IN VITRO CULTURES OF *CURCUMA MANGGA* VAL. FOR THE PRODUCTION OF (*E*)-LABDA-8(17), 12-DIENE-15, 16-DIAL

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ABSTRACT

Curcuma mangga Val., commonly known as the mango ginger has been used traditionally as a seasoning for food and as a treatment for stomach aches, fever, and cancer. Due to its medicinal importance, a systematic approach was taken to establish a rapidly growing suspension culture of *C. mangga*. In initiating callus, various responses were obtained from shoot bud explants cultured on MS basal medium supplemented with different concentrations of 2,4-D, NAA and IAA either alone or in combinations. Different concentrations of sucrose were also tested. Rapid growing friable callusobtained from shoot explants cultured on MS basal medium supplemented with 1 mg l^{-1} 2,4-D, 30 g l^{-1} sucrose and 2 g l^{-1} gelrite were selected for the initiation of suspension cultures based on histological morphology. From various medium screened, rapid growing suspension cultures were established by using MS liquid medium supplemented with 0.3 mg l^{-1} 2,4-D, 0.1 mg l^{-1} NAA, 30 g l^{-1} sucrose, 0.1 g l^{-1} malt extract, 0.5 mg l^{-1} d-Biotin, 100 mg l^{-1} glutamine, 5 mg l^{-1} ascorbic acid and citric acid respectively. Phenolic compounds production was most effectively controlled by the incorporation of ascorbic and citric acid as antioxidants.

(*E*)-labda-8(17),12-dien-15,16 dial is one of the bioactive compounds isolated from rhizomes of *C. mangga*. Recently, the cytotoxicity of this compound against cancer cells has been reported. (*E*)-Labda-8(17),12-dien-15,16 dial was extracted from cells, callus, rhizomes and shoots using solvent extraction method. Gas Chromatography (GC) and Gas Chromatography Flame Ionization Detector (GCFID) was used to compare the quantity of (*E*)-labda-8(17),12-dien-15,16 dial production in suspension cells and callus induced through various treatment and at different growth period.The presence of this compound in field grown rhizomes and *in vivo* shoot buds samples were also tested. The amount of (*E*)-labda-8(17),12-dien-15,16 dial in *in vivo*

sources was higher than the *in vitro* sources, but the presence can be enhanced through various method in future studies.

ABSTRAK

Curcuma mangga Val. atau lebih dikenali sebagai temu pauh, secara tradisinya digunakan sebagai perasa dalam makanan dan juga untuk merawat demam, sakit perut dan kanser. Disebabkan kepentingan perubatan spesis ini, langkah yang sistematik telah disusun untuk menghasilkan kultur sel ampaian yang aktif membiak dan membahagi. Dalam uji kaji menghasilkan kalus, pelbagai keputusan didapati daripada eksplan tunas pucuk yang dikultur di atas media pepejal MS yang dirawat dengan beberapa kepekatan dan kombinasi hormon 2,4-D, NAA dan IAA yang berbeza. Kesan kepekatan sukrosa yang berbeza turut diuji. Kalus rapuh (friable) yang giat membahagi diperolehi daripada media pepejal MS yang dirawat dengan 1 mg l^{-1}2,4-D, 30 g l^{-1}sukrosa dan 2 g l^{-1} fitagel. Kalus ini dipilih untuk memulakan kultur sel ampaian berdasarkan histologi kalus ini. Berbanding dengan pelbagai rawatan berbeza yang dikaji, sel ampaian yang aktif membahagi diperolehi daripada media cecair MS yang dirawat dengan 0.3 mg l^{-1} 2,4-D, 0.1 mg l^{-1} NAA, 30 g l^{-1} sukrosa, 0.1 g l^{-1} ekstrak malt, 0.5 mg l^{-1} d-Biotin, 100 mg l^{-1} glutamin, 5 mg l^{-1}asid askorbik dan 5 mg l^{-1}asid sitrik. Penghasilan sebatian fenolik berjaya dikawal melalui penggunaan asid askorbik dan asid sitrik sebagai antioksidan.

(*E*)-labda-8(17),12-dien-15,16 dial adalah salah satu sebatian bioaktif yang diekstrak daripada rizom *C. mangga*. Sitotoksiti sebatian ini terhadap sel-sel kanser telah dilaporkan baru baru ini. Dalam kajian ini, (*E*)-Labda-8(17),12-dien-15,16 dial telah diekstrak daripada sel ampaian, kalus, rizom dan tunas pucuk dengan menggunakan teknik “cold soak”atau pengekstrakan pelarut. Gas Kromatografi (GC) dan “Gas Chromatography Flame Ionization Detector” (GC-FID) digunakan untuk memeriksa dan membandingkan kuantiti (*E*)-labda-8(17),12-dien-15,16 dial yang dihasilkanoleh sel-sel suspensi dan kalus yang diperolehi daripada pelbagai rawatan media dan pada tempoh pertumbuhan yang berbeza dengan rizom biasa dan tunas pucuk. Keputusan menunjukkan kehadiran (*E*)-labda-8 (17),12-dien-15,16 dial dalam

sampel yang diuji. Kuantiti (*E*)- labda-8 (17),12-dien-15,16 dial dalam sumber *in vivo*di dapati lebih tinggi daripada sumber *in vitro*. Walaubagaimanapun, manipulasi faktor kimia dan fizikal dipercayai dapat mempertingkatkan kuantiti (*E*)- labda-8 (17),12-dien-15,16 dial dalam kajian pada masa depan.

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I FEEL TRULY BLESSED

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	Description
%	Percent
ºC	degree celsius
±	more less
:	is to
&	and
^{13}C NMR	carbon-13 nuclear magnetic resonance
2,4-D	2,4-dichlorophenoxyacetic acid
<	less than
>	more than
µl	microlitre
amp	ampere
cm	centimeter
C	carbon
$C_{20}H_{30}O_2$	(*E*)-labda-8(17),12-diene-15,16-dial
DCM	dichloromethane
DNA	deoxyribonucleic acid
e.g.	*exempli gratia* (for example)
et al.	*et alia* (and others)
Fig	Figure
g	gramme
g l^{-1}	gramme per litre
GC	gas chromatography
GC-FID	gas chromatography- flame ionisation detector

GC-MS	gas chromtography-mass spectrometry
H	hydrogen
HCl	hydrogen chloride
$HgCl_2$	mercury chloride
HOCl	hypochlorous acid
IAA	indole-3-acetic acid
kPa	kilopascal
LC	liquid chromatography
min	minute
mg	milligramme
mg l^{-1}	milligramme per litre
ml	millilitre
mm	millimetre
m/z	mass-to-charge ratio
M	Molar
MeOH	methanol
MS	Murashige and Skoog
MS	mass spectrometry
nm	nanometre
NAA	napthaleneacetic acid
NaOCl	sodium hypochlorite
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
O	oxygen
pH	potential hydrogen

ppm	part per million
pTLC	preparative thin layer chromatography
PGR	plant growth regulators
rpm	range per unit
R_f	retention factor
RNA	ribonucleic acid
sec	second
SCV	settle cell volume
S.D	standard deviation
S.E	standard error
TLC	thin layer chromatography
UV	ultra violet light
v/v	volume per volume
V	volt
w/v	weight per volume

LIST OF APPENDIX