability.

Previous studies have demonstrated the complementary selectivity and separation capabilities of HPLC and CCC techniques, making them suitable for obtaining pure saponin fractions. Therefore, these techniques will be used in the planned experiments. CCC is a unique liquid chromatography technique widely utilized for separating various components in natural mixtures, facilitating the subsequent identification of new structures. Prior to initiating the biological tests, appropriate solvent systems and other CCC separation parameters will be selected. The extracts and collected CCC and HPLC fractions will be analyzed using HPLC-ESI-MS/MS and/or UHPLC-CAD-QTOF-HRMS/MS, and the NMR techniques will be used to determine the new structures of saponins.

The cytotoxicity and anticancer selectivity of the extracts and pure saponins will be assessed using the colorimetric MTT *in vitro* test, which measures the activity of energy metabolism in mitochondria. The assessment of anticancer effectiveness will be conducted using various cell lines obtained from the American Type Culture Collection: VERO (CCL-81, monkey kidney), RKO (CRL-2577, human colon cancer), A-375 (CRL-1619, human malignant melanoma), MDA-MB-231 (HTB-26, human breast cancer), and A-549 (CCL-185, human carcinoma lung).

In conclusion, the research we propose will contribute to the development of a suitable methodology for isolating and separating bioactive triterpene saponins from *B. vulgaris*, aiming to assess their anticancer activity and explore their potential applications in medicine. It is important to highlight the scarcity of research findings in the scientific literature pertaining to the specific area covered by this project. Therefore, the obtained results will undoubtedly make a significant contribution to expanding our understanding of saponins and will be published in a reputable, high-quality journal, which will be considered a noteworthy achievement by our scientific team.