

Article ID: 9110  
DOI: 10.5586/asbp.9110

#### Publication History

Received: 2021-11-29  
Accepted: 2022-05-07  
Published: 2022-09-08

#### Handling Editor

Robert Konieczny; Jagiellonian University in Kraków, Poland;  
<https://orcid.org/0000-0002-4205-2656>

#### Authors' Contributions

AP: supervision of manuscript preparation, writing of Introduction and Conclusions sections, preparing Table 1; AB: writing of the section concerning investigations of *Plantago* spp.; JB: writing of the section concerning investigations of *Dionea muscipula*, *Drosera gigantea*, and *Primula veris*; HE: cowriting of the section concerning investigations of *Ammi majus*, *Ruta* spp., *Pastinaca sativa*, and *Schisandra* spp.; MJ: writing of the section concerning investigations on *Cynoglossum columnae* and *Rindera graeca*; AKA: cowriting of the section concerning investigations of *Drosea* and *Dionea* spp.; MK: cowriting of the section concerning investigations of *Erygium planum* and *Chaenomeles japonica*; MKB: writing of the section concerning investigations of *Phyllanthus amarus* and *Scutellaria barbata*; AKR: cowriting of the section concerning investigations of *Ammi majus*, *Dionea muscipula*, and selected *Drosea* spp.; ŁK: writing of the section concerning investigations of *Salvia* spp.; MŁ: cowriting of the section concerning investigations of *Genista tinctoria*; JM: cowriting of the section concerning investigations of *Cichorium intybus*, *Lactuca* spp., and *Scutellaria baicalensis*; AM: cowriting of the section concerning investigations of *Agastache rugosa*, *Moluccella laevis*, and *Chelidonium majus*; AS: cowriting of the section concerning investigations of *Cichorium intybus*, *Lactuca* spp., and *Scutellaria baicalensis*; KSB: writing of the section concerning investigations of hairy root cultures of *Taxus × media*; ASz: cowriting of the section concerning investigations of *Schisandra* spp.; WS: writing of the section

REVIEW in POLISH BOTANY CENTENNIAL

# Polish Achievements in Bioactive Compound Production From In Vitro Plant Cultures

Agnieszka Pietrosiuk <sup>1\*</sup>, Anna Budzianowska <sup>2</sup>, Jaromir Budzianowski <sup>2</sup>, Halina Ekiert <sup>3</sup>, Małgorzata Jeziorek <sup>1</sup>, Anna Kawiak <sup>4</sup>, Małgorzata Kikowska <sup>2</sup>, Mirosława Krauze-Baranowska <sup>5</sup>, Aleksandra Królicka <sup>4</sup>, Łukasz Kuźma <sup>6</sup>, Maria Łuczkiwicz <sup>5</sup>, Janusz Malarz <sup>7</sup>, Adam Matkowski <sup>8</sup>, Anna Stojakowska <sup>7</sup>, Katarzyna Sykłowska-Baranek <sup>1</sup>, Agnieszka Szopa <sup>3</sup>, Wojciech Szypuła <sup>1</sup>, Barbara Thiem <sup>2</sup>, Sylwia Zielińska <sup>8</sup>

<sup>1</sup>Department of Pharmaceutical Biology and Medicinal Plant Biotechnology, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland

<sup>2</sup>Department of Pharmaceutical Botany and Plant Biotechnology, Poznań University of Medical Sciences, Św. Marii Magdaleny 14, 61-861 Poznań, Poland

<sup>3</sup>Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Kraków, Poland

<sup>4</sup>Intercollegiate Faculty of Biotechnology UG and MUG, University of Gdansk, Abrahama 58, 80-307 Gdańsk, Poland

<sup>5</sup>Department of Pharmacognosy with Medicinal Plant Garden, Faculty of Pharmacy, Medical University of Gdańsk, al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

<sup>6</sup>Department of Biology and Pharmaceutical Botany, Faculty of Pharmacy, Medical University of Lodz, Muszyńskiego 1, 90-151 Łódź, Poland

<sup>7</sup>Maj Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

<sup>8</sup>Department of Pharmaceutical Biotechnology and Department of Pharmaceutical Biology and Botany, Faculty of Pharmacy, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland

\* To whom correspondence should be addressed. Email: [agnieszka.pietrosiuk@wum.edu.pl](mailto:agnieszka.pietrosiuk@wum.edu.pl)

## Abstract

Plant cell and organ cultures are potential sources of valuable secondary metabolites that can be used as food additives, nutraceuticals, cosmeceuticals, and pharmaceuticals. Phytochemical biosynthesis in various in vitro plant cultures, in contrast to that in planta, is independent of environmental conditions and free from quality fluctuations.

Pharmaceutical application of plant biotechnology is of interest to almost all departments of the Faculty of Pharmacy and Institute of Pharmacology in Poland with a botanical profile (Pharmaceutical Botany, Pharmacognosy, and Pharmacology).

This study discusses the advances in plant biotechnology for the production of known metabolites and/or biosynthesis of novel compounds in plant cell and organ in vitro cultures in several scientific centers in Poland.

## Keywords

bioreactors; biotransformation; elicitation; hairy roots; multiple shoots; plant tissue cultures; secondary metabolites; undifferentiated cultures

concerning investigations of *Huperzia selago*; BT: cowriting of the section concerning investigations of *Erygium planum* and *Chaenomeles japonica*; SZ: cowriting of the section concerning investigations of *Agastache rugosa*, *Moluccella laevis*, and *Chelidonium majus*

#### Funding

This work did not involve funding.

#### Competing Interests

No competing interests have been declared.

#### Copyright Notice

© The Author(s) 2022. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits redistribution, commercial and noncommercial, provided that the article is properly cited.

## 1. Introduction

### 1.1. Significance and Major Advantages of Plant In Vitro Cultures

Plants are the exclusive source of flavors, fragrances, dyes, pigments, pesticides, food additives, and life-saving drugs for the majority of the global population. More than 80% of the approximately 30,000 known natural products are of plant origin. The use of plant cells for the production of natural or recombinant compounds of commercial interest has gained attention over the past few decades. Secondary metabolites (also called specialized metabolites) play a major role in the adaptation of plants to their environment and are an important source of pharmaceuticals (Alamgir, 2017; Hussain et al., 2012).

Most pharmaceutically important natural products are isolated from wild or cultivated plants, because of their highly complex structures that make their chemical synthesis not economically feasible (Oksman-Caldentey & Inzé, 2004). However, such methods consume more plant resources than nature could actually regenerate. Additionally, obtaining sufficient active substances from plants is challenging because their content is generally low, plant growth is often slow and requires special cultivation conditions, and some species is restricted to certain climatic zones.

Plant in vitro cultures, since their first use (Haberlandt, 1943), have opened new opportunities for their use and have become a tool in both basic and applied research. They have enabled mass clonal propagation of the most valuable or endangered species independent of their natural life-cycle under strictly controlled conditions. They have also been used as tools for introducing genetic modifications. Moreover, they have increased the acquisition of biologically active compounds (Altpeter et al., 2016; Debnath et al., 2006; Karuppusamy, 2009; Thorpe, 2007).

Biotechnological production of active compounds in plant cell and organ cultures is an attractive alternative; however, the commercial success is limited till date because of insufficient understanding of their biosynthesis mechanism. Advances in plant genomics and metabolite profiling offer possibilities for studying the complexity of the biochemical capacity of plants. Genomics tools can be used to enhance the production of known target metabolites or synthesize novel compounds using combinatorial biochemistry in cultivated plant cells (Oksman-Caldentey & Inzé, 2004).

Despite some limitations, research in plant tissue and organ culture technology has produced many bioactive metabolites (alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids) for new therapeutics under defined conditions (Alamgir, 2017; Hussain et al., 2012).

Since the 1960s, intensive research has introduced in vitro tissue culture-derived secondary metabolites, including scopolamine, rosmarinic acid (RA), shikonin, and berberine. Paclitaxel is a flagship example of using in vitro cultures to extract valuable therapeutic substances. Obtaining paclitaxel from naturally growing yew trees is economically and ecologically disadvantageous due to the long life-cycle of *Taxus* spp. Additionally, the chemical synthesis of paclitaxel is difficult and results in significant yield losses at almost every stage. Biotechnological studies reported biosynthesis of both paclitaxel and its precursor baccatin III from *Taxus brevifolia* suspension cultures. The in vitro synthesis of bioactive compounds or their precursors not only controls microbiological purity, but also allows the production of the given compound independently of its natural sources as shown in the example above. Furthermore, biosynthesis of secondary metabolites is carried out in a predictable, reproducible, and tightly controlled manner. The application of elicitors, appropriate culture medium, genetic modifications, and selection of suitable culture methods, including cell immobilization or large-scale farming in bioreactors, improves the efficiency of biosynthesis (Michalak, 2021).

Unfortunately, the number of secondary metabolites commercially extracted from tissue and cell cultures for pharmaceutical purposes remains low primarily due to the high demands of the industry, both in terms of the efficiency of the processes, as well as the purity and quality of the ingredients and products. However, this does

not mean that there is little interest in plant in vitro culture. Innovations and developments in other industries, particularly cosmetics, increase the demand for plant origin components. Increasing consumer awareness, concern for sustainability and the environment, and the desire to use effective and safe products, often with ingredients of exotic origins (including endemic plants), will expand the use of in vitro plant cultures (Fonseca-Santos et al., 2015).

This review presents the achievements of in vitro plant culture studies for the production of bioactive compounds in scientific centers in Poland with a pharmaceutical profile. The main research findings are summarized in Table 1.

**Table 1** Summary of secondary metabolites obtained from various in vitro-cultured plant tissues.

Family/ Species	Type of in vitro culture	Secondary metabolite	Research center*	References
<b>Apiaceae</b>				
<i>Ammi majus</i>	Callus	Umbelliferone, bergapten, xanthotoxin, isopimpinellin, imperatorin	3	Ekiert, 1993, 2004; Ekiert & Gomółka, 2000a
<i>Ammi majus</i>	Callus	Umbelliferone	4	Królicka et al., 2001
<i>Ammi majus</i>	Cell suspension	Umbelliferone	4	Królicka et al., 2001
<i>Ammi majus</i>	Hairy roots	Umbelliferone	4	Królicka et al., 2001
<i>Ammi majus</i>	Callus	Scopoletin, dehydrogeijerin	4	Staniszewska et al., 2003
<i>Eryngium planum</i>	Micropropagated plants (shoots and roots), shoot culture, callus, cell suspension	Rosmarinic acid, chlorogenic acid, caffeic acid	2	Kikowska et al., 2012, 2015; Thiem et al., 2013
<i>Eryngium planum</i>	Micropropagated plants (shoots and roots), root culture, callus, cell suspension	Triterpenoid saponins: 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-O-acetyl, 22-O-angeloyl-R1-barrigenol [ES1], 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-O-angeloyl-R1-barrigenol [ES2], 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-O-angeloyl-A1-barrigenol	2	Kikowska et al., 2019; Kowalczyk et al., 2014
<i>Pastinaca sativa</i>	Callus	Umbelliferone, bergapten, xanthotoxin, isopimpinellin	3	Ekiert, 2004; Ekiert & Gomółka, 2000b
<b>Asteraceae</b>				
<i>Cichorium intybus</i>	Hairy roots	8-Deoxylactucin, lactucopicrin and crepidiaside B, sonchuside A, and zaluzanin-like guaianolide – ixerisoside D, (7S,8R)-3'-demethyl-dehydrodiconiferyl alcohol-3'-O-β-glucopyranoside, 3,5-DCQA and 8-deoxylactucin glucoside, hydroxycinnamates	7	Malarz et al., 2002, 2007; Malarz, Stojakowska, & Kisiel, 2013; Malarz, Stojakowska, Szneler, & Kisiel, 2013;

Continued on next page

## 1.2. The History of In Vitro Cultures in Polish Scientific Centers With the Pharmaceutical Profile

Scientific research on using plant in vitro cultures to the study of medicinal plants was initiated at the Pharmaceutical Faculties in Warsaw, Poznań, Kraków, Łódź, and Gdańsk in the 1970s. Prof. Mirosława Furmanowa (1927–2014) was the first in Poland to introduce this field of research to the Department of Pharmaceutical

Table 1 Continued.

