ANTIOXIDANT AND CYTOTOXIC INVESTIGATIONS OF *BETA VULGARIS L.*

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ABSTRACT

Beta vulgaris has been commonly consumed and traditionally used for various medicinal purposes. The present study scientifically evaluates the antioxidant and cytotoxic potential of the plant using various bioassays. The root part of *Beta vulgaris* was collected and extracted using methanol and then fractionated with hexane, ethyl acetate and water. The fresh root of *Beta vulgaris* was juiced and then subjected to evaporation under reduced pressure to form an extract. Chemical investigations were then directed to the ethyl acetate fraction and juice extract; fraction ET1, ET2 and ET3 were obtained from ethyl acetate fraction by isolation using high performance liquid chromatography (HPLC) technique while fraction purple and yellow were isolated from the juice extract using the same technique.

Antioxidant activity of the juice extract, crude methanolic, fractionated extracts (hexane, ethyl acetate and water) and sub-fractions [(ET1, ET2, ET3 – from ethyl acetate fraction), (purple and yellow fractions – from juice extract)] of the plant were evaluated. The antioxidant assays that measured the first line of defense mechanism were TBARS assay and metal chelating assay. The second line antioxidant defense capacity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, reducing power assay, superoxide dismutase (SOD) activity assay and Folin-Ciocalteu Assay. Third line of defense was evaluated by β-carotene bleaching assay and tyrosinase inhibitory assay (monophenolase and diphenolase activity). Only the crude extracts were studied for their cytotoxic activity based on the Neutral Red assay.

For the DPPH radical scavenging assay, the ethyl acetate fraction exhibited the highest activity (IC_{50} = 0.31 mg/ml), followed by the methanol extract, juice extract, water and hexane fractions. As for the sub-fractions, the purple fraction exhibited the highest

activity (IC_{50} = 0.13 mg/ml), followed by ET2, ET1, yellow and ET3. For the reducing power assay, the ethyl acetate fraction again showed the highest antioxidant activity with the highest potential of reduction in converting ferricyanide complex to ferrous form. This was followed by butylated hydroxyanisole (BHA), water fraction, methanol extract, hexane fraction and juice extract. Among the fractions, the purple fraction had the highest activity followed by ET1, ET3, ET2 and the yellow fraction. In the β-carotene bleaching assay, the hexane fraction showed the highest antioxidant activity followed by BHA, ethyl acetate fraction, ascorbic acid, juice extract, methanol extract and water fraction. In the metal chelating assay, all the extracts and sub-fractions had lower percentage of inhibition as compared to the standard (EDTA). In the SOD activity assay, the highest antioxidant activity was exhibited by ethyl acetate fraction (IC_{50} = 0.71 mg/ml), methanol extract, water fraction, juice extract and hexane fraction. As for the sub-fractions, excellent activities were reported by ET1 (IC_{50} = 0.21 mg/ml) and ET2 (IC_{50} = 0.24 mg/ml). Moderate activity was shown by the purple fraction. The yellow fraction and ET3 had low SOD activity. In the lipid peroxidation assay (TBARS), the inhibition rate of lipid peroxidation was highest for methanol extract followed by hexane fraction, juice extract, water fraction and ethyl acetate fraction. As for the sub-fractions, only the yellow fraction had higher inhibition towards lipid oxidation as compared to standards. In the tyrosinase inhibitory assay, monophenolase activity using L-tyrosine as substrate was tested. Crude extracts and fractions did not show higher activity than kojic acid but sub-fractions such as ET1, the purple and yellow fractions showed better activity than kojic acid. Tyrosinase inhibitory assay which used L-Dopa as the substrate (diphenolase activity) showed that the water fractions and juice extract at 20.00 mg/ml had better activity than kojic acid but all the other extracts had lower percentage of inhibition compared to the standard. All the sub-

fractions showed better activity than kojic acid at all the tested concentrations. Folin-Ciocalteau Assay which measures the reducing capacity of the extracts and fractions showed that ethyl acetate fraction had the highest reducing capability. This may be due to high phenolic content in this particular fraction. The lowest activity was exhibited by the juice extract. Hence, *Beta vulgaris L.* exhibited stronger antioxidant activities in comparison to the standards used in the reducing power assay, β-carotene bleaching assay, SOD assay, TBARS assay and tyrosinase inhibitory assay.

The crude and fractionated extracts were investigated for their effect on the cancer cell lines namely, hormone-dependent human breast (MCF7), human lung (A549), human colon (HCT116), human cervical (CasKi) and the human colon (HT29) carcinoma cell lines, using the Neutral Red Cytotoxicity assay. All the extracts showed poor inhibition towards the cancer cell lines tested (IC_{50} > 100.00 µg/ml) and the ethyl acetate fraction exhibited the lowest IC_{50} value of 69.50 µg/ml.

