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ESSENTIAL OIL PRODUCTION IN CELL CULTURES OF *ARTEMISIA VULGARIS*
VAR. *INDICA*, *CUMINUM CYMINUM*, *FORTUNELLA JAPONICA*, AND *POGOSTEMON CABLIN*

Mrs. Supawan Bunrathep

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the Degree of Doctor of Philosophy Program in Pharmaceutical Chemistry and Natural Products

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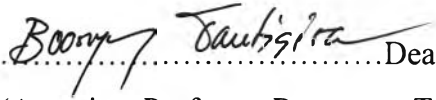
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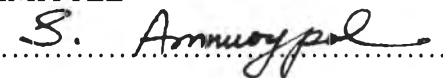
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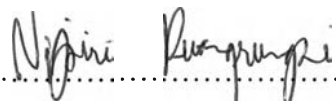
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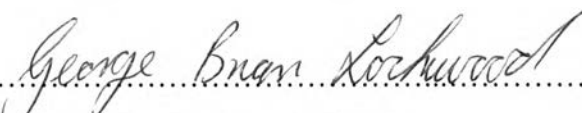

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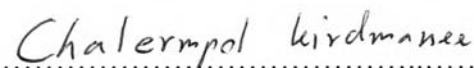
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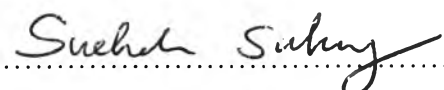

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ศุภวรรณ บุญระเทพ : การสร้างน้ำมันระเหยในเซลล์เพาะเลี้ยงของโกลจุพาลำพาจิน ยี่หระ่า กิมกิด และพิมเสน (ESSENTIAL OIL PRODUCTION IN CELL CULTURES OF *ARTEMISIA VULGARIS* VAR. *INDICA*, *CUMINUM CYMINUM*, *FORTUNELLA JAPONICA*, AND *POGOSTEMON CABLIN*) อ.ที่ปรึกษา : รศ.ดร.นิจศิริ เรืองรังษี, อ.ที่ปรึกษาร่วม : ดร.ธนภัทร ทรงศักดิ์, 199 หน้า. ISBN 974-17-5547-3

การศึกษาองค์ประกอบเคมีของน้ำมันระเหยของโกลจุพาลำพาจิน ยี่หระ่า กิมกิด และพิมเสน ทำได้โดยกลั่นชิ้นส่วนต่างๆ ของพืชด้วยไอน้ำและนำน้ำมันระเหยที่ได้มาวิเคราะห์ด้วยวิธี Gas Chromatography-Mass Spectrometry พบว่าน้ำมันระเหยของพืชดังกล่าวประกอบด้วยสารประกอบเทอร์ปีนอยด์หลายชนิด และมี (+)-davanone (71.59 %), cuminaldehyde (36.30 %), β -pinene (47.44 %), d-limonene (87.07 %) และ patchouli alcohol (60.30 %) เป็นองค์ประกอบเคมีหลักในใบโกลจุพาลำพาจิน ผลยี่หระ่า ใบกิมกิด เปลือกผลกิมกิด และใบพิมเสน ตามลำดับ เพื่อที่จะศึกษาองค์ประกอบเคมีของน้ำมันระเหยของเซลล์เพาะเลี้ยงของพืชเหล่านี้ ชิ้นส่วนของพืชชนิดต่างๆ จึงถูกนำมาฆ่าเชื้อที่ผิวและชักนำให้เกิดเป็นแคลลัสบนอาหารเพาะเลี้ยงกึ่งแข็งชนิด MS ที่ประกอบด้วยสารควบคุมการเจริญเติบโตชนิดต่างๆ แล้วจึงเพาะเลี้ยงในสภาวะที่เหมาะสมสำหรับพืชแต่ละชนิด หลังจากนั้นเปลี่ยนถ่ายแคลลัสที่สมบูรณ์ลงในอาหารเหลวชนิดเดียวกัน เพื่อชักนำให้เกิดเป็นเซลล์เพาะเลี้ยงแขวนลอย การศึกษาองค์ประกอบเคมีของน้ำมันระเหยของเซลล์เพาะเลี้ยงโกลจุพาลำพาจิน ยี่หระ่า กิมกิด และพิมเสนทำได้โดยนำเซลล์เพาะเลี้ยงแต่ละชนิดมาสกัดด้วย dichloromethane และนำสารสกัดที่ได้ไปวิเคราะห์องค์ประกอบเคมีด้วยวิธี Gas Chromatography และ Gas Chromatography-Mass Spectrometry ผลการทดลองแสดงว่าน้ำมันระเหยจากเซลล์เพาะเลี้ยงเหล่านี้มีองค์ประกอบเคมีหลักที่เหมือนกับต้นจริง แต่มีปริมาณน้อยมาก และประกอบด้วยองค์ประกอบเคมีย่อยชนิดต่างๆ ในปริมาณเล็กน้อย ดังนั้นการทดลองนี้จึงศึกษาหาวิธีเพิ่มปริมาณองค์ประกอบเคมีหลักของน้ำมันระเหย และศึกษาการเปลี่ยนแปลงทางชีวภาพของสารเทอร์ปีนอยด์ต่างๆ ในเซลล์เพาะเลี้ยงพืช รวมทั้งหาวิธีชักนำให้เกิดเป็นอวัยวะเพาะเลี้ยงเพื่อให้เป็นแหล่งสะสมน้ำมันระเหยในเซลล์เพาะเลี้ยงเหล่านี้ ผลการทดลองแสดงว่าวิธีที่สามารถเพิ่มปริมาณองค์ประกอบเคมีหลักได้คือการเติมสารตั้งต้นของกระบวนการชีวสังเคราะห์ และการใช้โคโคซานเป็นสารกระตุ้นการสร้างสารทุติยภูมิ ส่วนวิธีอื่นๆ สามารถเพิ่มปริมาณองค์ประกอบเคมีย่อยได้บ้างเล็กน้อย