Family/ Species	Type of in vitro culture	Secondary metabolite	Research center*	References
<i>Lactuca aculeata</i>	Callus	Mono- and dicaffeoylquinic acids, 1,5-di- <i>O</i> -caffeoylquinic acid, 1,5-DCQA, (+)-5-methoxybalanophonin, syringaresinol, (+)-buddlenol A, (+)-balanophonin-9- <i>O</i> - $\beta$ -glucopyranoside, (–)-dihydrodehydrodiconiferyl alcohol-4- <i>O</i> - $\beta$ -glucopyranoside, (–)-dihydrodehydrodiconiferyl alcohol-9- <i>O</i> - $\beta$ -glucopyranoside	7	Stojakowska & Malarz, 2017
<i>Lactuca virosa</i>	Hairy roots	8-Deoxylactucin, jacquinelin, crepidiaside B and picriside A, macrocliniside A, ixerin F and scorzoside, lactuside A, lactucopicrin, 5- <i>O</i> -caffeoylquinic acid, 3,5-di- <i>O</i> -caffeoylquinic acid	7	Kisiel et al., 1995; Stojakowska et al., 2012
<i>Lactuca virosa</i>	Callus and cell suspensions	Neolignans, caffeic acid derivatives	7	Stojakowska & Kisiel, 2000; Stojakowska et al., 2012
<b>Boraginaceae</b>				
<i>Cynoglossum columnae</i>	Anatomical roots	Rinderol, cynoglosol	1	Jeziorek et al., 2019
<i>Rindera graeca</i>	Hairy roots	Rinderol	1	Graikou et al., 2021
<b>Byblidaceae</b>				
<i>Byblis liniflora</i>	Plantlets	Acteoside, isoacteoside, desrhamnosylacteoside, desrhamnosylisoacteoside	2	Schlauer et al., 1994
<b>Droseraceae</b>				
<i>Dionaea muscipula</i>	Plantlets	Plumbagin, 8,8'-biplumbagin, 3-chloroplumbagin, maritinone, ellagic acid, 3- <i>O</i> -methylellagic acid, 3,3'-di- <i>O</i> -methylellagic acid, 3,3'-di- <i>O</i> -methylellagic acid 4- <i>O</i> -glucoside, 3,3'-di- <i>O</i> -methylellagic acid 4,4'-di- <i>O</i> -glucoside, kaempferol 3- <i>O</i> -glucoside and galactoside, quercetin 3- <i>O</i> -glucoside and galactoside, 2''- <i>O</i> -galloyl esters of quercetin 3- <i>O</i> -glucoside and galactoside; 1- <i>O</i> -galloylglucose. Plumbaside A	2	Kukulczanka & Budzianowski, 2002; Pakulski & Budzianowski, 1996a, 1996b
<i>Dionaea muscipula</i>	Adventitious shoots	Plumbagin	4	Krolicka et al., 2008
<i>Dionaea muscipula</i>	Transformed teratomas	Plumbagin	4	Makowski et al., 2021
<i>Drosera capensis</i> var. <i>alba</i>	Transformed teratomas	Ramentaceone	4	Krolicka et al., 2010
<i>Drosera capensis</i>	Adventitious shoots	Ramentaceone	4	Krolicka et al., 2008
<i>Drosera gigantea</i>	Leaf rosettes	Plumbaside A, plumbagin, droserone (3,5-dihydroxy-2-methyl-1,4-naphthoquinone), hydroxydroserone (3,5,8-trihydroxy-2-methyl-1,4-naphthoquinone), droserone 5- <i>O</i> -glucoside, hydroxydroserone 5- <i>O</i> -glucoside		Budzianowski, 2000

Continued on next page



Table 1 Continued.

Family/ Species	Type of in vitro culture	Secondary metabolite	Research center*	References
<i>Drosera intermedia</i>	Leaf rosettes	Myricetin 3-O-rhamnoside, quercetin 3-O-rhamnoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, plumbaside A (2-methyl-1,4,5-trihydroxynaphthalene 4-O-β-glucopyranoside), rossoliside, plumbagin, 7-methyljuglone	2	Budzianowski, 1996; Budzianowski et al., 1993
<i>Drosera rotundifolia</i>	Leaf rosettes	Rossoliside	2	Budzianowski, 1996
<i>Drosera spatulata</i>	Leaf rosettes	Myricetin 3-O-rhamnoside, quercetin 3-O-rhamnoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, rossoliside (7-methyl-1,4,5-trihydroxynaphthalene 4-O-β-glucopyranoside)	2	Budzianowski, 1995; Budzianowski et al., 1993
<i>Drosera spatulata</i>	Leaf rosettes	Triterpene 3-O-acetylaleuritic acid (3-O-AAA)	2	Toton et al., 2020
<b>Drosophyllaceae</b>				
<i>Drosophyllum lusitanicum</i>	Shoots, callus	5-hydroxy-4-methoxy-2- naphthalenecarboxylic acid 5-O-β-glucoside (drosophylloside) and 5-hydroxy-4-methoxy-2- naphthalenecarboxylic acid methyl ester; 5-hydroxy-4-methoxy-2- naphthalenecarboxylic acid (ancistronephthoic acid B), hydroplumbagin 4-O-glucoside (plumbaside A), plumbagin, 3-chloroplumbagin, C-glycosylflavones: vitexin, isovitexin, orientin, isoorientin	2	Budzianowski et al., 2002
<b>Euphorbiaceae</b>				
<i>Phyllanthus amarus</i>	Non- transformed roots	Lignan (7'-oxocubebin dimethyl ether)	5	Sparzak et al., 2015
<b>Fabaceae</b>				
<i>Cyclopia genistoides</i>	Microshoots and callus	Benzophenones and xanthenes	5	Kokotkiewicz et al., 2009
<i>Cyclopia subternata</i>	Cell suspension	Xanthenes (mangiferin derivatives), benzophenones, flavones, flavanes and isoflavones	5	Kokotkiewicz et al., 2009
<i>Cyclopia subternata</i>	Suspension cultures in a stirred-tank bioreactor	7-O-β-glucoside of calycosin, 7-O-glucoside of pseudobaptygenin and 7-O-β-glucoside of formononetin	5	Kokotkiewicz, Luczkiewicz, Kowalski, et al., 2013; Kokotkiewicz, Luczkiewicz, Pawlowska, et al., 2013
<i>Genista tinctoria</i>	Callus	Isoflavonoids (14 metabolites)	5	Luczkiewicz et al., 2014; Łuczkiwicz & Głód, 2003
<i>Genista tinctoria</i>	Shoot/root co cultures	Derivatives of genistin and daidzin	5	Łuczkiwicz & Głód, 2005; Łuczkiwicz & Kokotkiewicz, 2005a
<i>Genista tinctoria</i>	Transformed roots	Isoliquiritigenin	5	Łuczkiwicz & Kokotkiewicz, 2005b
<i>Genista tinctoria</i>	Cell suspension	Isoflavones with genistin as a main component	5	Luczkiewicz & Kokotkiewicz, 2012

Continued on next page

Table 1 Continued.

Family/ Species	Type of in vitro culture	Secondary metabolite	Research center*	References
<b>Lamiaceae</b>				
<i>Agastache rugosa</i>	Shoot cultures	Methyl chavicol (estragole), menthone, isomenthone, limonene, pulegone, $\alpha$ -pinene, piperitone, $\beta$ -caryophyllene, $\alpha$ -humulene, germacrene D	8	Zielińska et al., 2011, 2017
<i>Agastache rugosa</i>	Shoot cultures	Rosmarinic acid, rosmarinic acid methyl ester, cryptochlorogenic acid, feruoylquinic acid, two ferulic acid glucoside isomers, apigenin derivative	8	Zielińska et al., 2019
<i>Moluccella laevis</i>	Callus and shoot cultures	Caffeic acid, ferulic acid, chlorogenic acid, rosmarinic, cyanidin 3-O-galactoside, cyanidin 3-O-malonylglucoside	8	Zielińska et al., 2020
<i>Salvia austriaca</i>	Hairy roots	Taxodione, 15-deoxy-fuerstione, royleanone, taxodone and 7-2'-oxohexyl-taxodione	6	Kuźma, Wysokińska, Różalski, Krajewska, & Kisiel, 2012
<i>Salvia sclarea</i>	Hairy roots	Salvipipone, ferruginol, aethiopinone, 1-oxoaethiopinone, ursolic acid, oleanolic acid, 2 $\alpha$ ,3 $\alpha$ -dihydroxy-urs-12-en-28-oic acid, 2 $\alpha$ ,3 $\alpha$ ,24-trihydroxy-urs-12-en-28-oic acid, $\beta$ -sitosterol, stigmasterol, campesterol	6	Kuźma et al., 2006
<i>Scutellaria barbata</i>	Non-transformed roots	Flavone glycosides (wogonoside, baicalin), phenylethanoid – acteoside	5	Wilczańska-Barska et al., 2011, 2012
<i>Scutellaria lateriflora</i>	Hairy roots	Flavone glycosides (wogonoside, baicalin), phenylethanoid – acteoside	5	Wilczańska-Barska et al., 2011, 2012
<i>Scutellaria baicalensis</i>	Hairy roots	Flavones – glycosides of: baicalein, wogonin, oroxylin A, chrysin, norwogonin, 5,7,2'-trihydroxyflavone, 5,2'-dihydroxy-6,7,8-trimethoxyflavone and 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone	7	Stojakowska & Malarz, 2000; Stojakowska et al., 2001
<b>Lycopodiaceae</b>				
<i>Huperzia selago</i>	Sporophytes	Huperzine A	1	Szypuła et al., 2005
<i>Huperzia selago</i>	Gamethophytes, prothallus	Huperzine A	1	Szypuła et al., 2020
<b>Papaveraceae</b>				
<i>Chelidonium majus</i>	Callus and shoot cultures	Chelidonine, sanguinatinine, chelerythrine, protopine, coptisine, berberine	8	Zielinska et al., 2018
<b>Plantaginaceae</b>				
<i>Plantago lanceolata</i>	Plants, micropropagated plants, callus	Acteoside (verbascoside), plantamajoside, lavandulifolioside, leucosceptoside A, martynoside, desrhamnosylisoacteoside, plantainoside D, desrhamnosylacteoside, lancetoside, i.e., 2-(4-hydroxyphenyl)ethyl O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)-[trans- or cis-p-coumaroyl-( $\rightarrow$ 4)]- $\beta$ -D-glucopyranoside	2	Budzianowska et al., 2004
<i>Plantago media</i>	Micropropagated plants (shoots and roots)	Acteoside, plantamajoside	2	Budzianowska et al., 2019

Continued on next page

Table 1 Continued.

