Production of mouse interleukin-2 by genetic engineering technique was carried out. The IL-2 gene showed 450 base pairs in length with eight repeating CAG codon. Recombinant mouse interleukin-2 (rmIL-2) consisted 161 amino acids with 6x His tag at the N-terminus for protein purification by Ni-NTA resin. Analysis of the rmIL-2 by SDS-PAGE illustrated monomeric and dimeric forms of the protein with the molecular weights of 19 and 38 kDa, respectively. The rmIL-2 was able to react with mouse IL-2 specific monoclonal antibody and induce proliferation of splenocytes. This result was confirmed by immunofluorescence exhibiting the binding signal to the receptor of IL-2.

Development of highly sensitive ELISA technique for investigation of mouse IL-2 in vitro using cell culture of splenocytes. The technique was then applied screening of the immunostimulants from red holy basil (Ocimum sanctum Linn.). Comparison of ELISA four techniques gradually decrease in the sensitivity by these technique, triple antibody sandwich ELISA > indirect inhibition ELISA > indirect ELISA > indirect ELISA combine with biotin/streptavidin system, respectively. The least concentration of rmIL-2 that could be detected by triple antibody sandwich ELISA was 152.59 pg/ml.

Extraction of the immunostimulants from red holy basil (Ocimum sanctum Linn.) was done by column chromatography using semi-preparative column HPLC for the purification of these compounds. The chemical structure of the substances had been investigated by UV-visible spectroscopy, infrared spectroscopy (IR) and nuclear magnetic resonance (\( ^1H \) NMR, \( ^{13}C \) NMR and \( ^1H-^1H \) COSY NMR). Two compounds were isolated which included CF7 compound (C\(_2\)H\(_{19}\)O\(_{10}\)) or 3-{3-(3,4-dihydroxy-phenyl)-2-[3-(3,4-dihydroxy-phenyl)-acryloyloxy]-1-hydroxy-propoxy}-acrylic acid) by IUPAC name and its derivative, CF8 compound, but the analysis of its structure could not be accomplished. Both compounds showed the increase of IL-2 production from Con A stimulated-splenocytes by 800 and 19,000 pg/ml, respectively.

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