Isolation and characterization of coagulansin-A

Plant material was collected, sorted out for any foreign material, diseased or deteriorated parts and was processed for isolation as reported previously (Ihsan-Ul-Haq et al. 2013). Briefly, it was shade dried with continuous agitation every 6 hourly and then crushed in a grinding mill. Total 10 kg of shade dried and crushed aerial parts without fruits (leaves and stems only) were taken and extracted (by maceration) in 30 liters of solvent (mixture of chloroform and methanol (1:1)) by occasional shaking for 3 days. Filtrate of extraction was dried by vacuum distillation to a constant weight (750 g). This crude extract was suspended in 2500 ml hot water and hot water suspension was defatted three times with n-hexane (2500 ml x 3) using a separating funnel. Aqueous layer was again extracted three times with ethyl acetate (2500 ml x 3). This ethyl acetate layer was dried in rotary evaporator at 35°C and 130 g ethyl acetate fraction (WCE) was obtained.

WCE was subjected to further purification and normal phase column chromatography was used. Briefly the fraction was dissolved in appropriate solvent (ethyl acetate and methanol mixture (5:1)) and was adsorbed on 125 g silica gel 60 (70-230 mesh, Merck, Germany). Gravitational glass column was packed with 1000 g silica gel 60 (230-400 mesh, Merck, Germany) and the dried sample was loaded on the top. The column was eluted with gradient change in mobile phase; starting from 20% acetone in n-hexane to 50% acetone in n-hexane and finally 1000 ml of n-hexane, acetone and methanol (1:1:0.5). Each fraction of 250 ml was collected and dried in rotary evaporator at 35°C. Total 19 fractions (WCE1- WCE19) were collected. On the basis of TLC analysis WCE18-19 were combined and subjected to normal phase column chromatography (silica gel 60, 230-400 mesh) using the mobile phase n-Hex:
EA/3:1-0:1. Total 27 fractions (250 ml each) were obtained of which the fraction 09 was crystallized and re-crystallize to get pure Coagulansin-A (cog-A; 500 mg). The compound was characterized by performing 2D NMR and LC–MS experiments. Purity of cog-A was analysed on RP-HPLC (Agilent 1200 series with DAD; Germany) by using C8 (Zorbax RX-C8) 4.6 x 250 mm and 5 µm particle size. A gradient method was employed by using mobile phase A (H2O) and mobile phase B (CH3OH). A linear gradient of 0-20 min 50-100% B was optimized and compound was detected at λ 225 nm. The purity of the compound was calculated as 98.6% on the basis of percent peak area (Ihsan-Ul-Haq et al. 2013).