Family/ Species	Type of in vitro culture	Secondary metabolite	Research center*	References
<b>Primulaceae</b>				
<i>Primula veris</i>	Plantlets	3'-hydroxy-4',5'-dimethoxyflavone, 3'-methoxy-4',5'-methylenedioxyflavone, 3',4'-dimethoxyflavone, 2',5'-dimethoxyflavone, flavone, 2'-hydroxyflavone, 2'-methoxyflavone, 3'-methoxyflavone, 3',4',5'-trimethoxyflavone, 5,6,2',6'-tetramethoxyflavone (zapotin)	2	Budzianowski et al., 2005
<b>Rosaceae</b>				
<i>Chaenomeles japonica</i>	Micropropagated plants (shoots and roots), callus, cell suspension	Pentacyclic triterpenoids (ursolic acid, oleanolic acid, betulinic acid), polyphenols [chlorogenic acid, (–)-epicatechin, quercetin-3-hexoside]	2	Kikowska et al., 2018, 2019
<b>Rutaceae</b>				
<i>Ruta graveolens</i>	Shoot cultures	Psoralen, bergapten, xanthotoxin, isopimpinellin	3	Ekiert, 2004; Ekiert et al., 2001; Ekiert & Czygan, 2005, 2007; Ekiert & Gomółka, 1999; Ekiert & Kisiel, 1997
<i>Ruta graveolens</i>	Shoots co-culture with <i>Ammi majus</i> hairy roots	Xanthotoxine	4	Sidwa-Gorycka et al., 2003
<i>Ruta graveolens</i> ssp. <i>divaricata</i>	Shoot- differentiating callus cultures	Bergapten, xanthotoxin, isopimpinellin	3	Ekiert, 2004; Ekiert et al., 2005; Ekiert & Czygan, 2005, 2007
<b>Schisandraceae</b>				
<i>Schisandra. chinensis</i> , <i>Schisandra chinensis</i> 'Sadova'	Agar, stationary liquid agitated and bioreactor microshoot cultures	Dibenzocyclooctadiene lignans (e.g., deoxyschisandrin, schisandrin and gomisin A)	3	Szopa & Ekiert, 2011, 2014
<i>Schisandra henryi</i>	Agar microshoot and callus cultures	Dibenzocyclooctadiene lignans (schisandrin, gomisin G, schisantherin A and B, deoxyschisandrin and schisandrin C), dibenzylbutane lignans (henricine B), and aryltetralin lignans (wulignan A1 and A2, epiwulignan A1, enshicine, epienschicine and dimethylwulignan A1)	3	Jaferník et al., 2020
<i>Schisandra rubriflora</i>	Agar microshoot cultures (♂ and ♀ lines)	Dibenzocyclooctadiene lignans (schisantherin A and B, schisandrin, schisandrin C, gomisin A, D, G, J, N, O, 6-O-benzoylgomisin O, schisandrin A, rubrisandrin A, epigomisin O, schisanhenol, interiotherin C, angeloylgomisin H and O), aryltetralin lignan (wulignan A1), dibenzylbutane lignans (pregomisin, mesodihydroguaiaretic acid), tetrahydrofuran lignan (fragransin A2), and neolignans (licarin A and B)	3	Szopa, Dziurka, et al., 2018
<b>Taxaceae</b>				
<i>Taxus ×media</i>	Hairy roots	Paclitaxel	1	Furmanowa & Sykłowska- Baranek, 2000

Continued on next page

Table 1 Continued.

Family/ Species	Type of in vitro culture	Secondary metabolite	Research center*	References
<i>Taxus</i> × <i>media</i>	ATMA hairy root line carrying taxadiene synthase transgene	Paclitaxel, baccatin III	1	Syklowska-Baranek, Grech-Baran, et al., 2015; Syklowska-Baranek, Pilarek, et al., 2015
<i>Taxus</i> × <i>media</i>	ATM hairy root line carrying taxadiene synthase transgene	Paclitaxel, baccatin III	1	Syklowska-Baranek, Grech-Baran, et al., 2015; Syklowska-Baranek, Pilarek, et al., 2015
<i>Taxus</i> × <i>media</i>	KT hairy root line	Paclitaxel	1	Syklowska-Baranek et al., 2019
<i>Taxus</i> × <i>media</i>	ATMA hairy root line carrying taxadiene synthase transgene	Paclitaxel, baccatin III	1	Syklowska-Baranek et al., 2019

\* 1 – Department of Pharmaceutical Biology and Medicinal Plants Biotechnology, Faculty of Pharmacy, Medical University of Warsaw; 2 – Department of Pharmaceutical Botany and Plant Biotechnology, Poznań University of Medical Sciences; 3 – Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum; 4 – Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG & MUG, University of Gdańsk; 5 – Department of Pharmacognosy with Medicinal Plant Garden, Faculty of Pharmacy, Medical University of Gdańsk; 6 – Department of Biology and Pharmaceutical Botany, Medical University of Łódź; 7 – Maj Institute of Pharmacology, Polish Academy of Sciences; 8 – Department of Pharmaceutical Biotechnology and Department of Pharmaceutical Biology and Botany, Faculty of Pharmacy, Wrocław Medical University.

Biology and Botany at the Faculty of Pharmacy of the Medical Academy in Warsaw (presently the Department of Pharmaceutical Biology and Medicinal Plant Biotechnology, Faculty of Pharmacy, Medical University of Warsaw). The initial achievements of the Department in in vitro secondary metabolite biosynthesis were connected with the tissue cultures of plant species alien to Polish flora. Liriodenine, an antifungal alkaloid, was found in *Liriodendron tulipifera* L. callus cultures. Tingenone, a cytotoxic triterpene, was detected in the callus tissue of the tropical plant *Maytenus wallichiana* R. et B. In addition, diosgenin, a steroidal sapogenin, was found in *Dioscorea deltoidea* Wall. and *Trigonella foenum-graecum* L. callus and cell suspension cultures.

Dr. Jadwiga Halina Supniewska (1913–1984) initiated research on secondary metabolism in *Solanum laciniatum* Ait., *Ammi visnaga* Lam., *Strophanthus intermedius* Pax., and *Tecoma stans* Juss. callus and cell suspension cultures in the Plant Tissue Culture Laboratory at the Department of Phytochemistry, Maj Institute of Pharmacology, PAS, Kraków, in the late 1960s.

Prof. Stanisław Kohlmünzer, the Head of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Medical Academy in Kraków (since 1993, Faculty of Pharmacy, Collegium Medicum of Jagiellonian University), initiated the idea for establishment of a laboratory for plant biotechnology in the 1970s. Dr. Elżbieta Cyunel and Prof. Halina Ekiert started studies on secondary metabolite production in in vitro cultures. The first object of interest was in vitro cultures of *Scrophularia scopoli* Hoppe, *Phytolacca* sp., *Basella alba* L., and *Nicotiana tabacum* var. Samsun. Some investigations were performed in cooperation with Dr. Barbara Dohnal from the Maj Institute of Pharmacology, PAS [in vitro cultures of *Holarrhena antidysenterica* (Roxb.) Wall., *Catharanthus roseus* (L.) G. Don.] and *Phytolacca* sp. (Ekiert et al., 2017).

The first experiments on in vitro plant cultures in the Department of Pharmacognosy, Medical University of Gdańsk, including phytochemical analysis of in vitro cultures of *Rudbeckia hirta* L., *Vaccinium corymbosum* 'Bluecrop' and *Caryopteris incana* (Thunb.) Miq., were performed in early 90s under the mentorship of Prof. Wojciech Cisowski.

Department of Plant Protection and Biotechnology (later on Department of Biotechnology) IFB UG and MUG headed by Prof. Ewa Łojkowska was the second institution in Gdańsk to start studying in vitro plant cultures in 1994, including in

in vitro micropropagation of endangered plants such as *Drosera* sp. and *Dionaea muscipula* and several species of Polish orchids: *Cypripedium calceolus* L., *Dactylorhiza majalis*, *Epipactis atrorubens*, *Epipactis palustris*, and *Orchis morio*. Later studies focused on coumarin and furanocoumarin elicitation from in vitro *Ammi majus* and *Ruta graveolens* cultures. Studies have focused on the induction of secondary metabolite production from coumarins and naphthoquinones with antibacterial, antioxidative, and cytotoxic activities.

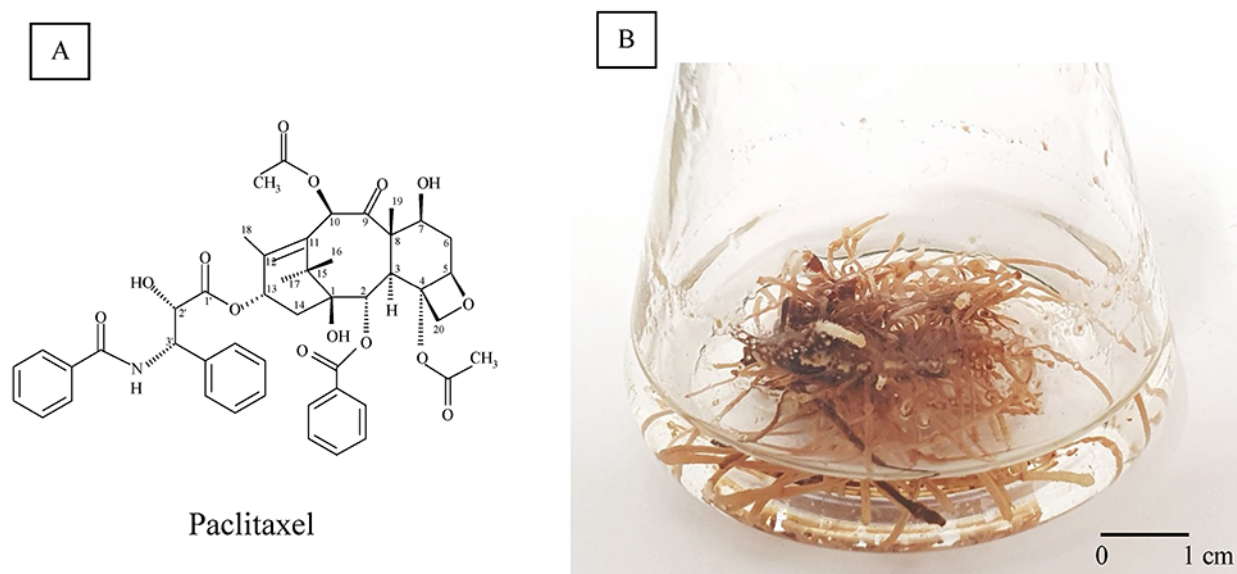
In 1979, at the Department of Pharmaceutical Botany of the Medical Academy in Poznań (currently Poznań University of Medical Sciences), Prof. Lutosława Skrzypczak, the head of this unit, Dr. Maria Wesołowska, and Prof. Barbara Thiem, initiated research in in vitro plant culture in relation to phytochemical analysis, such as the search for new biotechnological sources of secondary metabolites with a biological effect, including *Fagopyrum esculentum* Moench., *Plantago lanceolata* L. and in vitro androgenetic cultures of *Hyoscyamus niger* L. and *Hyoscyamus albus* L.

## 2. In Vitro Plant Culture Studies for Specific Bioactive Compounds

### 2.1. Paclitaxel in *Taxus ×media* Hairy Root Cultures

Paclitaxel (Figure 1A), originally named Taxol, is a well-established antineoplastic drug (Yared & Tkaczuk, 2012). The bark of various *Taxus* species, evergreen, low-growing plants of the Taxaceae family, is the natural source of paclitaxel, but other methods for their production have been elaborated because of its low concentration [0.02% dry weight (DW)] and the necessity of whole plant destruction.

The medical importance of paclitaxel has prompted efforts to develop highly productive *Taxus* spp. in vitro. Hairy root cultures of *Taxus ×media* (Figure 1B) were established via yew transformation with *Agrobacterium rhizogenes* LBA 9402 (Furmanowa & Sykłowska-Baranek, 2000). The paclitaxel content of these roots was 69 µg/g DW, which increased to 210 µg/g DW by elicitation with methyl jasmonate (MJ). Furthermore, combined L-phenylalanine (PHEN) and MJ treatment improved the yield up to 319.7 µg/g DW (Syklowska-Baranek et al., 2009). Two new hairy root lines carrying the taxadiene synthase transgene, ATMA and ATM, were also obtained. Elicitation and precursor feeding strategy (MJ, PHEN) improved paclitaxel yield in ATMA and ATM root line cultures to 10.78 and 1.63 mg/L,



**Figure 1** (A) Paclitaxel structure. (B) *Taxus ×media* hairy roots obtained after transformation with *Agrobacterium rhizogenes* LBA 9402.



respectively (Sykłowska-Baranek, Grech-Baran, et al., 2015). Next, the ATMA root line was cultivated in hybrid two-phase perfluorodecalin-supported cultures (Sykłowska-Baranek, Pilarek, et al., 2015). The degassed perfluorodecalin and MJ increased paclitaxel production to 1,440.08  $\mu\text{g/g}$  DW. Further, the single elicitation with MJ, PHEN, and sodium nitroprusside has been the most efficient in stimulating paclitaxel biosynthesis (2,473.29  $\mu\text{g/g}$  DW) when combined with degassed perfluorodecalin (Sykłowska-Baranek et al., 2019). Significant progress has been made in the development of biotechnological approaches for the production of paclitaxel and other taxanes via *Taxus* spp. cell cultures on a commercial scale. Nevertheless, the observed increase in demand for taxanes for therapeutic purposes creates a space for developing new stable and efficient culture systems for their production.

