INTISARI

Insidensi kanker rahim menduduki peringkat kedua terbanyak yang dialami oleh para wanita di dunia, dan menduduki peringkat pertama di negara-negara Afrika, Asia, dan Amerika Selatan. Oleh sebab itulah penelitian untuk mencari obat kanker menjadi sangat penting. *Elephantopus scaber* L. merupakan salah satu tanaman yang memiliki potensi untuk dikembangkan menjadi agen kemoprefentif, terkait dengan *isodeoxyelephantopin* yang dikandungnya. *Isodeoxyelephantopin* merupakan senyawa aktif hasil isolasi dari fraksi etil asetat ekstrak etanolik daun *E. scaber*.

Penelitian ini bertujuan untuk menetapkan kandungan *Isodeoxyelephantopin* di dalam ekstrak etanolik, fraksi etil asetat dan subfraksi etil asetat daun *E. scaber* secara Kromatografi Cair Kinerja Tinggi (KCKT) dengan detektor UV. Melihat korelasi kandungan *isodeoxyelephantopin* dengan efek sitotoksik ekstrak etanolik, fraksi etil asetat dan subfraksi etil asetat daun *E. scaber* terhadap sel HeLa dan menyelidiki mekanisme molekuler yang memperantarai efek sitotoksik tersebut.

Penetapan kadar *isodeoxyelephantopin* dilakukan dengan KCKT. Metode analisa yang dilakukan mencakup linearitas, presisi dan akurasi. Uji aktivitas sitotoksik dilakukan dengan metode MTT. Pengecatan DNA dilakukan dengan cara *double stainning* dilanjutkan dengan imunositokimia.

Kadar *isodeoxyelephantopin* dalam subfraksi etil asetat sebesar 5985,88 ppm. Persentase dalam fraksi etil asetat dan ekstrak etanolik sebesar 7,8% dan 0,06%. Linearitas baku *isodeoxyelephantopin* memiliki korelasi 0,998. Presisi Metode dari pengukuran sampel memiliki nilai RSD 1,83% (RSD $\leq$ 2%). IC_{50} ekstrak etanolik, fraksi etil asetat, subfraksi etil asetat, dan *isodeoxyelephantopin* sebesar 229, 130, 82 dan 27 µg/ml. Subfraksi etil asetat pada konsentrasi 50 dan 100 µg/ml mengindikasikan terjadinya apoptosis. Pengamatan ekspresi protein secara imunositokimia memberikan ekspresi negatif untuk p53 dan ekspresi positif untuk Bax.

KCKT merupakan metode analisa yang cukup baik untuk penetapan kadar *isodeoxyelephantopin*, meskipun akurasinya tidak begitu bagus. Berdasarkan hasil uji sitotoksik terlihat bahwa semakin murni senyawa uji memiliki nilai IC_{50} yang semakin kecil. Hasil pengecatan imunositokimia mengindikasikan bahwa mekanisme apoptosis yang terjadi akibat perlakuan subfraksi etil asetat diperantarai oleh peningkatan ekspresi Bax sebagai salah satu protein proapoptosis melalui jalur yang *independent* p53.

Kata kunci: Penetapan kadar, *E.scaber* , *Isodeoxyelephanthopin*, MTT, *Double stainning*, Apoptosis

ABSTRACT

Cervical cancer is the second most frequent cancer among women worldwide and the most frequent cancer among women in Africa, Asia, and South America, therefore researches to develop cancer drugs are important. *Elephantopus scaber* L. is one of the potential plant that can be developed as a cancer chemopreventive agent, related to the *isodeoxyelephantopin* as the chemical active compound that has been isolated from ethyl acetate fraction of the ethanolic leaf extract.

The aim of this research is to determine *isodeoxyelephantopin* in the ethanolic leaf extract, ethyl acetate fraction and ethyl acetate subfraction of the *E. scaber* by HPLC (High Performance Liquid Chromatography) with UV detector, cytotoxic activity againts HeLa cell line (cervixs cancer) and also to investigate the molecular mechanism of the activity.

Isodeoxyelephantopin was measured by using HPLC. HPLC analitycal method consisted of linearity, repeatability (precision) and accuracy (recovery). Cytotoxic activities has been conducted by using the MTT assay. While the doublestaining has been conducted to examine the presence of apoptotic cell, continued by imunocytochemistry.

The concentration of *isodeoxyelephantopin* in ethyl acetate subfraction was 5985,88 ppm. The percentage of *isodeoxyelephantopin* in ethyl acetate fraction and in ethanolic leaf extract was 7,8% and 0,06% respectively. Linearity was measured by assaying five concentrations of *isodeoxyelephantopin* standards with correlation between two variables 0.998. Repeatability expressed as the percentage of relative standard deviation (RSD) 1,83% (≤ 2%), and accuracy expressed as the percentage of recovery that was only 89%. MTT assay was applied to measure the growth inhibitory effect of ethanolic extract, ethyl acetate fraction, ethyl acetate subfraction, and *isodeoxyelephantopin* compound againt HeLa cell line. The results showed that ethanolic extract, ethyl acetate fraction, ethyl acetate subfraction, and *isodeoxyelephantopin* possess IC_{50} 229 µg/ml, 130 µg/ml, 82 µg/ml and 27 µg/ml respectively. Ethyl acetate subfraction induced apoptotic at concentration 50 and 100 µg/ml. The ethyl acetate subfraction-treated HeLa cells displayed typical morphological apoptotic characteristics by using imunocytochemistry, the results indicate that ethyl acetate subfraction increased the expression of Bax, but not p53.

These results indicated that HPLC was the good enough analytical method to measure the *isodeoxyelephantopin* in ethyl acetate subfraction although the accuracy was not good. Based on cytotoxic examination, the more pure sample the less IC_{50} that they have. Ethyl acetate subfraction induces efficient cell apoptosis by up-regulating Bax expression through p53 independent pathway.

Key words: Concentration measurement, *E.scaber* , *Isodeoxyelephanthopin* MTT, Double stainning, Apoptotic