World Academy of Science, Engineering and Technology
International Journal of Pharmacological and Pharmaceutical Sciences
Vol:9, No:05, 2015

Isolation and Structural Elucidation of 20 Hydroxyecdystone from Vitex doniana Sweet Stem Bark

Authors : Mustapha A. Tijjani, Fanna I. Abdulrahman, Irfan Z. Khan, Umar K. Sandabe, Cong Li

Abstract : Air dried sample V. doniana after collection and identification was extracted with ethanol and further partition with chloroform, ethyl acetate and n-butanol. Ethanolic extract (11.9g) was fractionated on a silica gel accelerated column chromatography using solvents such as n-hexane, ethyl acetate and methanol. Each eluent fractions (150ml aliquots) were collected and monitored with thin layer chromatography. Fractions with similar Rf values from same solvents system were pooled together. Phytochemical test of all the fractions were performed using standard procedure. Complete elution yielded 48 fractions (150ml/fraction) which were pooled to 24 fractions base on the Rf values. It was further recombined and 12 fractions were obtained on the basis on Rf values and coded Vd1 to Vd12 fractions. Vd8 was further eluted with ethylacetate and methanol and gave fourteen sub fractions Vd8-a, -Vd8-m. Fraction Vd8-a (56mg) gave a white crystal compound coded V1. It was further checked on TLC and observed under ultraviolet lamp and was found to give a single spot. The Rf values were calculated to be 0.433. The melting point was determined using Gallenkamp capillary melting point apparatus and found to be 241-243°C uncorrected. Characterization of the isolated compound coded V1 was done using FT-infra-red spectroscopy, HNMR, 13CNMR(1and 2D) and HRESI-MS. The IR spectrum of compound V1 shows prominent peaks that corresponds to OHstr (3365cm-1) and C=0 (1652cm-1) etc. This spectrum suggests that among the functional moiety in compound V1 are the carbonyl and hydroxyl group. The 1H NMR (400 MHz) spectrum of compound V1 in DMSO-d6 displayed five singlet signals at δ 0.72 (3H, s, H-18), 0.79 (3H, s, H-19), 1.03 (3H, s, H-21), 1.04 (3H, s, H-26), 1.06 (3H, s, H-27) each integrating for three protons indicating the five methyl functional groups present in the compound. It further showed a broad singlet at δ 5.58 integrated for 1 H due to an olefinic H-atom adjacent to the carbonyl carbon atom. Three signals at δ 3.10 (d, J = 9.0 Hz, H-22), 3.59 (m, 1H, 2H-a) and 3.72 (m, 1H, 3H-e), each integrating for one proton is due to oxymethine protons indicating that three oxymethine H-atoms are present in the compound. These all signals are characteristic to the ecdysteroid skeletons. The 13C-NMR spectrum showed the presence of 27 carbon atoms, suggesting that may be steroid skeleton. The DEPT-135 experiment showed the presence of five CH3, eight CH2, and seven CH groups, and seven quaternary C-atoms. The molecular formula was established as C27H44O7 by high resolution electron spray ionization-mass spectroscopy (HRESI-MS) positive ion mode m/z 481.3179. The signals in mass spectrum are 463, 445, and 427 peaks corresponding to losses of one, two, three, or four water molecules characteristic for ecdysterone skeleton reported in the literature. Based on the spectral analysis (HNMR, 13CNMR, DEPT, HMQC, IR, HRESI-MS) the compound V1 is thus concluded to have ecdysteriod skeleton and conclusively conforms with 2β, 3β 14α, 20R, 22R, 25-hexahydroxy-5 β cholest-7-ene-6- one, or 2, 3, 14, 20, 22, 25 hexahydroxy cholest-7-ene-6-one commonly known as 20-hydroxyecdysone.

Keywords : vitex, phytochemical, purification, isolation, chromatography, spectroscopy
Conference Title : ICPMPS 2015 : International Conference on Pharmacology, Medicinal and Pharmaceutical Sciences
Conference Location : Rome, Italy
Conference Dates : May 05-06, 2015