## 2.2. Huperzine A in Various In Vitro *Huperzia selago* Cultures

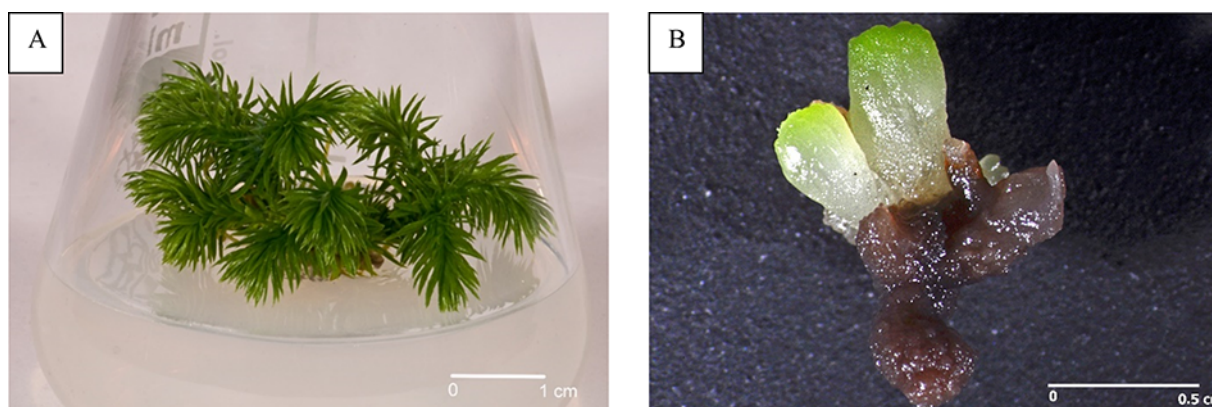
Among the alkaloids identified only in Lycopodiaceae s. l., quinolizine, pyridine, and  $\alpha$ -pyridone-type alkaloids are potent and reversible acetylcholinesterase inhibitors (Ma & Gang, 2004; Muszyński, 1955; Szypuła & Pietrosiuk, 2021). Moreover, huperzine A (HupA, selagine) and huperzine B (HupB) easily cross the blood–brain barrier, are highly acetylcholinesterase-specific inhibitors, and inhibit butyrylcholinesterase at high concentrations (Ferreira et al., 2016; Szypuła & Pietrosiuk, 2021).

With the growing interest in the therapeutic applications of HupA, the demand for club mosses as a source of HupA and other alkaloids is increasing rapidly, and studies on in vitro cultures of Lycopod are gaining importance (Szypuła & Pietrosiuk, 2021).

Till date, procedures for establishing and maintaining in vitro cultures of *Huperzia selago* sporophytes (Figure 2A) and gametophytes (Figure 2B) have been developed using various starting materials, including shoot fragments, vegetative propagules, somatic embryos, and spores (Szypuła & Pietrosiuk, 2021; Szypuła et al., 2005, 2013, 2020).

The first studies on HupA biosynthesis were conducted by Szypuła et al. (2005, 2011, 2013). The establishment of *H. selago* sporophyte in vitro cultures initiated from shoot explants and vegetative propagules (bulbils) allowed approximately tenfold reduced developmental stages with the omission of the gametophyte stage, thus increasing the sporophyte mass tenfold within 6 months. The sporophytes regenerating from somatic embryos produced the highest reported Lycopod yield of HupA till date (3.33 mg/g DW).

Using spores from sporophytes procured from natural populations is an effective method for establishing in vitro axenic cultures of *H. selago* gametophytes (Szypuła et al., 2020). Prothalli were obtained after 7–18 months, and optimal results were achieved on the medium originally developed by Whittier and Storchova (2007) and on Moore medium as modified by Freeberg and Wetmore (1957). Using *H. selago*



**Figure 2** *Huperzia selago* (A) sporophyte and (B) gametophyte in in vitro culture.

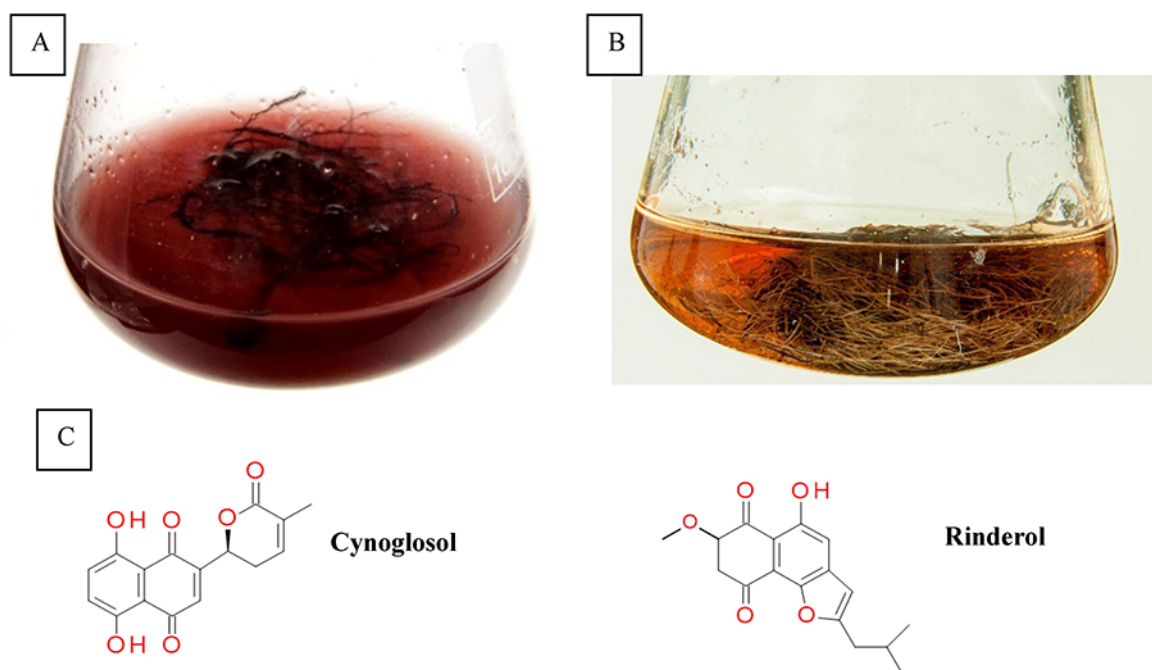
gametophytes can increase HupA biosynthesis by approximately 42% than sporophyte culture, with its yield being 35-fold higher than that of *Hydrangea serrata*, used in the pharmaceutical industry (Szypuła et al., 2020). HupA content in *H. selago* prothallus is 0.74–4.73 mg/g DW, which is the maximum reported for club mosses (Szypuła et al., 2020).

### 2.3. Naphthoquinone Derivatives From In Vitro *Cynoglossum columnae* and *Rindera graeca* roots

Shikonin derivatives present multidirectional pharmacological activities including wound healing, anti-inflammatory, antimicrobial, and anticancer properties (Papageorgiou et al., 1999, 2006, 2008). In addition, the simultaneous use of shikonin derivatives and classical cancer therapies has a promising synergistic effect (He et al., 2016; Jeziorek et al., 2019; Wang et al., 2014; Zhao et al., 2015).

Naphthoquinone pigments with chemotaxonomic and pharmaceutical significance have been derived from *Cynoglossum columnae* Ten. in vitro root cultures (Figure 3A). The root extracts and postculture media were subjected to detailed chromatographic analysis, which allowed to isolate two novel naphthoquinone derivatives, rinderol and cynoglosol (Jeziorek et al., 2019) (Figure 3C). The cytotoxicity of both compounds was tested against HL-60, HeLa, and HCT-116 cancer cell lines. Rinderol showed the highest activity against HL-60 cells ( $IC_{50} = 2 \mu\text{g/mL}$ ), while cynoglosol was selectively active against HL-60 cells ( $IC_{50} = 4.3 \mu\text{g/mL}$ ). Rinderol also demonstrated an antimicrobial activity against *Staphylococcus epidermidis* ATCC 12228 (MIC = 3.91  $\mu\text{g/mL}$ ) and *Staphylococcus epidermidis* MRSE 456 (MIC = 7.81  $\mu\text{g/mL}$ ) (Jeziorek et al., 2019).

Rinderol was detected in *Rindera graeca* (A. DC.) Bois. & Heldr. hairy root cultures (Figure 3B) obtained via *Agrobacterium rhizogenes* ATCC 15834 infection (Graikou et al., 2021; Pietrosiuk et al., 2006; Sykłowska-Baranek et al., 2008, 2012). The isolated rinderol exhibited cytotoxic activity against two human lung cancer cell lines NSCLC-N6-L16 ( $IC_{50} = 1.2 \mu\text{g/mL}$ ) and A549 ( $IC_{50} = 0.9 \mu\text{g/mL}$ ).

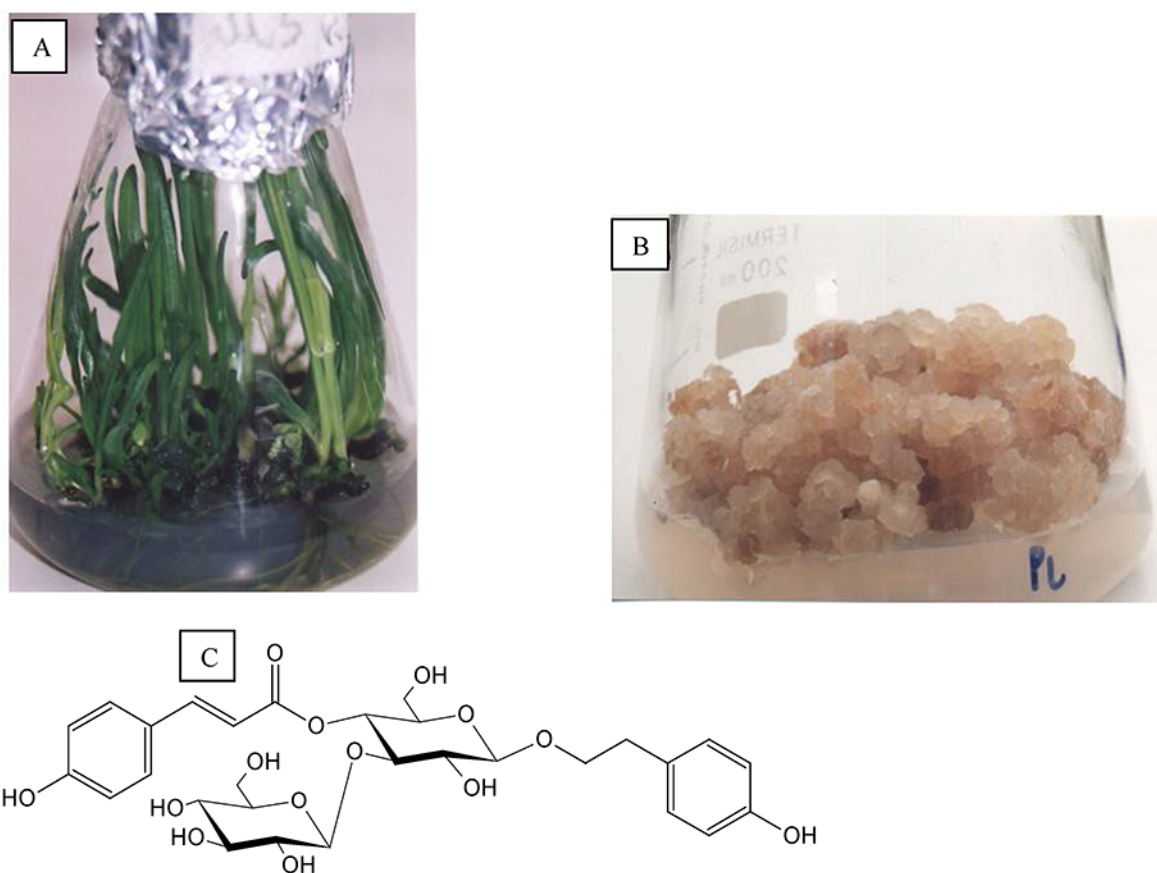


**Figure 3** In vitro (A) *Cynoglossum columnae* and (B) *Rindera graeca* root cultures (photo: I. Rudnicki). (C) Rinderol and cynoglosol structures.

