ABSTRACT

Three selected taxonomically identified *Diospyros* species namely *Diospyros graciliflora* Hiern., *D. discolor* Willd. and *D.lanceifolia* Roxburgh. were studied for their anti-tumour promoter activity. For *D. graciliflora* and *D. discolor*, the plant parts used were stem and stem bark, whereas in the case of *D. lanceifolia* only the stem was used. The dried powdered samples were extracted with petroleum ether (PE), chloroform and methanol respectively to yield all together 15 crude extracts. These crude extracts were preliminary screened for bioactive compounds using brine shrimp (*Artemia salina* Leach.) lethality procedure. The PE extracts of *D. discolor* and *D. lanceifolia* stem and the PE extract of *D. discolor* stem bark exhibited the LC_{50} values of 122.4, 196.5 and 146.7 ppm respectively. The *D. graciliflora* stem exhibited a lower LC_{50} value of 301.7 ppm. The other extracts were not active against brine shrimp with LC_{50} values of above 3 500 ppm.

The three crude extracts positive for brine shrimp lethality (LC_{50} < 200 ppm) and one with low activity (LC_{50} 307.1 ppm) were further tested for their anti-tumour promoter activities by observing their inhibitory effect on 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induced Epstein-Barr virus early antigen (EBV EA) in Raji cells. The crude PE extract of *D. discolor* stem exhibited inhibitory effect with IC_{50} of 16.43 µg/ml. The other cude extracts, did not exhibit any EBV EA inhibitory effects.

Phytochemical studies were also carried out on these four crude extracts. The results of phytochemical analysis showed that the major secondary metabolites were terpenes. Separation of compounds from the crude PE extract of *D. discolor* stem was then conducted using column chromatography and preparative TLC method. From this procedure, three pure compounds were yielded and labelled as Dd P2, Dd P3 and Dd P6. Dd P2, Dd P3 and Dd P6 were identified as lupeol, betulin and betulinic acid respectively, identified by comparison with authentic compounds and spectroscopic analysis. Experiments for anti-tumour promoter activities were further conducted on these pure compounds. Only lupeol which was labelled as Dd P2 exhibited inhibitory activity against EBV EA induction with IC_{50} value of 0.009 μg/ml.

Five plants from the family of Euphorbiaceae which were known for their tumour promoter activity were also collected from University of Malaya Botanical Garden, Rimba Ilmu. They were *Euphorbia hirta* L., *E. tirucalli* L., *E. splendes*, *Jatropha podagrica* Hook. and *Pedilanthus tithymaloides* (L.) Poitt.. These plants were extracted with PE in order to obtain natural phorbol esters. Experiments for the anti-tumour promoter activity were then conducted on lupeol, betulin and betulinic acid with the phorbol esters from the Euphorbiaceae plants, used in place of TPA, to induced EBV EA in Raji cells. Of these three compounds, only lupeol showed strong inhibitory activity while betulin and betulinic acid were ineffective as anti-tumour promoter in the same treatment system. The IC_{50} values of *E. hirta*, *E. tirucalli*, *E. splendes*, *J. podagrica* and *P. tithymaloides* induced EBV EA treated with lupeol were 0.012, 0.014, 0.013, 0.016 and 0.015 μg/ml respectively.

In conclusion, PE extract of *D. discolor* stem was found to be effective as an anti-tumour promoter. Three triterpenes namely lupeol, betulin and betulinic acid were isolated from the PE extract of *D. discolor* stem. Lupeol demonstrated the strongest anti-tumour promoter activity whereas betulin and betulinic acid were not active against EBV EA induction in Raji cells. Natural phorbol esters obtained from the Euphorbiaceae plants could be used in place of TPA as after induction with the phorbol esters, the equivalent values of inhibitory action by the PE extracts was obtained in Raji cells.

Abstrak

Kajian bagi aktiviti anti-penggalak tumor telah dilakukan ke atas tiga spesies terpilih *Diospyros* yang telah dikenalpasti ciri-ciri taksonomi mereka, iaitu *Diospyros graciliflora* Hiern., *Diospyros discolor* Willd. dan *Diospyros lanceifolia* Roxburgh. Bahagian batang dan kulit batang bagi spesies *D graciliflora* dan *D. discolor* telah digunakan untuk kajian, manakala bagi spesies *D. lanceifolia* pula, hanya bahagian batang yang digunakan. Sampel-sampel dalam bentuk serbuk kering telah diekstrak menggunakan larutan petroleum eter (PE), diikuti dengan klorofom dan akhir sekali dengan metanol bagi menghasilkan sejumlah 15 ekstrak mentah. Ekstrak-ekstrak mentah ini telah digunakan di dalam ujian awal bagi mengesan kehadiran sebatian-sebatian bioaktif dengan menggunakan kaedah kematian anak udang (*Artemia salina* Leach). Ekstrak mentah PE bagi bahagian batang *D. discolor* dan *D. lanceifolia*, juga kulit batang *D.discolor* masing-masing memberikan nilai LC_{50} 122.4, 196.5 dan 146.7 ppm. Ekstrak batang *D. graciliflora* pula, menunjukkan nilai LC_{50} yang lebih rendah iaitu 301.7 ppm. Ekstrak-ekstrak mentah lain adalah tidak aktif terhadap anak udang dengan nilai LC_{50} melebihi 3 500 ppm.

