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PRODUCTION OF ESSENTIAL OIL FROM *ARTEMISIA DUBIA* TISSUE CULTURE

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การเพาะเลี้ยงแคลลัสของโกฐจุฬาลำพา (*Artemisia dubia* Wall. ex Bess.) ซึ่งเป็นพืชในวงศ์ Asteraceae (Compositae) เริ่มต้นจากการนำชิ้นใบที่ผ่านการฆ่าเชื้อแล้ว วางบนอาหารเพาะเลี้ยงกึ่งแข็งชนิด MS ที่ประกอบด้วย 2,4-dichlorophenoxyacetic acid 1 มิลลิกรัมต่อลิตร และ kinetin 0.1 มิลลิกรัมต่อลิตร เพื่อชักนำให้เกิดเป็นแคลลัส หลังจากนั้นนำแคลลัสที่ได้เปลี่ยนถ่ายลงในอาหารเหลวชนิดเดียวกัน เพื่อเพาะเลี้ยงให้เป็นเซลล์เพาะเลี้ยงแขวนลอย เมื่อใช้วิธี Gas Chromatography (GC) และ Gas Chromatography-Mass Spectrometry (GC-MS) วิเคราะห์และเปรียบเทียบองค์ประกอบเคมีของน้ำมันระเหยที่ได้จากต้นจริง และจากการเพาะเลี้ยงเนื้อเยื่อพืช พบว่า น้ำมันระเหยที่ได้จากแคลลัสและเซลล์เพาะเลี้ยงแขวนลอยมีองค์ประกอบเคมีหลักเหมือนกับต้นจริง คือ สารกลุ่ม sesquiterpenes ชื่อ davanone แต่ปริมาณของ davanone ที่วิเคราะห์ได้นั้นน้อยมากเมื่อเปรียบเทียบกับต้นจริง จึงได้นำกลวิธีทางเทคโนโลยีชีวภาพมาใช้เพื่อเพิ่มความสามารถของเซลล์ในการสร้าง davanone ได้แก่ การใช้ตาข่ายไนล่อนเพื่อตรึงเซลล์ ร่วมกับการเติม geranyl acetate ความเข้มข้นต่างๆ เพื่อเป็นสารตั้งต้นในกระบวนการเปลี่ยนแปลงทางชีวภาพ และการใช้สารดูดซับเพื่อช่วยดูดซับน้ำมันระเหยที่ถูกขับออกมาจากเซลล์ ซึ่งจะสามารถวัดปริมาณของ davanone ได้สูงขึ้นหลังจากการนำกลวิธีทางเทคโนโลยีชีวภาพดังกล่าวมาใช้

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Callus cultures of *Artemisia dubia* Wall. ex Bess., the member of the family Asteraceae (Compositae), were initiated by placing sterilised leaf explants on semi-solid basal MS media, containing 1 mg/l 2,4-dichlorophenoxyacetic acid and 0.1 mg/l kinetin. Suspension cultures were also initiated by using callus cultures on the same media (except without the agar). The chemical constituents of essential oil from callus and suspension cultures were identified and compared to essential oil from intact plant leaves by using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). It was found that callus and suspension cultures produced the same major chemical constituent as the intact plants, sesquiterpenes namely davanone, but it was very low level of davanone recovered from their cultures. Some biotechnological techniques were used for improving the cell capacity to produce davanone. Nylon meshes were used for immobilising cell whilst various concentrations of geranyl acetate were fed for biotransformation process and adsorbent was also used for adsorbing the essential oil excreted from the cells. The level of davanone was increased after using these techniques.

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LIST OF ABBREVIATIONS

%	=	Percent (part per 100); percentage
μg	=	Microgram
μl	=	Microlittre
/	=	Per
2,4-D	=	2,4-Dichlorophenoxyacetic acid
AOAC	=	Association of Official Analytical Chemists
B5	=	Gamborge B5 medium
$^{\circ}$C	=	Degree Celsius
cm	=	Centimeter(s)
cont.	=	Continued
DW	=	Dry weight
ed(s)	=	Editor(s)
e.g.	=	For example
EO	=	Essential oil
et al.	=	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
l	=	Liter(s)
M	=	Molar
mg/l	=	Milligram per liter
min	=	Minute(s)
ml	=	Milliliter(s)

LIST OF ABBREVIATIONS (CONT.)

mm	=	Millimeter(s)
MS	=	Murashige and Skoog's media
NIST	=	National Institute of Standard and Technology
pH	=	The negative logarithm of the concentration of hydrogen ions
ppi	=	Pore per inch
ppm	=	Part per million
rpm	=	Revolution per minute
SD	=	Standard deviation
v/v	=	Volume over volume
w/v	=	Weight over volume