#### 2.4. Phenylethanoid glycosides in *Plantago media* and In Vitro *Plantago lanceolata* Cultures

The in vitro *Plantago lanceolata* L. cultures involved shoots developed on leaves (Figure 4A), root explants, and two root- and callus-leaf-derived lines (Figure 4B). A total of 16 phenylethanoid glycosides were isolated, including those new to the species and a novel natural product, lancetoside (Figure 4C). Plantamajoside and acteoside are the main constituents of calli and leaves, respectively (Budzianowska et al., 2004).

*Plantago media* L. was cultured on Murashige and Skoog (MS) (1962) medium and the best medium was supplemented with 2.0 mg/L 6-benzyladeninpurine (BA) and 0.1 mg/L indolyl-3-acetic acid (IAA). A TLC video densitometric assay showed high acetoside and plantamajoside content in shoots and roots, respectively (Budzianowska et al., 2019).



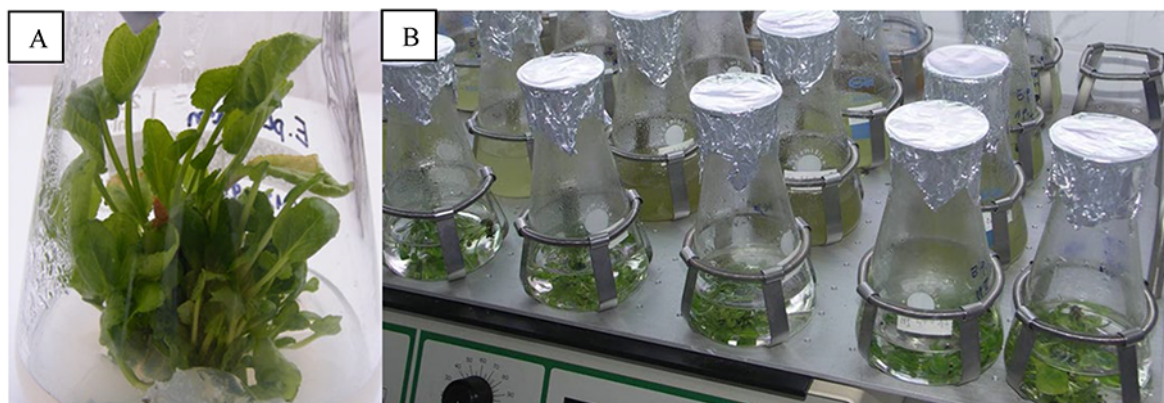
**Figure 4** In vitro *Plantago lanceolata* (A) leaf rosettes, and (B) callus cultures. (C) Lancetoside structure.

#### 2.5. Phenolic Acids and Triterpenoid Saponins in In Vitro *Eryngium planum* Cultures

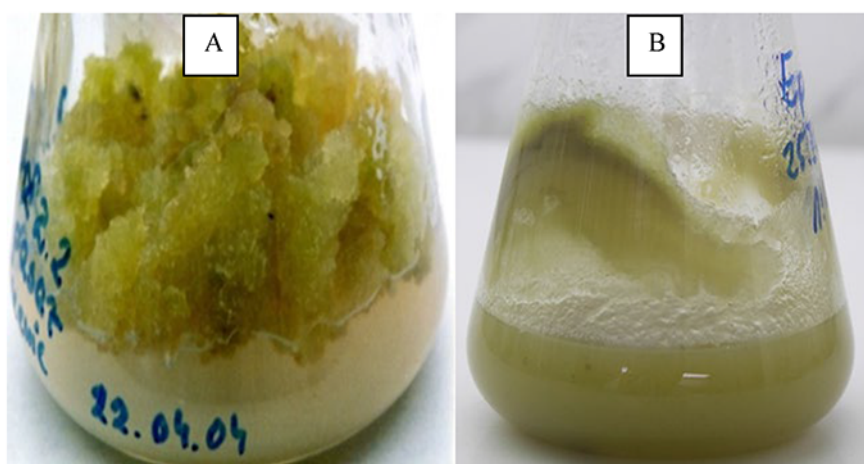
*Eryngium planum* L. from the Apiaceae family, a species native to Poland, contains a rich set of secondary metabolites (Kikowska, 2014). The biomass for phytochemical analysis has been obtained using clonal propagation (Figure 5A). The total content of selected phenolic acids (RA, chlorogenic acid, caffeic acid) as well as that of triterpenoid saponins was higher in micropropagated plantlet shoots and roots than those in analogous soil-grown plant organs (Kikowska et al., 2019; Thiem et al., 2013).

The influence of elicitor type and elicitation duration on phenolic acid accumulation in *E. planum* shoots has been studied (Figure 5B). MJ solution, high concentration





**Figure 5** (A) Shoot multiplication as a step of *Eryngium planum* micropropagation. (B) Agitated *E. planum* shoots.



**Figure 6** (A) Callus and (B) cell suspension culture of *Eryngium planum*.

sucrose (S) solution, and elicitation with yeast extract (YE) increased the RA content in shoots by up to 5.5, 4.7, and 2.3 times, respectively (Kikowska et al., 2015).

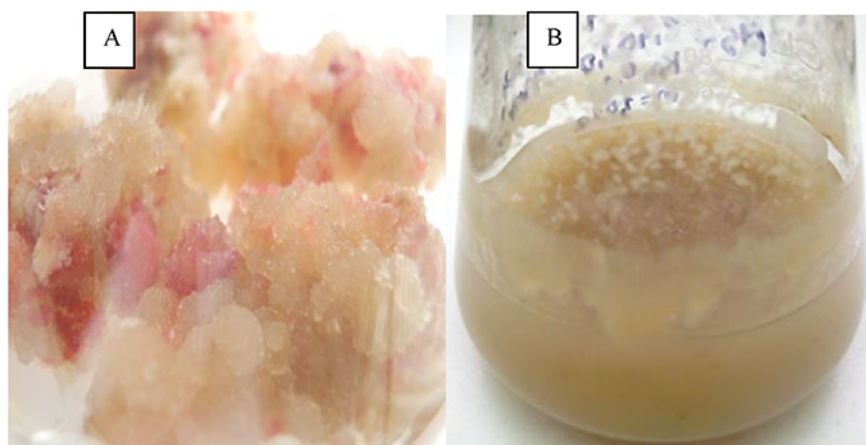
Bioactive compounds can also be produced in *E. planum* callus and cell suspension cultures (Figure 6). An MJ-treated suspension increased the total phenolic acid content by 1.65–2.08-fold (Kikowska et al., 2012). Adding high concentration S or MJ solution to the medium enhanced saponin accumulation (up to 1.7 and 1.2 times, respectively). Therefore, elicitation may be effective for enhancing phenolic acid and triterpenoid saponin accumulation in flat sea-holly cultures (Kikowska et al., 2019).

We have previously isolated and characterized triterpenoid saponins from *E. planum* roots, which quantitatively comprised different in vitro *E. planum* cultures (Kikowska et al., 2019; Kowalczyk et al., 2014).

*Eryngium planum* shows amoebicidal activity against pathogenic *Acanthamoeba castellanii* (Derda et al., 2013), neuroprotective activity (Ożarowski et al., 2015), apoptotic activity (Kikowska, 2014), and antimicrobial activity (Kikowska et al., 2016).

## 2.6. Pentacyclic Triterpenoids in Various In Vitro *Chaenomeles japonica* Cultures

Homogenous and uniformly stabilized callus and cell suspension cultures (Figure 7A,B) of *Chaenomeles japonica* (Thunb.) Lindl. ex Spach (Rosaceae), a dwarf shrub used in traditional medicine in the Far East, have been established (Kikowska et al., 2019).



**Figure 7** *Chaenomeles japonica* (A) callus and (B) cell suspension cultures.

Microscopic observations of fibroblast viability, morphology, and proliferation ratio proved that the callus extract significantly affected fibroblasts. Pentacyclic triterpenoid-rich *Ch. japonica* callus extract may be a potential source of cosmetic ingredients (Kikowska et al., 2018).

## 2.7. Rossoliside, Plumbaside A, and Related Compounds in In Vitro Carnivorous Species Cultures

The isolated phenolic glycosides rossoliside, new for *Drosera spatulata* Labill and known for *Drosera rotundifolia* L. (Droseraceae), and plumbaside A from *Drosera intermedia* L., are easily hydrolyzed and oxidized with air oxygen to 7-methyljuglone (ramentaceone) and plumbagin, respectively (Budzianowski, 1995, 1996). Moreover, rossoliside is oxidized to an unknown 2-methylnaphthazarin 5-O-glucoside (Budzianowski, 1997). Plumbaside A has also been isolated from in vitro cultures of (i) *Dionaea muscipula* Ellis (Kukulczanka & Budzianowski, 2002), which yields other phenolics as well (Pakulski & Budzianowski, 1996a, 1996b), (ii) *Drosophyllum lusitanicum* Link (Drosophyllaceae), together with two new naphthalene derivatives (Figure 8) and other phenolics (Budzianowski et al., 2002), and (iii) *Drosera gigantea* Lindley with droserone and hydroxydroserone 5-O-glucosides, which contribute to the maroon color of this plant (Budzianowski, 2000).

The triterpene 3-O-acetylaleuritic acid, a novel compound isolated from *D. spatulata* leaf rosettes (Figure 9), inhibits HeLa, HT-29, and MCF7 cell growth and migration (Toton et al., 2020).

In turn, in vitro *Byblis liniflora* Salisb (Byblidaceae) cultures yielded four new phenylethanoid glycosides. The presence of acteoside supports the placement of Byblidaceae in the order *Scrophulariales* and subclass *Asteridae* (Schlauer et al., 1994).

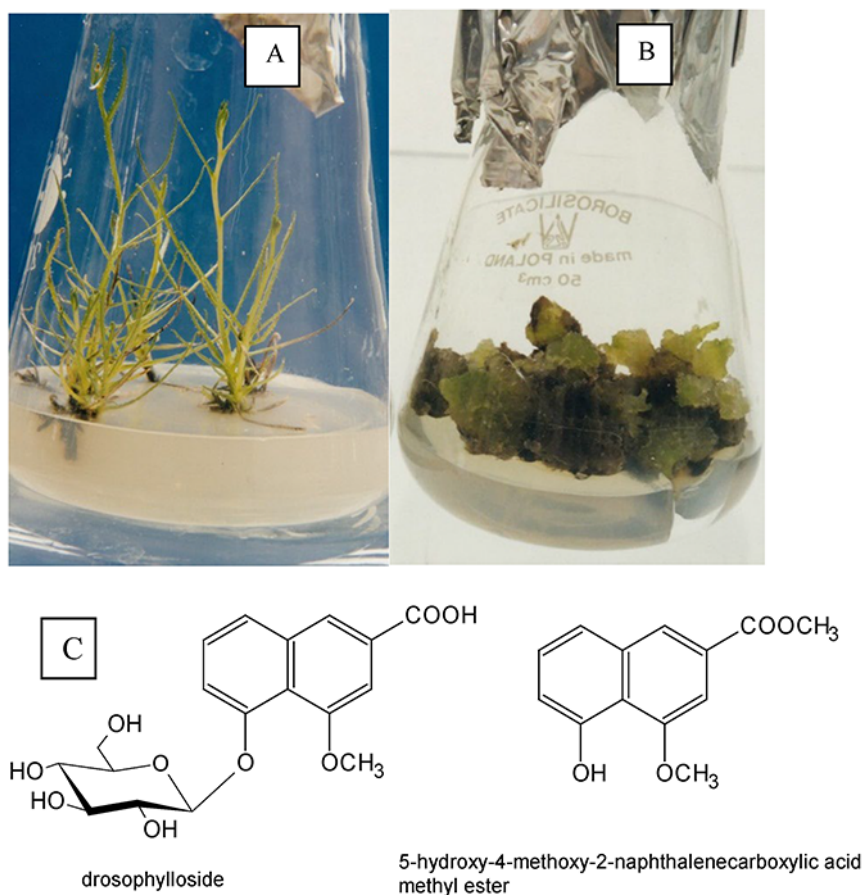
## 2.8. Lipophilic Flavones in *Primula veris* Shoot Cultures

The in vitro propagated *Primula veris* L. (Primulaceae) contains 10 lipophilic flavones, similar to those isolated from field-cultivated plants, including new compounds (Figure 10) (Budzianowski et al., 2005). Among them, zapotin has been investigated for the mechanism of anticancer activities (Toton et al., 2012, 2016).

