**Abstract**

Phytochemicals are increasingly being used in cancer treatment due to affordability and potential anticancer ef-fect with minimal adverse reactions compared to chemotherapy. Markhamia tomentosa is a medicinal plant used traditionally to treat cancer. In this study, the antiproliferative compounds from M. tomentosa were isolated using bioactivity-guided approach and characterized using various spectroscopic techniques. Through bioassay-guided fractionation of the crude ethanolic leaf extract, the dichloromethane (Mdf) and ethyl-acetate (Mef) fractions ex-hibited potent cytotoxicity activity against HeLa cells with IC50 values of 83.26 and 104.5 µg/ml respectively in the MTT assay. Trypan blue assay and cell cycle analysis showed that Mdf fraction demonstrated cytotoxic effect with more extensive cell death and induced G0/G1 phase cell cycle arrest with concomitant decrease in S phase. Mef fraction showed reduced percentage of stained dead cells as compared to Mdf fraction and induced G2/M phase with increase in the size of sub-G1 phase, corresponding to apoptosis. From the isolation and purification of Mdf and Mef fractions by repeated column chromatography, followed by identification by 1D and 2D NMR spectroscopy and by comparison of the NMR data with values reported in literature, sitosterol 1,mollicacid2, phytol 3 and oleanolic acid 4 were isolated for the first time from M. tomentosa.Mollicacid2 exhibited more po-tent cytotoxic activity compared to compounds 1, 3 and 4. The results from these findings suggest that mollic acid 2 isolated from Mef which exhibited apoptotic cell death may be responsible for the earlier reported apoptosis induction capability of M. tomentosa against cervical cancer cell line HeLa.