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ISOLATION AND PURIFICATION OF COMPOUNDS FROM SILYBUM MARIANUM

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Abstract: Milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)], also known as Saint Mary's Thistle, is used globally as a hepatoprotective, for mushroom poisoning, and recent studies show the chemoprevention of prostate cancer as well. In addition, it displays anti-inflammatory, immunomodulatory and antioxidant effects [1,2]. The commercial milk thistle extract is called silymarin, a complex mixture of flavonolignans and polyphenolic molecules obtained from milk thistle seeds. The composition of silymarin includes the flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, and silydianin) and a flavonoid (taxifolin) [3,4]. The objective of this research was to isolate and purify the diastereoisomers of silymarin, in gram scale, providing enough material for in vivo and in vitro studies. This research also involves finding more efficient methods for the isolation of these compounds and discovering new compounds that could present potential activities. Large scale preparations were accomplished with a CombiFlash Rf200 flash chromatography system (Teledyne-Isco) using an Isco reverse-phase C-18 column (130g). The HPLC system used was a Varian Prostar equipped with Prostar 210 pumps and a ProStar 320 photodiode array (PDA) detector with data collected and analyzed with Galaxie Chromatography Workstation software. For preparative HPLC, Atlantis T3 OBD (5µm; 250x19mm) and Phenomenex Luna PFP (5µm; 250x21.2mm) columns were used with specific flow rates for each compound. The purity of the isolates was determined via a Waters UPLC system using HSS-T3 and HSS-PFP columns (1.8µm; 100x2.1mm), and both CH_3OH-H_2O and CH_3CN-H_2O gradients, producing separations under four individual chromatography conditions. Chromatograms were observed at 288 nm, purity analyzed under all four conditions, and the retention times compared to standards for identification. In conclusion, through the use of the multi column UPLC system the best conditions to isolate and purify each compound was determined, allowing for gram quantities of each diastereoisomer to be purified. Furthermore, during the purification of isosilychristin and silychristin two new unknowns were discovered and purified.

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