## 2.9. Linear Furanocoumarins in Various In Vitro *Ammi majus*, *Pastinaca sativa*, *Ruta graveolens*, and *Ruta graveolens* ssp. *divaricata* Cultures

Linear furanocoumarins (LF) are used in photochemotherapy for skin diseases characterized by distorted pigmentation and excessive cell proliferation. They can also be used in cardiology and neurology (Bethea et al., 1999; Bohuslavizki et al.,



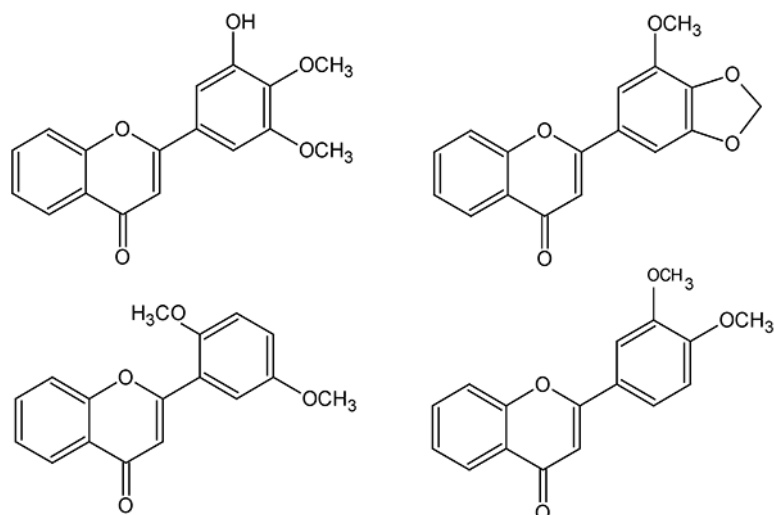


**Figure 8** *Drosophyllum lusitanicum* (A) shoot and (B) callus cultures. (C) New naphthalene derivatives.



**Figure 9** In vitro *Drosera spatulata* culture.

1994; Ekiert, 2004; Härmälä et al., 1992; Matern, 1999; Wolf, 2016). The most important LF used in therapy include bergapten, xanthotoxin, isopimpinellin, and imperatorin. Umbelliferone is a biogenic precursor of these compounds. *Ammi majus* L. and *Pastinaca sativa* L. fruits are rich LF sources for pharmaceutical applications.

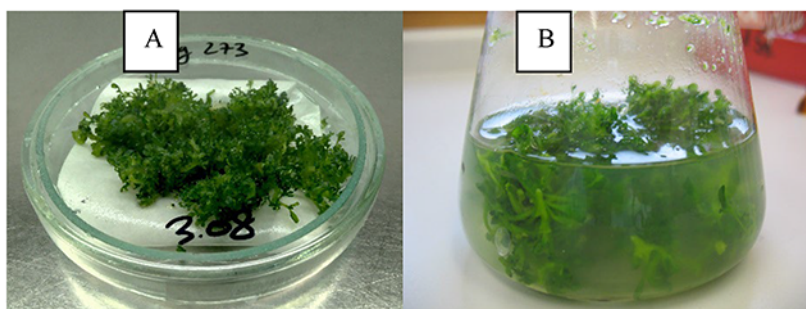


**Figure 10** New nature lipophilic flavones in in vitro propagated *Primula veris*.

The best conditions for LF production (113.83 mg/100 g DW) in the established *Ammi majus* callus cultures were LS variant (10 mg/L) 6-benzylaminopurine (BAP) and 10 mg/L 2,4-D), and white light (900 lx). The maximum isopimpinellin and imperatorin contents were equal to or exceeded those in the parent plant fruits. These investigations were among the first biotechnological studies from a pharmaceutical perspective in Poland (Ekiert, 1993).

The next stage of research involved the optimization of callus culture conditions for *Ammi majus* (cultures of other origin) and *Pastinaca sativa*. The total LF and umbelliferone content in *Ammi majus* cultures on 12 agar LS medium variants was 40.95–871.00 mg/100 g DW. The LS variant with 0.1 mg/L BAP and 0.1 mg/L 1-naphthylacetic acid (NAA) was the best “production” medium. High imperatorin, xanthotoxin, and umbelliferone levels have also been reported (Ekiert, 2004; Ekiert & Gomółka, 2000a). In contrast, isopimpinellin (90.20–238.90 mg/100 g DW) was the main metabolite accumulated in *Pastinaca sativa* callus cultivated on 10 LS variants. The total LF and umbelliferone content was 115.70–408.50 mg/100 g DW (Ekiert, 2004; Ekiert & Gomółka, 2000b).

In Western Europe, *Ruta graveolens* L. has been proposed as a rich LF source in pharmacology and cosmetology. In the established *Ruta graveolens* stationary liquid shoot cultures the ability to produce psoralen, bergapten, xanthotoxin, and isopimpinellin was documented (Figure 11A) (Ekiert & Kisiel, 1997). In contrast to the cultures under UV-C light or in darkness, the shoots cultured under white light (900 lx) produced equal or higher amounts of LF (1,022.2 mg/100 g DW) than plants growing in different habitats (575.0–1,033.0 mg/100 g DW) (Ekiert & Gomółka,



**Figure 11** *Ruta graveolens* L. (A) stationary liquid and (B) agitated shoot cultures.

1999). Moreover, *Ruta graveolens* shoot cultures in 12 LS medium variants have also been tested. The total LF ranged between 254.2 and 939.7 mg/100 g DW, with xanthotoxin being the main metabolite. The media with 0.1 mg/L BAP and NAA and 2 mg/L BAP and NAA were the best “production” media. Thus, these LS variants were chosen for studying the dynamics of LF accumulation in stationary liquid and agitated cultures (42 days growth cycles) of *Ruta graveolens* and *Ruta graveolens* ssp. *divaricata* (the culture obtained in the framework of cooperation with the Institute of Natural Sciences of the University in Würzburg).

The total LF and umbelliferone content in stationary cultures of rue and its subspecies increased 2,3-fold and 2,3-fold over 48 days and reached 966.0 and 283.4 mg/100 g DW on twenty-eighth and thirty-fifth culture day, respectively (Ekiert, 2004; Ekiert et al., 2001, 2005; Ekiert & Czygan, 2005, 2007). Cultures of both plants produced high bergapten and xanthotoxin amounts. The total LF content in agitated cultures of both plants increased by 4.8- and 2.0-fold over 48 days and reached 580.8 and 64.0 mg/100 g DW on forty-second and thirty-fifth culture day, respectively (Figure 11B). Xanthotoxin and bergapten were the main metabolites in both plant cultures.

In addition, studies on LF biogenesis have been conducted in cooperation with the Institute of Pharmaceutical Biology, University in Marburg/am Lahn, which resulted with isolation and characterization of bergapten *O*-methyltransferase from elicited in vitro *Ammi majus* cultures (of yet another origin). The isolated enzyme participates in bergapten (5-methoxypsoralen) biosynthesis from bergapten (5-hydroxypsoralen) (Hermann et al., 2004).

All presented results documented that in the investigated in vitro culture systems, the production of LF could be high and that biotechnological strategies could develop problems with imports and/or with obtaining high-quality plant material in Europe from in vivo conditions. The scale-up of maintaining in vitro cultures (in large-scale laboratory installations) is recommended in future investigations.

## 2.10. Dibenzocyclooctadiene Lignans in Various In Vitro *Schisandra* sp. Cultures

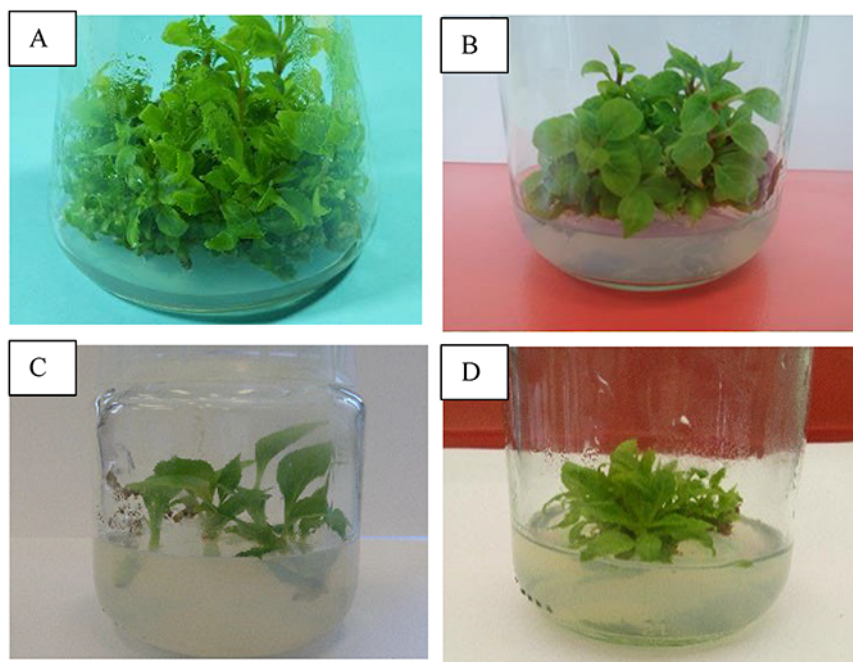
Dibenzocyclooctadiene lignans constitute the main secondary metabolites specific to the *Schisandra* genus (Szopa, Ekiert, & Ekiert, 2017). *Schisandra chinensis* Turcz. Baill. is the best-known of these species (World Health Organization, 2007, pp. 296–313). These compounds exhibit hepatoprotective, antioxidant, and anti-inflammatory activities (Szopa, Barnaś, & Ekiert, 2019; Szopa, Ekiert, & Ekiert, 2017).

Szopa, Klimek, and Ekiert (2016) aimed to investigate lignan production in in vitro *Schisandra* genus cultures using different comprehensive and globally innovative biotechnological methods. Experiments have been conducted using *Schisandra chinensis*, *Schisandra chinensis* ‘Sadova’, *Schisandra henryi* C. B. Clarke, and *Schisandra rubriflora* (Franch.) Rehd. et Wils cultures (Figure 12).

Optimization of *S. chinensis* agar microshoot culture conditions increased deoxyschisandrin, schisandrin, and gomisin A levels. The maximum total tested lignan content (486.78 mg/100 g DW) in microshoot agar cultures is 1.3- and 3.8-fold higher than that in the parent plant fruits and leaves, respectively (Szopa & Ekiert, 2011, 2014).

The next step of the research involved the optimization of lignan production in different bioreactors (balloon, spray, and column with shoot immobilization system) and temporary immersion systems RITA and PlantForm. Stationary liquid, agitated, and agar cultures have also been studied. The maximum contents of 14 tested lignans in agar, agitated, and PlantForm bioreactor cultures are 237.86, 195.15, and 546.98 mg/100 g DW, respectively (Szopa, Kokotkiewicz, et al., 2016, 2017, 2019).

