TISSUE CULTURE AND BIOLOGICAL ACTIVITIES OF

GARDENIA JASMINOIDES ELLIS

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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I dedicate this thesis to my deceased father, who taught me the best kind of knowledge and my lovely mother for her love and measureless support.

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Abstrak

Gardenia jasminoides Ellis adalah sejenis tumbuhan renek malar hijau dari keluarga *Rubiacea*. Spesies ini adalah salah satu tumbuhan ubatan dan terkenal dalam perubatan tradisional Cina. Buah *Gardenia jasminoides* Ellis mengandungi antioksidan yang tinggi, sementara pigmen tumbuhan ini digunakan dalam industri makanan.

Kajian ini dijalankan untuk saringan aktiviti antibakteria dan antioksidan menggunakan ekstrak *in vivo* dan *in vitro*, juga untuk mendapatkan kaedah propagasi *in vitro* tumbuhan *Gardenia jasminoides* yang efisien dengan menggunakan pelbagai media kultur tisu yang ditambah dengan hormon auksin dan sitokinin pada kepekatan yang berbeza.

Bagi tujuan ini, eksplan daun daripada *G. jasminoides* telah dikultur pada media MS dan WPM yang mengandungi 3% sukrosa dan hormon auksin (NAA, IBA, IAA, 2-4, D) serta sitokinin (TDZ & KN) pada kepekatan yang berbeza. Kepekatan hormon yang digunakan adalah pada julat 0 -5 mg l^{-1} untuk mendapatkan kalus, serta pembentukan pucuk dan akar. Selepas 4 minggu kalus telah terbentuk, pada kedua-dua jenis media yang telah ditambah dengan pelbagai hormon pada kepekatan yang berbeza. Hasil kajian menunjukkan bahawa 100% kalus telah terhasil pada MS dan WPM yang ditambah dengan 2, 2.5 dan 3 mg l^{-1} hormon NAA. Tiada perbezaan yang signifikan dalam pembentukan kalus yang diperhatikan di antara pelbagai jenis auksin pada media WPM. Sebaliknya, media MS menunjukkan perbezaan yang signifikan pada hormon IAA dibandingkan dengan auksin yang lain. Selain itu, Media MS yang ditambah dengan hormon Kinetin dan TDZ menunjukkan perbezaan dari segi statistik berbanding dengan media WPM.

Eksplan daun juga telah dikultur pada media MS dan media WPM yang ditambah dengan kombinasi semua auksin dengan TDZ dan Kn, tetapi tiada pembentukan pucuk diperhatikan. Pengakaran telah mula terbentuk pada kedua-dua media yang ditambah dengan pelbagai auksin selepas minggu kelima. Media MS yang ditambah dengan (1.5 dan 2 mg l^{-1}) NAA telah menunjukkan respons yang lebih tinggi untuk pembentukan akar (14.8 dan 13.4 cm) dan IAA (4.5 dan 5 mg l^{-1}) yang telah ditambah pada media WPM telah menunjukkan pembentukan akar terpanjang (18.3 dan 18.7cm). Analisis data menunjukkan perbezaan yang signifikan dalam media WPM ditambah dengan IAA, NAA, dan IBA masing-masing. Walau bagaimanapun, media MS menunjukkan perbezaan signifikan apabila ditambah dengan hormon NAA berbanding hormon IAA dan IBA.

Kalus segar telah terbentuk pada media MS yang ditambah dengan NAA (3 mg l^{-1}) dan Kn(5 mg l^{-1}), masing-masing dengan berat 34.23g dan 3.39 g. Di samping itu, kalus basah yang terbentuk pada media WPM yang ditambah dengan NAA (2.5 mg l^{-1}) dan Kn (4 mg l^{-1}) mempunyai berat basah 30.04g dan 3.78g. Lebih banyak kalus terbentuk daripada eksplan daun *Gardenia jasminoides* Ellis pada kedua-dua media MS dan media WPM yang ditambah dengan hormon NAA dan IBA pada kepekatan yang berbeza berbanding hormon auksin yang lain.

Kalus yang terbentuk pada kedua-dua media MS dan WPM yang ditambah dengan hormon NAA menunjukkan aktiviti antibakteria terhadap *Escherichia coli* dan *Bacillus cereus*, iaitu bakteria gram negatif dan gram positif. Ekstrak *in vivo* dan in vitro yang lain tidak menunjukkan zon antibakteria terhadap semua bakteria yang diuji.

