MICROPROPAGATION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *ASPARAGUS OFFICINALIS* cv. MARY WASHINGTON *IN VIVO* AND *IN VITRO*

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This Thesis is dedicated to my Parents
Who supported me for each and every day of my life since birth,
Enabling such a study to take place today

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Abstract

In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. *Asparagus officinalis* is a herbaceous perennial plant that belongs to Liliaceae family, a valued vegetable for its medicinal properties.

The present study was carried out in order to establish an efficient *in vitro* propagation protocol for *Asparagus officinalis*. For this purpose, the nodal explants of *Asparagus officinalis* Cv. Mary Washington were cultured on MS medium containing 3% sucrose and different concentrations of NAA and BAP or IBA and Kn, mixed or separately with range of 0-1.5 mg/l for BAP/Kn and 0-0.5 mg/l for NAA/IBA in order to obtain callus, shoot and root formation. In this study, indirect organogenesis was tested under 2 different conditions (In light and in dark) for the regeneration of *Asparagus officinalis in vitro*. After 6 weeks of culture the results showed that 100% of callus formation in 17 of treatments under dark and 3 treatments under light condition. So between dark and light condition, dark condition was found to be more efficient than light condition in promoting callus formation. Also among the two groups of hormones (BAP + NAA, Kn + IBA concentrations), Kn + IBA was reported to be more efficient that BAP + NAA in promoting callus formation. Results also showed that the highest average number of shoots (4.25) of size 4 mm or more per explant, formed under dark condition using 1.5 mg/l BAP mixed with 0.05 mg/l NAA. The formed shoots under dark condition were less developed, with abnormal thick and yellow color compared with the shoots produced under light condition. In light condition the highest average numbers of shoots (3.63) of size 4 mm or more per explant were found on the MS medium supplemented with 0.8 mg/l BAP alone, not in combination with NAA. Rooting was best induced in shoots excised from shoot cultures which were proliferated on MS medium supplemented with an optimal concentration of 0.4 mg/l IBA (2 roots per explant).

In the second part of the study the antioxidant and antibacterial activities of ethanolic extracts of *in vivo* grown *Asparagus officinalis* cv. Mary Washington were investigated using superoxide dismutase, erythrocyte haemolysis and 2,2- diphenyl-1-picrylhydrazil free radical scavenging methods. The measured antioxidant and antimicrobial potential were then compared with the activities shown by the ethanolic extracts of *in vitro* grown *A. officinalis* as well as ethanolic extract of undifferentiated callus cells of *A. officinalis* produced on Murashige and Skoog medium containing 1.5 mg/l 6-benzylaminopurine combined with 0.5 mg/l naphthalene acetic acid. The highest antioxidant capacity was obtained from the *in vivo* grown plant extract followed by *in vitro* grown plant extract in all three examined assays. Although, no antibacterial activity was detected from both *in vivo* and *in vitro* grown plant extracts in the disc diffusion antimicrobial assay, ethanolic extract of *A. officinalis* offered antibacterial activity against *Bacillus cereus*.

Abstrak

Sejak kebelakangan ini terdapat minat atau kecenderungan baru terhadap ubat-ubatan semulajadi yang diperolehi dari bahagian-bahagian tumbuhan atau ekstrak tumbuhan. *Asparagus officinalis* adalah tumbuhan herba saka yang tergolong dalam keluarga Liliaceae, sayur-sayuran penting yang mempunyai nilai perubatan. Kajian ini telah dijalankan dalam usaha untuk menghasilkan regenerasi dan propagasi pesat tumbuhan *Asparagus officinalis.* Untuk tujuan ini eksplan nod *Asparagus officinalis* cv. Mary Washington telah dikultur di dalam media MS (Murashige & Skoog, 1962) yang mengandungi 3% sukrosa serta NAA dan BAP atau IBA dan kinetin, samada dalam kombinasi atau secara berasingan dengan kepekatan antara 0-1.5 mg/l BAP/Kn beserta 0-0.5 mg/l NAA/IBA untuk mendapatkan kalus, pucuk dan pembentukan akar. Dalam kajian ini, organogenesis secara tidak langsung diuji dibawah dua keadaan berbeza iaitu gelap dan cahaya untuk memperolehi regenerasi *Asparagus officinalis in vitro.* Selepas enam minggu dikultur, keputusan menunjukkan 100% pembentukan kalus dari 17 perlakuan dibawah keadaan gelap dan 3 perlakuan sahaja dibawah keadaan cahaya. Oleh itu, antara keadaan gelap dan cahaya, didapati keadaan gelap lebih efisien berbanding dibawah cahaya dalam pembentukan kalus bagi spesies ini. Diperhatikan juga, antara dua kumpulan hormon (BAP dan NAA; Kn dan IBA), Kn dan IBA adalah lebih berkesan daripada BAP dan NAA dalam mempromosi pembentukan kalus. Hasil keputusan juga menunjukkan bahawa bilangan purata pucuk yang paling tinggi (4.25) bersaiz 4 mm atau lebih bagi setiap eksplan terbentuk di bawah keadaan gelap dengan penambahan 1.5 mg/l BAP yang dicampur dengan 0.05 mg/l NAA kedalam media. Pucuk yang terbentuk di bawah keadaan gelap kurang sempurna atau tidak berkembang dengan baik, dengan warna yang tidak normal serta tebal dan kuning berbanding dengan pucuk yang dihasilkan di bawah keadaan cahaya.