These studies were extended by the successful establishment of microshoot cultures of the high-yield cultivar *S. chinensis* ‘Sadova’. The maximum total contents of lignans were also high, reaching 574.42, 375.11, and 313.51 mg/100 g DW in agar, agitated, and PlantForm bioreactor cultures, respectively (Szopa, Klimek-Szczykutowicz, et al., 2018; Szopa, Kokotkiewicz, et al., 2019).



**Figure 12** Agar microshoot cultures of (A) *Schisandra chinensis*, (B) *S. chinensis* 'Sadova', (C) *S. henryi*, and (D) *S. rubriflora*.

The presence of dibenzocyclooctadiene, dibenzylbutane, and aryltetralin lignans has been confirmed in *S. henryi* microshoot and callus solid cultures. The total amount of the detected lignans is 873.71 mg/100 g DW. The main compounds are schisantherin A and B (Jafernik et al., 2020).

Biotechnological studies on *S. rubriflora* involved the  $\sigma$  and  $\varphi$  lines of microshoot cultures. The presence of dibenzocyclooctadiene, aryltetralin, dibenzylbutane, tetrahydrofuran, and neolignans has been confirmed in the culture extracts. The maximum total content for the detected lignans is 250.92 and 220.70 mg/100 g DW for the  $\sigma$  and  $\varphi$  lines, respectively. The main compounds are schisantherin B, angeloylgomisin H, and O (Szopa, Dziurka, et al., 2018).

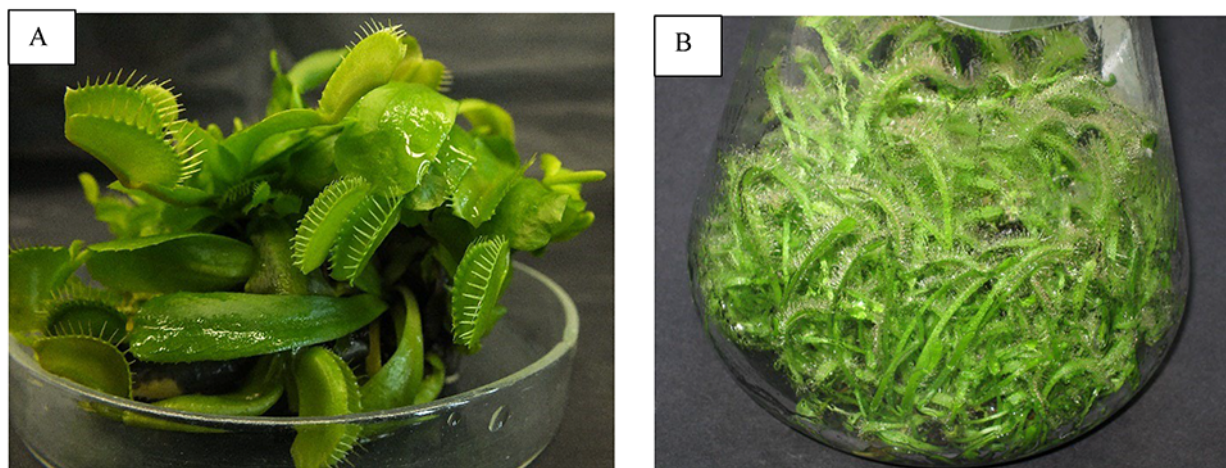
All the presented results document the high biosynthetic potential of cells from in vitro cultures of different *Schisandra* species and cultivars. The high lignin production could be a valuable alternative for the import of *Schisandra* fruit from China.

#### 2.11. Naphthoquinones in Various In Vitro *Drosera* and *Dionaea* Plant Cultures

Plants from the *Drosera* and *Dionaea* genera are valuable sources of biologically active naphthoquinones (NQs), with the most pharmacologically significant including plumbagin (PL), ramentaceone (RAM), and 3-chloroplumbagin (3ChPL). These plants are micropropagated through adventitious shoot regeneration from leaf explants and shoot tips (Kawiak et al., 2003). Optimal adventitious *Drosera aliciae* shoot regeneration was obtained from leaf explants grown on 1/2 MS medium supplemented with 0.4  $\mu$ M 6-benzyladenine. These growth conditions yielded the highest micropropagation coefficient and increased survival rate by 42%. The genetic fidelity of the shoots, assessed with RAPD, confirmed the genetic homogeneity of the regenerants (Kawiak et al., 2011). Elicitation was employed to increase the secondary metabolite content in *Drosera* and *Dionaea* cultures, and was performed by the application of biotic and abiotic elicitors. Biotic elicitation of *Drosera* and *Dionaea* cultures with *Agrobacterium rhizogenes* lysates increased PL content by 2.6-fold from 1.1 mg/g fresh weight (FW) in *Dionaea muscipula* and RAM content by 1.9-fold from 1.2 mg/g FW in *Drosera capensis* (Krolicka et al., 2008).

*Dionaea muscipula* (Figure 13A) and *Drosera capensis* var. *alba* (Figure 13B) has been transformed for increasing NQ production. *Dionaea muscipula* has been





**Figure 13** Transformed tissue (teratomas) of carnivorous plants (A) *Dionea muscipula* and (B) *Drosera capensis* var. *alba*.

successfully transformed using two wild-type *Rhizobium rhizogenes* strains to form teratoma (transformed shoot) cultures without hairy roots.

HPLC analysis revealed an increase in secondary metabolite content, with a 1.7-fold increase in PL content in transformed tissue (106 mg/g DW) (Makowski et al., 2021). *Drosera capensis* var. *alba* transformation formed teratomas without hairy root formation. The growth index increased 3.7-fold and the RAM level in the transformed teratomas increased by 1.6-fold to 410 µg/g DW (Krolicka et al., 2010).

The biological activity of the NQs derived from in vitro cultured plants have been evaluated. RAM isolated from in vitro *D. aliciae* cultures displayed antibacterial activity, with the highest effects on *Staphylococcus aureus*. Both the susceptible and antibiotic-resistant strains were sensitive to RAM (MBC 0.05 mg/mL). The mode of RAM activity toward bacterial cells was determined as DNA and RNA synthesis inhibition (Krolicka et al., 2009). RAM also displays cytotoxic activity against human cancer cells. The highest activity of RAM was exerted toward leukemic cells HL-60 (IC<sub>50</sub> = 8.7 µM) and U937 (IC<sub>50</sub> = 3.2 µM), with the cytotoxic activity toward U937 cells being similar to that of etoposide (Kawiak et al., 2011). The mechanism of RAM action is associated with ROS-mediated apoptosis induction in HL-60 cells (Kawiak et al., 2012).

PL isolated from *Drosera binata* has high cytotoxic activity toward a panel of cancer cells, including HL-60 cells (IC<sub>50</sub> = 2.7 µM). PL exerts its activity in HL-60 cells by stabilizing the topoisomerase II-DNA complex and inducing DNA cleavage, which triggers apoptosis (Kawiak et al., 2007). PL also modulates the activity of chemotherapeutics, including paclitaxel. Moreover, PL downregulates MAPK/ERK signaling in breast cancer cells, which is upregulated in paclitaxel-resistant cells. PL, through decreasing p-ERK levels, overcomes the resistance of breast cancer cells to paclitaxel (Kawiak, Domachowska, Krolicka, et al., 2019; Kawiak, Domachowska, & Lojkowska, 2019).

The 3ChPL found in *D. binata* has higher bactericidal efficacy than PL. Specifically, it has high activity against *S. aureus* (MBC = 8 µg/mL). 3ChPL exerts synergistic activity with silver nanoparticles (AgNPs), which further enhances the antimicrobial activity of 3ChPL. Bacterial cell membrane disruption is supposedly the mechanism underlying the synergistic effects of 3ChPL and AgNPs. Synergistic interactions with AgNPs increased the efficacy of reduced 3ChPL concentrations, at which they are non-toxic to human keratinocytes (Krychowiak et al., 2018). Further studies have shown that AgNPs overcome intrinsic microbial resistance to NQs (Krychowiak-Maśnicka et al., 2021). 3ChPL also has high activity toward cancer cells, particularly breast cancer cells (IC<sub>50</sub> = 0.6–2 µM). 3ChPL induces apoptosis in breast cancer cells by downregulating the anti-apoptotic Bcl-2 family protein Mcl-1 (Kawiak, Domachowska, Krolicka, et al., 2019).



## 2.12. Coumarins and Furanocoumarins in Various In Vitro *Ammi majus* and *Ruta graveolens* Cultures

*Ammi majus* and *Ruta graveolens* are sources of coumarins (umbelliferone) and furanocoumarins (psoralen, xanthotoxin, bergapten, and imperatorin). *Ammi majus* callus, cell suspension, and hairy root cultures obtained using two agropine *R. rhizogenes* strains have been established as potential coumarin and furanocoumarin sources. Umbelliferone was detected in all cultures, with the highest level (19 µg/g DW) determined in hairy root cultures (Królicka et al., 2001). Umbelliferone content further increased 96-fold in *A. majus* callus cultures (9.6 mg% DW) and 1.2-fold in hairy root cultures (2.3 mg% DW) with biotic elicitation (Staniszewska et al., 2003). Further studies used *A. majus* and *R. graveolens* to establish a co-culture of these plants for furanocoumarin synthesis in *R. graveolens* cultures utilizing umbelliferone substrate released by *A. majus* cultures. The most efficient co-culture model included *A. majus* hairy roots and *R. graveolens* shoots. A 2.5-fold increase in furanocoumarin and xanthotoxine (38.1 mg/g DW) synthesis was reported (Sidwa-Gorycka et al., 2003).

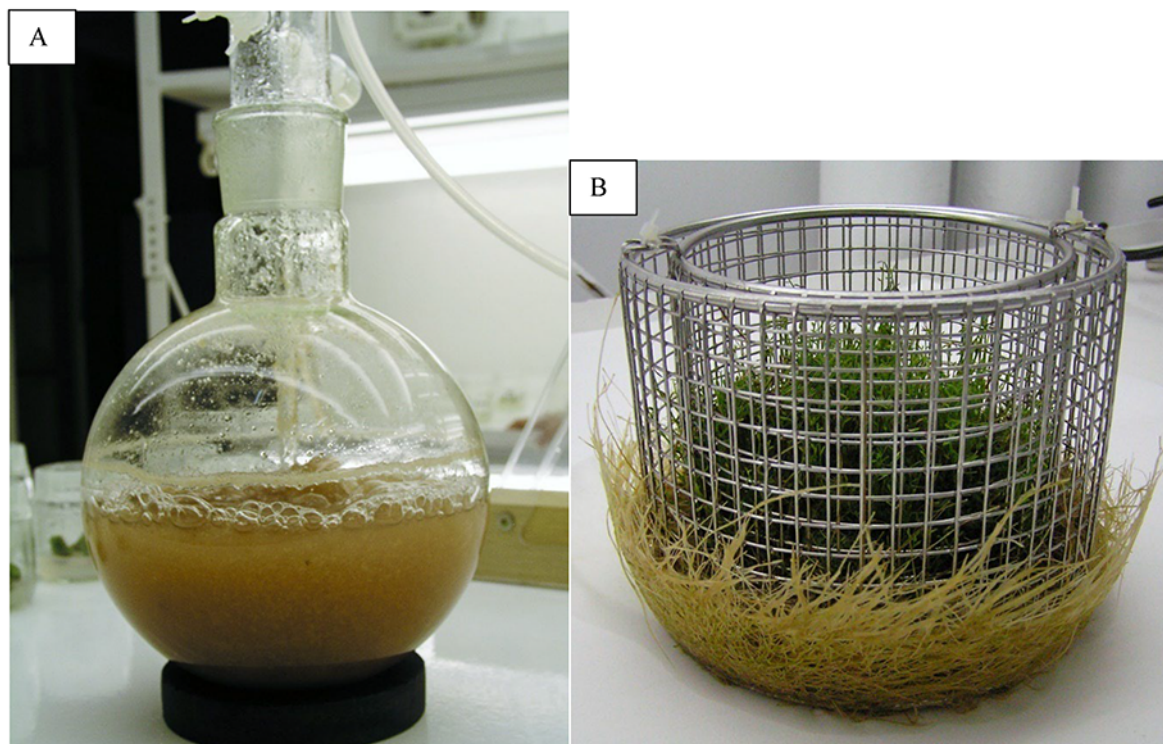
## 2.13. Isoflavonoids in Various In Vitro *Genista tinctoria* Cultures

At the Department of Pharmacognosy (Medical University of Gdańsk), in vitro *Genista tinctoria* L. cultures have been obtained as a source of phytoestrogens used in hormone-dependent tumor treatment and mitigate the effects of menopause. Callus cultures of the species [Schenk and Hildebrandt (SH) medium with 2,3,5-triiodobenzoic acid (TIBA) and kinetin] selectively produced 14 isoflavonoids (10,474.23 mg/100 g DW) (Łuczkiwicz et al., 2014; Łuczkiwicz & Głód, 2003), without a set of toxic quinolizidine alkaloids. Cultivating the biomass as suspension and after elicitor treatment (MJ and chitosan) obtained 11,138.95 mg isoflavones from 100 g DW (the main metabolite – genistin – 9,414.7 mg/100 g DW), of which 80% was released from the plant matrix into the medium [permeabilization with dimethyl sulfoxide (DMSO)] (Łuczkiwicz & Kokotkiewicz, 2012). The suspension may be considered an in vitro plant system for profitable estrogen/mimetic isoflavone production (Figure 14A).