The identification of components in the sub-fractions through liquid chromatography - mass spectrometry - mass spectrometry (LC-MS-MS) showed the presence of known compounds such as betavulgarin, betanin and isobetanin in ET1; 2,15,17-tridecarboxybetanin and betagarin in ET2; ET3, betagarin in ET3. The yellow fraction showed the presence of vulgaxanthine I whilst the purple fraction contains betanin, isobetanin, neobetanin and decarboxylated betanin.

Thus, it can be concluded that *Beta vulgaris L.* is excellent as an antioxidant in the first, second and third line of defense mechanisms but did not show good *in vitro* cytotoxic ability. With its strong antioxidant properties, *Beta vulgaris L.* can be recommended for chemoprevention and regularly consumed in our diet to maintain good health.

ABSTRAK

Beta vulgaris L. kerap diambil dalam diet dan digunakan secara tradisional untuk pelbagai keperluan kesihatan. Dalam kajian ini, aktiviti antioksidan dan keupayaan sitotoksik dikaji secara saintifik dengan menggunakan pelbagai esei. Bahagian akar *Beta vulgaris L*. dikutip dan diekstrak dengan menggunakan metanol dan difraksikan dengan menggunakan heksana, etil asetat dan air. Bahagian akar *Beta vulgaris L*. yang masih segar juga dijus dan dijadikan sebagai ekstrak. Proses pengasingan dengan menggunakan teknik Kromatografi Cecair Prestasi Tinggi (HPLC) juga dilakukan pada fraksi etil asetat dan ekstrak jus. Penyiasatan kimia dilakukan kepada fraksi etil asetat dan juga ekstrak jus; fraksi ET1, ET2 dan ET3 diperoleh daripada fraksi etil asitat daripada proses pengasingan HPLC manakala, fraksi ungu dan fraksi kuning pula diperolehi daripada ekstrak jus.

Aktiviti antioksidan esktrak jus, ekstrak metanol, fraksi-fraksi heksana, etil asetat dan air dan juga fraksi-fraksi yang telah diasingkan [(ET1,ET2,ET3 daripada fraksi etil asetat) dan (fraksi ungu dan fraksi kuning dari ekstrak jus)] seterusnya dikaji. Assai antioksidan yang mengkaji tentang benteng pertahanan pertama antioksidan adalah assai TBARS dan assai pengikatan ion besi. Benteng pertahanan kedua antioksidan pula dikaji berdasarkan assai DPPH, assai kuasa penurunan, assai aktiviti SOD dan assai Folin-Ciocalteu. Mekanisma benteng pertahanan ketiga pula dikaji oleh assai pelunturan beta karotena dan assai perencatan enzim tirosina (aktiviti monophenolase dan aktiviti diphenolase). Kajian sitotoksik dijalankan menggunakan assai ‘Neutral Red’ untuk ekstrak mentah.

Berdasarkan assai DPPH (1,1-difenil-2 pikrilhidrazil), fraksi etil asetat telah memaparkan aktiviti yang tertinggi (IC_{50} = 0.31 mg/ml), diikuti dengan ekstrak metanol,

ekstrak jus, fraksi air dan fraksi heksana. Bagi fraksi yang telah diasingkan, fraksi ungu telah menunjukkan aktiviti perencatan DPPH yang tertinggi (IC_{50} = 0.13 mg/ml) diikuti dengan fraksi ET2, ET1, kuning dan ET3. Dalam assai kuasa penurunan pula, fraksi etil asetat menunjukkan kuasa penurunan kompleks feriksianida kepada ion ferus yang tertinggi. Ini diikuti dengan fraksi air, ekstrak metanol, fraksi heksana, dan fraksi jus. Bagi fraksi yang diasingkan, penunjuk piawai menunjukkan aktiviti yang lebih baik. Antara fraksi, fraksi ungu memunjukkan aktiviti yang tertinggi. Dalam assai pelunturan beta karotena, fraksi heksana menunjukkan aktiviti tertinggi dan diikuti oleh fraksi etil asetat, ekstrak jus, ekstrak metanol dan fraksi air. Untuk fraksi yang diasingkan, aktiviti tidak begitu tinggi dan aktiviti piawai positif adalah lebih tinggi. Dalam assai pengikatan ion besi, semua ekstrak dan fraksi menunjukkan aktiviti lebih rendah berbanding piawai positif EDTA. Dalam assai aktiviti 'Superoxide Dismutase' SOD, aktiviti tertinggi ditunjukkan oleh fraksi etil asetat diikuti oleh ekstrak metanol, ekstrak jus dan fraksi heksana. Dalam fraksi yang diasingkan pula, fraksi-fraksi ET1 dan ET2 juga menunjukkan aktiviti yang amat baik. Aktiviti yang sederhana terhadap SOD ditunjukkan oleh fraksi ungu dan activiti yang lemah terhadap assai ini ditunjukkan oleh fraksi kuning. Dalam assai oksidasi lemak (TBARS) pula, perencatan terhadap oksidasi lemak yang tertinggi antara fraksi ekstrak dicatat oleh ekstrak metanol dan diikuti oleh fraksi heksana, ekstrak jus, fraksi air dan fraksi etil asetat. Bagi fraksi yang diasingkan pula, hanya fraksi kuning yang menunjukkan perencatan terhadap oksidasi lemak yang tinggi. Dalam assai perencatan enzim tirosinase, L-tyrosine digunakan sebagai substrat untuk menunjukkan aktiviti 'monophenolase' dan L-DOPA digunakan sebagai substrat untuk menunjukkan aktiviti 'diphenolase'. Ekstrak mentah tidak menunjukkan aktiviti yang lebih baik daripada penunjuk piawai, asid kojik, akan tetapi fraksi yang diasingkan; fraksi ET1, ungu dan kuning menunjukkan aktiviti yang

