

Materials and Methods for Isolation of Calycosin

The dry roots of Radix Astragali were extracted twice with 30L distilled water at 80°C for 2 hours, and then filtered to remove impurities. The water extraction was concentrated and applied to HPD600 macroporous resin column for the further separation. After eluting with ethanol for 10L, the A0 part was obtained by concentrating the elution. A0 part was mixed with silica gel (10-77 μm), applied to pre-filled silica gel (77-147 μm) column, and eluted with different polarity of solution (petroleum ether: ethyl acetate =9:1, 4:1, 2:1, 1:1, ethyl acetate, and methanol). Collection fraction size was 500 ml, and the solvent was recycled at 35°C using spin evaporator (BÜCHI, Switzerland). TLC was used for monitoring the status of elution. Fraction at petroleum ether: ethyl acetate =1:1 was collected. It was further separated with silica gel again with small size column. The solvent for elution was: chloroform, chloroform: acetone=20:1, 1:1, 1:3. Then, fraction at chloroform: acetone=20:1 part was collected. After re-crystallization, pre-HPLC (Agilent Technologies, USA) was used to do further purify the compound and methanol was used as mobile phase. Finally, pure compound was obtained and identified with NMR.