ABSTRACT

Benjakul (BEN), a Thai Traditional medicine preparation, is composed of five plants; *Piper chaba* fruit (PC), *Piper sarmentosum* root (PS), *Piper interruptum* stem (PI), *Plumbago indica* root (PL) *and Zingiber officinale* rhizome (ZO). It is a balanced health preparation in Thai traditional medicine. From selective interviews of folk doctors in Southern Thailand, it was found that Benjakul was used as the adaptogen drug for cancer patients. The objectives of this research were to study on biological and chemical fingerprints of ethanolic extract of Benjakul preparation, and also its stability for quality control of the extract. The biological fingerprint of Benjakul preparation and its ingredients were investigated by studied cytotoxicity activity against four human cancer cell lines, lung cancer cell lines (COR-L23), breast cancer cell lines (MCF-7), cervical cancer cell lines (Hela), liver cancer cell line (HepG2), and also tested one normal fibroblast cell (MRC-5) using by SRB assay. Benjakul preparation was also isolated for pure compounds and tested cytotoxic activity. The chemical fingerprints of Benjakul preparation was investigated by high performance liquid chromatography (HPLC) and also tested stability under accelerated condition. The results found that PC, PI, PL ZO and BEN showed high cytotoxic against COR-L23 (IC_{50} = 15.82, 18.40, 3.43, 7.90 and 19.80 µg/ml, respectively).

Bioassay guided fractionation was used for isolation of ethanolic extract of Benjakul preparation which showed the highest activity. Three pure compounds were found including piperine (BENS1), plumbagin (BENS1) and 6-gingerol (BENS3). These compounds were tested cytotoxic activities. The results exhibited that plumabagin showed the highest activity against Hela, HepG2, MCF-7 and COR-L23 with IC_{50} value of 0.78, 0.49, 0.43 and 0.48 µg/ml, respectively. Although plumbagin exhibited high cytotoxic activity against all types of cancer cell lines, but also showed cytotoxic activity against normal cell line (MRC-5). It showed that plumbagin had nonspecific cytotoxic activity.

The study on chemical fingerprint was carried out using Reverse Phase High performance liquid chromatography (RP-HPLC) and including the study on linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation

(LOQ) for validate the HPLC method. The results exhibited that HPLC method showed good linearity, precision, accuracy and lower LOD and LOQ. The stability of the ethanolic extract of Benjakul preparation was evaluated under accelerated condition (45±2°C with 75±5% RH for 4 months) and determined contents of piperine and plumbagin as marker compounds by using HPLC method. The results found that the amount of piperine was slightly reduced (84.91% after day 120) but plumbagin was greatly reduced (25.74% after day 120).

In summary, the ethanolic extract of Benjakul preparation showed high *in vitro* cytotoxic activity against lung cancer cells. Three pure compounds were isolated and plumbagin showed highest cytotoxic against all cancer cell lines. These results supported the use of Benjakul as a drug for cancer patients especially lung cancer. However plumbagin was unstable. Thus, Benjakul preparation should be kept in the freezer or -20 °C for use.