lebih baik daripada asid kojik. Bagi aktiviti diphenolase pula, fraksi air dan ekstrak jus menunjukkan aktiviti perencatan yang lebih tinggi daripada asid kojik pada kepekatan 20.00 mg/ml. Semua fraksi yang diasingkan mempunyai aktiviti yang lebih baik daripada penunjuk piawai bagi akitivi diphenolase. Dalam assai Folin-Ciocalteau yang memantau kuasa penurunan ekstrak, fraksi etil asetat menunjukkan kuasa penurunan yang tinggi. Ini mungkin mempunyai perkaitan yang bahawa fraksi ini mempunyai kandungan bahan kimia fenolik yang tinggi. Aktiviti terendah dalam assai ini dicatat oleh ekstrak jus. Oleh yang demikian, *Beta vulgaris L*. menunjukkkan aktiviti yang lebih baik daripada piawai positif dalam assai kuasa penurunan, assai pelunturan β-karotena, assai aktiviti SOD, assai TBARS dan assai perencatan enzim tirosina.

Assai sitotoksik Neutral Red digunakan untuk kajian sitotoksisiti. Semua ekstrak dan fraksi dikaji dengan sel kanser MCF7, HCT116, HT29, A549 dan CasKi. Hanya fraksi etil asetat menunjukkan aktiviti yang sederhana pada sel HT29 (sel karsinoma usus manusia) (IC_{50} = 69.50 µg/ml). Semua ekstrak dan fraksi lain menunjukkan perencatan yang rendah terhadap sel kanser yang dikaji.

Berdasarkan pengenalpastian menggunakan ‘Liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS), sebatian-sebatian yang telah dikenali seperti betavulgarin, betanin dan isobetanin dapat dikenalpasti dalam ET1. ET2 pula menunjukkan kehadiran 2,15,17-tridekarboksibetanin dan betagarin. Di ET3, betagarin juga didapati hadir. Dalam fraksi kuning, vulgaxanthine I dikenalpasti hadir dan dalam fraksi ungu, kehadiran betanin, isobetanin, neobetanin dan dekarboksibetanin (betanin terdekarboksilat) dikenalpasti.

Daripada kajian ini, kita dapat membuat kesimpulan bahawa *Beta vulgaris L.* mempunyai mekanisma pertahanan primer, sekunder dan tertier. Akan tetapi ia tidak dapat

merencatkan sel kanser. Jadi, *Beta vulgaris L.* adalah penting utnuk menghindarkan kanser dan untuk mengekalkan kesihatan badan yang baik.

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%	Percentage
±	plus-minus
µg	microgram
µl	microlitre
µm	micrometer
CO_2	carbon dioxide
e.g.	for example
etc	et cetera
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric Ion
$FeCl_2$	ferrous chloride
$FeCl_3$	ferric chloride
g	gram
IC_{50}	concentration that causes 50% inhibition
m	meter
mg	milligram
min	minute
ml	mililitre
mm	milimetre
ºC	degree Celcius
U	units
v/v	volume by volume
w/v	weight by volume
α	alpha
β	beta
Ω	ohm
ABTS	2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid
BHA	butylated hyroxyanisole
BHT	butylated hyroxytoluene
SOD	superoxide dismutase
CAT	catalase
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPPH	1,2-diphenyl-2-picrylhydrzyl
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immunosorbent assay
FBS	foetal bovine serum
FCR	Folin Ciocalteu Reagent
FRAP	ferric reducing antioxidant power
GSH	glutathione
GSH.Px:	glutathione peroxidase
GSSG	glutathione disulfide
GST	glutathione S transferase

HAT	hydrogen atom transfer
HPLC	high performance liquid chromatography
LC-MS-MS	liquid chromatography-mass spectrometry-mass spectrometry
LOOH	lipid hydroperoxides
mRNA	messenger ribonucleic acid
NBT	nitroblue tetrazolium
NR	neutral red
ORAC	oxygen radical absorbance capacity
PBS	phosphate buffered saline
RNS	reactive nitrogen species
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
rpm	revolution per minute
SD	standard deviation
SET	single electron transfer
TBA	thiobarbituric acid
TCA	trichloroacetic acid
TEAC	trolox equivalent antiradical capacity
UV	ultraviolet
XO	xanthine oxidase

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