สาขาวิชาเกษตรเคมีและผลิตภัณฑ์ธรรมชาติ

ปีการศึกษา 2548

ลายมือชื่อนิสิต ศุภวรรณ บุญระเทพ

ลายมือชื่ออาจารย์ที่ปรึกษา นิจศิริ เรืองรังษี

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม ดร.ธนภัทร ทรงศักดิ์

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 CELL CULTURES

SUPAWAN BUNRATHEP : ESSENTIAL OIL PRODUCTION IN CELL
 CULTURES OF *ARTEMISIA VULGARIS* VAR. *INDICA*, *CUMINUM CYMINUM*,
FORTUNELLA JAPONICA, AND *POGOSTEMON CABLIN*. THESIS
 ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-
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Study on chemical constituents of essential oils of *Artemisia vulgaris* var. *indica*, *Cuminum cyminum*, *Fortunella japonica*, and *Pogostemon cablin* was done by hydrodistillation on each explant and then analysed by Gas Chromatography-Mass Spectrometry. It was found that individual essential oil contained terpenoid compounds of which (+)-davanone (71.59 %), cuminaldehyde (36.30 %), β -pinene (47.44 %), d-limonene (87.07 %), and patchouli alcohol (60.30 %) are major constituents of leaves of *Artemisia vulgaris* var. *indica*, fruits of *Cuminum cyminum*, leaves of *Fortunella japonica*, peels of *Fortunella japonica* and leaves of *Pogostemon cablin*, respectively. In order to study chemical constituents of essential oil of these plant cell cultures, each explant was surface sterilised and callus cultures initiated on MS media containing various plant growth regulators, followed by incubation in suitable culture conditions. Cell suspension cultures were initiated by subculturing each cell cultures into new liquid media and maintained in the same conditions. Study on chemical constituents of essential oils produced by these cell cultures was done by extraction with dichloromethane and extracts analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry. The results showed that essential oil obtained from these cultures had contained same major constituents as in the intact plant but the level was low, and also contained a small amount of minor constituents. Methods for improving the major constituents of these essential oils, and biotransformation of terpenoids in individual plant cell cultures, including methods for organ culture initiation for use as accumulation sites in cultures had been studied in this experiment. It was found that feeding precursors of biosynthesis and elicitation with chitosan can improve the yield of major constituents successfully, whilst other methods can improve a small amount of minor constituents.

Field of study	Pharmaceutical Chemistry and Natural Products	Student's signature	Supawan Bunrathep
Academic year	2005	Advisor's signature	Nijsiri Ruangrungsi
		Co-advisor's signature	Thanapat Songsak

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ABBREVIATIONS

%	=	Percent (part per 100); percentage
µg	=	Microgram(s)
µl	=	Microlitre(s)
µm	=	Micrometre(s)
/	=	Per
2,4-D	=	2,4-Dichlorophenoxyacetic acid
AOAC	=	Association of Official Analytical Chemists
BA	=	6-Benzylaminopurine or N ⁶ -benzyladenine
°C	=	Degree Celsius
cm	=	Centimetre(s)
cm ²	=	Centimetre square(s)
CVS	=	Cell volume after sedimentation
DW	=	Dry weight
ed(s)	=	Editor(s)
e.g.	=	For example
EI-MS	=	Electron impact mass spectra
EO	=	Essential oil
<i>et al.</i>	=	Et alii
eV	=	Electron volt
FW	=	Fresh weight
FID	=	Flame ionization detector
Fig.	=	Figure
g	=	Gram(s)
GC	=	Gas Chromatography
GC-MS	=	Gas Chromatography-Mass Spectrometry
h	=	Hour(s)
IC ₅₀	=	50% Inhibitory concentration
Kn	=	Kinetin or 6-furfurylaminopurine
l	=	Litre(s)
M	=	Molar
MEJA	=	Methyl jasmonate

mg/l	=	Milligram per litre
min	=	Minute(s)
ml	=	Millilitre(s)
mm	=	Millimetre(s)
MS	=	Murashige and Skoog's media
NAA	=	Napthaleneacetic acid
PGR	=	Plant growth regulator
pH	=	The negative logarithm of the concentration of hydrogen ions
ppi	=	Pore per inch
ppm	=	Part per million
rpm.	=	Round per minute or revolution per minute
SD	=	Standard deviation
sec	=	Secound(s)
TDZ	=	Thidiazuron
v/v	=	Volume over volume or volume by volume
w/v	=	Weight over volume or weight by volume