Dalam keadaan cahaya, bilangan purata tertinggi bagi pucuk (3.63), bersaiz 4 mm atau lebih bagi setiap eksplan diperhatikan pada media MS yang ditambah dengan 0.8 mg/l BAP sahaja bukan dalam kombinasi dengan NAA. Pertumbuhan akar yang terbaik pada pucuk pula dihasilkan daripada kultur pucuk yang dikultur dalam media MS yang ditambah dengan IBA pada kepekatan optima iaitu 0.4 mg/l (2 akar per eksplan).

Dalam kajian seterusnya, antioksidan dan aktiviti antibakteria dari ekstrak etanolik dari tumbuhan *in vivo Asparagus officinalis* cv. Mary Washington telah dijalankan menggunakan superoxide dismutase, haemolisis eritrosit dan "2,2,- Diphenyl-1-picrylhydrazil free radical scavenging methods". Antioksidan yang diukur dan potensi antimikrobial kemudiannya dibandingkan dengan aktiviti-aktiviti yang ditunjukkan oleh ekstrak etanolik dari tumbuhan yang dikultur secara *in vitro* dan juga dari tisu kalus yang dihasilkan di atas MS media yang mengandungi 1.5 mg/l 6-benzylaminopurine bersama 0.5 mg/l asid naftalena asetik. Kapasiti antioksidan tertinggi telah diperolehi dari ekstrak tumbuhan yang ditanam secara *in vivo*, diikuti dengan ekstrak *in vitro* dalam ketiga-tiga sampel yang diperiksa. Walaupun tiada aktiviti antibakteria yang dikesan dari kedua-dua ekstrak tumbuhan yang ditanam secara *in vivo* dan *in vitro* menggunakan " disc diffusion antimicrobial assay", tetapi ekstrak etanolik dari *Asparagus officinalis* menunjukkan aktiviti antibakteria terhadap bakteria *Bacillus cereus*.

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2,4 D	2,4-Dichlorophenoxyacetic acid
ABTS	2,2’-azinobis[3-ethylbenzothiazoline-6-sulphonate
ANOVA	Analysis of variance
B	Boron
BAP	6-Benzylaminopurine
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
˚C	Degree celsius
Ca	Calcium
Cl	Chloroform
Cu	Copper
dm	decimeter
DPPH	2,2-diphenyl-1-picrylhydrazil
DNA	Deoxyribonucleotic acid
DMCT	Duncan’s Multiple Comparison Test
ESR	Electron Spin Resonance
FRAP	Ferric reducing ability of plasma
Fe	Iron
g	gram
Hcl	Hydrochloride acid
IAA	Indole-3-Acetic Acid
IBA	Indole-3-Butric Acid
K	Potassium
Kn	kinetin
KJ	Kilojoule
Kcal	Kilocalorie
L	Litter
Mg	Magnesium
mg	milligram
MH	Mueller Hinton broth
Mn	Manganese

MS	Murashige and Skoog
N	Nitrogen
Na	Sodium
P	Phosphate
PBS	phosphate buffer solution
PGRs	Plant Growth Regulators
pCPA	p-chlorophenoxyacetic acid
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TEAC	Trolox equivalent antioxidant capacity
Zn	Zinc