INVESTIGATION OF ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *PORTULACA OLERACEA*

ABSTRACT

Portulaca oleracea known as purslane in English or *Gelang Pasir* in Bahasa Melayu. Traditionally, this plant is used to alleviate pain and swelling. This plant is reported to exhibit many pharmacological effects such as antibacterial, analgesic, anti-inflammatory and wound healing. In the present study, the experimental approach was based on bioassay-guided fractionation. The crude methanolic and fractionated extracts were initially investigated for their antioxidant and cytotoxic activities. The cytotoxic effects of the extracts were tested against five human cell lines namely the hormone-dependent breast carcinoma cell line (MCF7), human nasopharyngeal cell line (KB), human cervical carcinoma cell line (Ca Ski), human lung carcinoma cell line (A549), human colon carcinoma cell line (HT-29), human colon carcinoma cell line (HCT 116) and non-cancer human fibroblast cell line (MRC5).

The antioxidant properties of the extracts were investigated using three assays: DPPH, reducing power and β-carotene bleaching assays. In the DPPH and reducing power assays, the ethyl acetate fraction of *Portulaca oleracea* exhibited the highest antioxidant activity. This may be due to the presence of antioxidant compounds that can donate electrons to the DPPH radicals. For the reducing power assay, the ethyl acetate fraction of *Portulaca oleracea* may contain high amounts of reductones that can react with free radicals to stabilize and terminate oxidation chain reactions. In the β-carotene bleaching assay, it is the water fraction that exhibited the highest antioxidant

activity followed by the ethyl acetate fraction, hexane fraction and methanol extract. For the β-carotene bleaching assay, the water fraction which showed the highest activity may be due to the presence of antioxidative component that can reduce the extent of β-carotene degradation by neutralizing the linoleate free radicals and other radicals in the system.

In the neutral red cytotoxicity assay, all extracts of *Portulaca oleracea* exerted no damage to MRC5 normal cells. From the results of the cytotoxic activity, it was observed that the ethyl acetate fraction possessed the strongest biological activities compared to other fractions. Only the ethyl acetate fraction showed the highest inhibition capacity against HT-29 cell line. Thus, further chemical investigations were directed to the ethyl acetate fraction of *Portulaca oleracea*.

The analysis of hexane fraction of *Portulaca oleracea* by GC-MS showed that it contains methyl palmitate (11.10 %), methyl oleate (2.88 %), methyl linoleate (4.07 %), methyl linolenate (8.70 %), phytol (41.55 %), palmitic acid (7.86 %) and squalene (19.81 %). Further isolation of the ethyl acetate fraction yielded 4 mixtures. Mixture (I) and (II) did not exhibit cytotoxic activity against HT-29 cells. Whilst, mixture (III) and (IV) exhibited weak cytotoxic activity against HT-29 cells. It was observed that the semi-purified form has lost its activity in comparison to the crude form. This observation can be explained through the effect of synergism. The ethyl acetate fraction of *Portulaca oleracea* contain a combination of active constituents which interact within themselves to enhance (synergize) the therapeutic effect. Synergism among the components in the mixture contributed to the overall cytotoxic activity which depended not only on the structure and interaction among the components, but also the concentration of certain components. Purification led to loss of biological activity indicating that synergistic activities occured between the components.

The findings of *Portulaca oleracea* in the present study provide scientific validation on the use of *Portulaca oleracea* for cancer treatment.