Data yang dianalisis dari kajian ini telah menunjukkan potensi antioksidan pada tisu kalus *in vitro* yang menggunakan NAA, IBA, TDZ, dan Kn, dan kajian ini telah

menunjukkan bahawa aktiviti antioksidan kalus mempunyai perbezaan ketara dibandingkan dengan tumbuhan *in vivo*.

Abstract

In order to establish an efficient *in vitro* propagation protocol for *Gardenia jasminoides* Ellis, Murashige and Skoog (MS) and woody plant medium (WPM) with different concentrations and types of auxin and cytokinin were used.

The leaf explants of *G. jasminoides* were cultured on MS and WPM medium containing 3% sucrose and different concentrations of NAA, IBA, IAA, 2,4-D as auxin and TDZ & Kn as cytokinin with range of 0-5 mg l^{-1} in order to obtain callus, shoot and root formation. After 2 weeks, the callus was formed on both types of media supplemented with various hormones at different concentrations. The results showed that 100% callus was formed on MS and WPM supplemented with 2, 2.5 and 3 mg l^{-1} of NAA. No significant difference in callus formation was observed between various auxins on WPM media, in contrast, MS media showed significant differences between IAA to other auxins. In addition, Kinetin and TDZ supplemented to MS media showed statistical differences compare to WPM media.

When leaf explants were cultured on MS and WPM media supplemented with a combination of all used auxins with TDZ and Kn, no shoot formation was observed. Rooting started on both media supplemented with various auxins after the fifth weeks. MS medium supplemented with (1.5 and 2 mg l^{-1}) NAA showed higher response for root length (14.8 and 13.4 cm) and IAA (4.5 and 5 mg l^{-1}) supplemented to WPM media showed the longest root length (18.3 and 18.7 cm), respectively. Data analyses showed significant differences in WPM medium supplemented with IAA, NAA and IBA, respectively. However, MS media showed statistical differences when supplemented with NAA compare to IAA and IBA.

Fresh grown callus on MS medium supplemented with NAA (3 mg l^{-1}) and Kn (5 mg l^{-1}) were 34.23 g and 3.39 g respectively. In addition, 30.04 g and 3.78 g were the fresh weight of the callus, cultured on WPM media supplemented with (2.5 mg l^{-1}) NAA and (4 mg l^{-1}) Kn. A high amount of callus was formed from leaf explants of *G. jasminoides* Ellis on both MS and WPM media supplemented with different concentrations of NAA and IBA.

Extracts from callus grown on both MS and WPM supplemented with NAA showed antibacterial activity against *Escherichia coli* (gram-negative) and *Bacillus cereus* (gram-positive). The other extracts from *in vivo* and *in vitro* calluses showed no antibacterial zone against the rest of the bacteria tested.

The *in vitro*-grown callus derived using NAA, IBA, TDZ, and Kn disclosed the antioxidant activity of the callus extract which is significantly different from the intact plant.

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2, 4-D	2, 4-Dichlorophenoxyacetic acid
2iP	6-Gamma, gamma-Dimethyl allylamino purine
ANOVA	Analysis of variance
ACC	1- aminocylopropane-1- carboxylic acid
B	Boron
BAP	6-Benzylaminopurine
˚C	Degree of Celsius
Ca	Calcium
CFU	Colonies-Forming Units
cm	Centimeter
Cu	Copper
DMCT	Duncan's Multiple Comparison Test
DPPH	2, 2-diphenyl-1-picrylhydrazil
DTPA	Diethylenetriaminepentaacetic acid
Fe	Iron
g	gram
Hcl	Hydrochloride acid
IAA	Indole-3-Acetic Acid
IBA	Indole-3-Butric Acid
K	Potassium
Kn	Kinetin

L	Litter
LS	Linsmaier & skoog medium
nm	Nano meter
Mg	Magnesium
mg	Milligram
mg l^{-1}	Milligram per liter
MH	Mueller Hinton broth
Mn	Manganese
MS	Murashige and Skoog Medium
mm	Millimeter
ml	Milliliter
N	Nitrogen
Na	Sodium
NAA	Naphthalene acetic acid
P	Phosphate
PBS	Phosphate buffer solution
pH	Hydrogen ion concentration
psi	Per Square Inch
PGR	Plant Growth Regulator
SOD	Superoxide Dismutase
UV	Ultra Wave
V/V	Volume per volume
WPM	Woody Plant Media

W/V	Weight per Volume
TDZ	Thidiazuron
Zn	Zinc
µg	Microgram
µl	Micro liter