Ujian bagi menentukan aktiviti anti-penggalak tumor telah dijalankan ke atas tiga ekstrak mentah yang positif terhadap ujian kematian anak udang (LC_{50} < 200 ppm) dan satu ekstrak beraktiviti rendah (LC_{50} 301.7 ppm) dengan menggunakan kaedah perencatan ke atas antigen awal virus Epstein-Barr yang digalakkan oleh 12-O-tetradecanoyl-phorbol-

13-acetate (TPA) di dalam sel Raji. Ekstrak mentah PE bagi bahagian batang *D. discolor* menunjukkan kesan perencatan dengan IC_{50} bernilai 16.43 μg/ml. Ekstrak-ekstrak mentah lain tidak memberikan sebarang kesan perencatan.

Kajian fitokimia telah juga dijalankan ke atas ke empat-empat ekstrak mentah tersebut. Keputusan analisis fitokimia menunjukkan bahawa sebatian kimia utama adalah daripada kelas terpen. Kaedah kromatografi turus dan persediaan kromatografi lapisan nipis telah digunakan untuk mengasingkan sebatian-sebatian di dalam ekstrak mentah PE bagi bahagian batang *D. discolor*. Tiga sebatian tulen telah diperoleh dan dilabelkan sebagai Dd P2, Dd P3 dan Dd P6. Pengecaman sebatian tersebut telah dilakukan melalui perbandingan dengan sampel autentik. Dd P2 telah dikenalpasti sebagai lupeol, manakala Dd P3 adalah betulin, Dd P6 pula adalah asid betulinik. Ujian bagi aktiviti anti-penggalak tumor juga dijalankan ke atas ke tiga-tiga sebatian tulen ini. Hanya lupeol yang dilabelkan sebagai Dd P2 menunjukkan aktiviti perencatan dengan nilai IC_{50} 0.009 μg/ml.

Lima jenis tumbuhan daripada family Euphorbiaceae yang telah diketahui menunjukkan aktiviti penggalak tumor telah juga dikutip dari Rimba Ilmu, University Malaya. Tumbuhan tersebut adalah *Euphorbia hirta* L., *E. tirucalli* L , *E. splendes, Jatropha podagrica* Hook. dan *Pedilanthus tithymaloides* (L.) Poitt. Tumbuhan tersebut telah diekstrak dengan menggunakan larutan PE untuk menghasilkan phorbol ester asli. Ujian bagi aktiviti anti-penggalak tumor telah dijalankan ke atas ke tiga-tiga sebatian tulen (lupeol, betulin dan asid betulinik) yang ditindakbalaskan bersama-sama phorbol ester

asli dari ke lima-lima tumbuhan tersebut yang bertindak sebagai pengganti kepada TPA. Keputusan ujikaji menunjukkan hanya lupeol memberikan kesan perencatan manakala betulin dan asid betulinik tidak menunjukkan sebarang aktiviti anti-penggalak tumor. Nilai IC_{50} bagi *E. hirta*, *E. tirucalli*, *E. splendes*, *J. podagrica* dan *P. tithymaloides* adalah 0.012, 0.014, 0.013, 0.016 dan 0.015 μg/ml.

Kesimpulannya, didapati bahawa ekstrak mentah PE bagi bahagian batang *D. discolor* adalah efektif sebagai anti-penggalak tumor. Tiga jenis triterpene iaitu lupeol, betulin dan asid betulinik berjaya diasingkan daripada ekstrak PE batang *D. discolor*. Lupeol menunjukkan aktiviti anti-penggalak tumor paling berkesan manakala betulin dan asid betulinik adalah tidak aktif. Phorbol ester asli yang diperolehi daripada tumbuhan Euphorbiaceae boleh digunakan sebagai pengganti kepada TPA kerana selepas induksi dengan phorbol ester tersebut, kesan perencatan yang setara dengan ekstrak-ekstrak mentah PE diperoleh dalam sel Raji.