KAJIAN AKTIVITI ANTIOKSIDAN DAN AKTIVITI KESITOTOKSIKAN *PORTULACA OLERACEA*

ABSTRAK

Portulaca oleracea dikenali sebagai purslane dalam Bahasa Inggeris dan Gelang Pasir dalam Bahasa Melayu. Dalam perubatan tradisional, tumbuhan ini digunakan untuk melegakan kesakitan dan bengkak. Tumbuhan ini dilaporkan menunjukkan banyak kesan farmakologi seperti antibakteria, analgesik, anti-inflamasi dan kebolehan memulihkan luka. Dalam kajian ini, pendekatan eksperimen yang digunakan adalah pemfraksikan berdasarkan bioaktiviti. Aktiviti antioksidan dan aktiviti kesitotoksikan ekstrak *Portulaca oleracea* dikaji dalam experimen ini. Penyaringan aktiviti kesitotoksikan dijalankan dengan menggunakan kesitotoksikan *in vitro* ke atas lima titisan sel karsinoma manusia iaitu titisan sel karsinoma payu dara yang melibatkan hormon (MCF7), titisan sel karsinoma epidermoid nasofarinks (KB), titisan sel karsinoma serviks (Ca Ski), titisan sel karsinoma paru-paru (A549), titisan sel karsinoma kolon (HT-29), titisan sel karsinoma kolon (HCT 116) dan titisan sel manusia bukan karsinoma (MRC5).

Aktiviti antioksidan bagi ekstrak *Portulaca oleracea* dikaji dengan menggunakan assai DPPH radikal, assai kuasa penurunan dan assai pelunturan β-karotena. Fraksi etil asetat *Portulaca oleracea* mencatatkan aktiviti antioksidan yang paling tinggi dalam assai DPPH radikal dan assai kuasa penurunan. Fraksi etil asetat mencatatkan aktiviti antioksidan yang tertinggi dalam assai DPPH radikal mungkin disebabkan oleh kehadiran sebatian-sebatian yang dapat menderma elektron kepada radikal DPPH. Untuk assai kuasa penurunan, kehadiran "reductone" yang banyak dalam fraksi etil asetat membolehkan fraksi ini menunjukkan kuasa penurunan yang sangat kuat. "Reductone" dapat bertindak balas dengan radikal bebas, menstabilkan dan

menamatkan reaksi rantai oksidasi. Untuk assai pelunturan β-karotena, fraksi akues mencatatkan aktiviti antioksidan yang paling tinggi diikuti oleh fraksi etil asetat, heksana dan ekstrak metanol. Aktiviti antioksidan yang ditunjukkan oleh fraksi akues mungkin disebabkan oleh kehadiran komponen antioksidatif yang dapat mengurangkan degradasi β-karotena. Komponen antioksidatif dapat meneutralkan radikal linoleat dan radikal bebas lain yang wujud dalam sistem tersebut.

Penyaringan aktiviti kesitotoksikan dilakukan dengan menggunakan assai neutral red. Kesemua fraksi *Portulaca oleracea* tidak menunjukkan ketoksikan terhadap titisan sel manusia bukan karsinoma (MRC5). Berdasarkan keputusan penyaringan aktiviti kesitotosikan, fraksi etil asetat menunjukan aktiviti kesitotoksikan yang paling tinggi berbanding dengan fraksi yang lain. Fraksi etil asetat menunjukkan aktiviti kesitotoksikan yang sangat signifikan terhadap titisan sel karsinoma kolon (HT-29). Justeru itu, kajian kimia ditujukan kepada fraksi etil asetat *Portulaca oleracea*.

Daripada analisis GC-MS, didapati bahawa fraksi heksana *Portulaca oleracea* mengandungi metil palmitat (11.10 %), metil oleat (2.88 %), metil linoleat (4.07 %), metil linolenat (8.70 %), fitol (41.55 %), asid palmitik (7.86 %) dan skualena (19.81 %). Kajian kimia fraksi etil asetat menghasilkan 4 campuran. Campuran (I) dan (II) tidak menunjukkan aktiviti kesitotoksikan terhadap sel karsinoma kolon HT-29. Manakala campuran (III) dan (IV) menunjukkan aktiviti kesitotoksikan yang lemah terhadap sel karsinoma kolon HT-29. Didapati bahawa fraksi dalam keadaan semi-penulenan kehilangan aktiviti kesitotoksikannya jika dibandingkan dengan fraksi asal etil asetat. Keadaan ini terjadi disebabkan oleh kesan sinergistik. Kombinasi komponen-komponen dalam fraksi etil asetat *Portulaca oleracea* membolehkan komponen-komponen berinteraksi sesama mereka dan menghasilkan kesan sinergistik. Kesan sinergistik bukan sahaja bergantung kepada struktur dan interaksi antara komponen, tetapi juga

kepekatan sesuatu komponen. Kehilangan aktiviti kesitotoksikan melalui penulenan menunjukan berlakunya kesan sinergistik.

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