Further research resulted in plant system development as intra-species (shoot/hairy root) *G. tinctoria* co-cultures (Łuczkiwicz & Kokotkiewicz, 2005a). Liquid plant microshoot monocultures (SH medium with IBA) produce phytoestrogens which belong to the genistein metabolic pathway with relatively low daidzein derivative production (Łuczkiwicz & Głód, 2005). *Genista tinctoria* hairy roots produce only isoliquiritigenin, which is a direct daidzein precursor, not found in the intact plant (Łuczkiwicz & Kokotkiewicz, 2005b). Both biomass types were combined in a co-culture system (basket-bubble bioreactor, SH medium with IBA). Isoliquiritigenin is translocated from the roots to the medium only after supplementing the plant system with abscisic acid, allowing its bioconversion to daidzein and daidzin in the shoots (Figure 14B). The system synthesized genistein derivatives at the level of microshoot monocultures, “enriched” with the additional daidzin and daidzein concentration. Productivity and specific productivity of the system in terms of genistin (1,381 mg/L and 34.53 mg/day, respectively) and daidzin (434.90 mg/L and 8.0 mg/day, respectively) accumulation indicates that it may be used for the industrial production and bioconversion of selected phytoestrogens (Łuczkiwicz & Głód, 2005).

## 2.14. Xanthonones and Polyphenolic Compounds in Various In Vitro *Cyclopia subternata* and *Cyclopia genistoides* Cultures

Microshoots and calli from selected African honeybush plants (*Cyclopia* genus) have been used to develop in vitro plant systems designed to produce xanthonones and polyphenolic compounds with antioxidant, anti-inflammatory, and estrogen/mimetic activities (Kokotkiewicz et al., 2009). *Cyclopia subternata* suspension selectively accumulates three isoflavones absent in the intact plant, while *Cyclopia genistoides* microshoots synthesize benzophenones and xanthonones identical

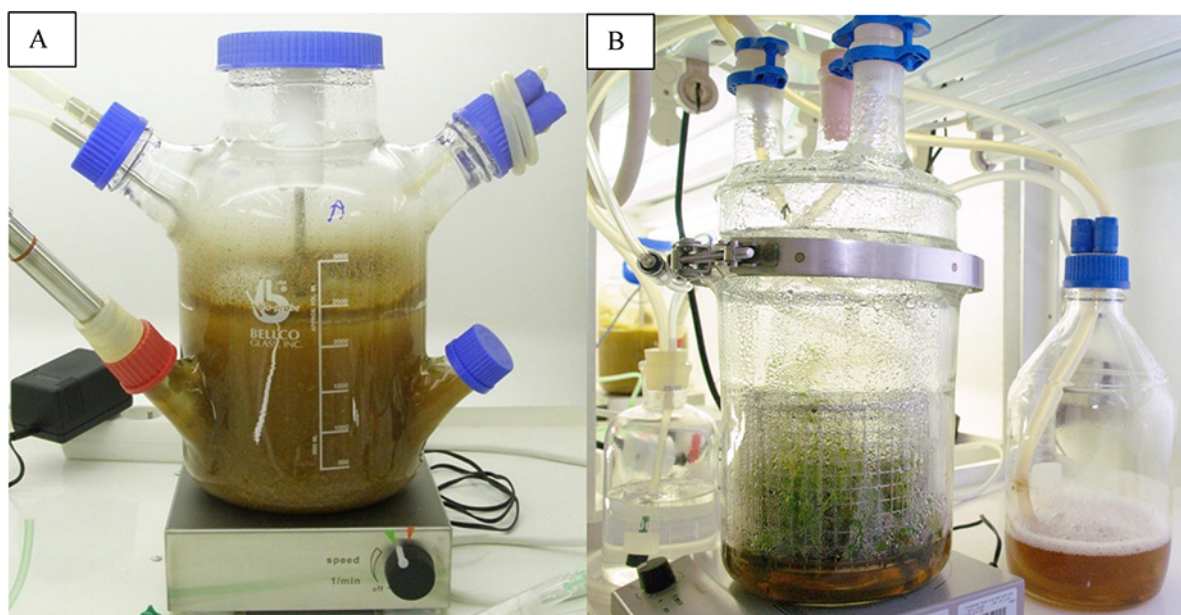


**Figure 14** *Genista tinctoria* (A) suspension and (B) shoot/hairy root co-cultures.

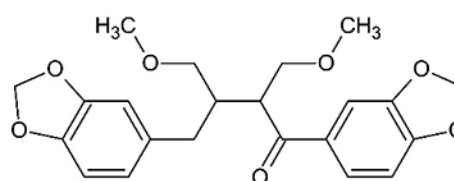
to those found in the intact plant (Kokotkiewicz et al., 2009). After increasing isoflavone and selected xanthone and benzophenone accumulation in these cultures (retrobiosynthetic strategies, elicitation, and immobilization), both the suspension and microshoots were upgraded for growth in prototype bioreactors designed at the Department of Pharmacognosy, Medical University of Gdańsk (Kokotkiewicz, Luczkiewicz, Kowalski, et al., 2013; Kokotkiewicz, Luczkiewicz, Pawlowska, et al., 2013). *Cyclopia subternata* suspension cultures were grown in a stirred-tank bioreactor (MS medium with 4-CPPU, 2,4,5-T, and 5% v/v coconut water) and synthesized 7-*O*- $\beta$ -glucoside in calycosin, 7-*O*-glucoside of pseudobaptygenin, and 7-*O*- $\beta$ -glucoside of formononetin (their respective productivities were 0.96, 0.44, and 0.22 mg/L/day, respectively) (Figure 15A) (Kokotkiewicz, Luczkiewicz, Pawlowska, et al., 2013). *Cyclopia genistoides* microshoots produce high xanthone and benzophenone concentrations (Kokotkiewicz, Luczkiewicz, Kowalski, et al., 2013) and have been used for growth in a temporary immersion bioreactor (SH medium with IBA) (Figure 15B). They produced mangiferin, isomangiferin, hesperidin, 3-*C*- $\beta$ -glucoside iriflophenone, and maclurine and the productivity calculated (5.48, 1.58, 0.72, 3.04, and 0.06 mg/L/day, respectively) indicate the potential to use the system for continued production of these metabolites, independent of environmental conditions (Kokotkiewicz, Luczkiewicz, Kowalski, et al., 2013). The compounds isolated from *C. genistoides* cultures have been screened for pro-apoptotic activity in TNF- $\alpha$ -stimulated synovial cells isolated from rheumatoid arthritis patients. The strongest effect, measured as the apoptotic cell percentage, has been recorded for isomangiferin (75%), followed by iriflophenone-3- $\beta$ -glucoside (71%), and mangiferin (65%), which is an indication for further biological research into their application in rheumatoid arthritis treatment.

#### 2.15. Lignans in Non-Transformed *Phyllanthus amarus* Root Cultures

The roots and aerial parts of many species of the genus *Phyllanthus* are a source of medicinal plant materials, including lignans, which are the main biologically active compounds. In our study, non-transformed *Phyllanthus amarus* root cultures (Figure 16) have been established and cultivated on SH medium with IBA and 1/2



**Figure 15** (A) *Cyclopia subternata* suspension cultures and (B) *C. genistoides* microshoots.



**7'-oxocubebin dimethyl ether**

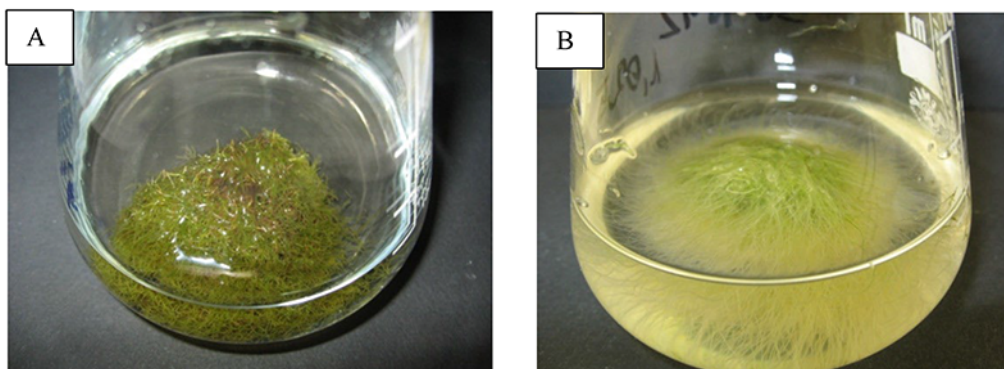
**Figure 16** Non-transformed root culture of *Phyllanthus amarus*.

macro and micro salt concentration as a selective source of only one lignan, 7'-oxocubebin dimethyl ether (Figure 16). This is a new compound in the plant kingdom and has strong cytotoxic activity against the HeLa cell line ( $IC_{50} = 3.8 \mu\text{g/mL}$ ) (Sparzak et al., 2015).

#### 2.16. Flavone Glycosides and Phenylethanoid in *Scutellaria* sp. Hairy and Non-Transformed Root Cultures

Many species of the genus *Scutellaria* are medicinal plants that contain biologically active flavones. Their concentration is the highest in *Scutellaria baicalensis* roots after cultivating for approximately 6 years. Two in vitro root cultures have been established, the non-transformed *Scutellaria barbata* roots (B5 with IBA and SH with IBA; Figure 17A,B) and *Scutellaria lateriflora* hairy roots (Figure 18), which mainly accumulates flavone glycosides (Wilczańska-Barska et al., 2011, 2012). The flavone productivity in *Scutellaria lateriflora* hairy roots (478 mg/L) was 2.5-fold lower than





**Figure 17** *Scutellaria barbata* root cultures cultivated in IBA-supplemented (A) B5 and (B) SH medium.



**Figure 18** Hairy roots of *Scutellaria lateriflora*.

that in *S. barbata* root culture; however, both cultures accumulated more flavones than *S. baicalensis* hairy roots. Using *Pectobacterium carotovorum* suspension, a plant pathogen, changed the production profile and stimulated the selective aglycone-wogonin accumulation in hairy roots (3% DM). *Scutellaria lateriflora* hairy roots produce can be a rich source of wogonin with antioxidant, anti-inflammatory, and neuroprotective activities as a phytoalexin (Wilczańska-Barska et al., 2012).

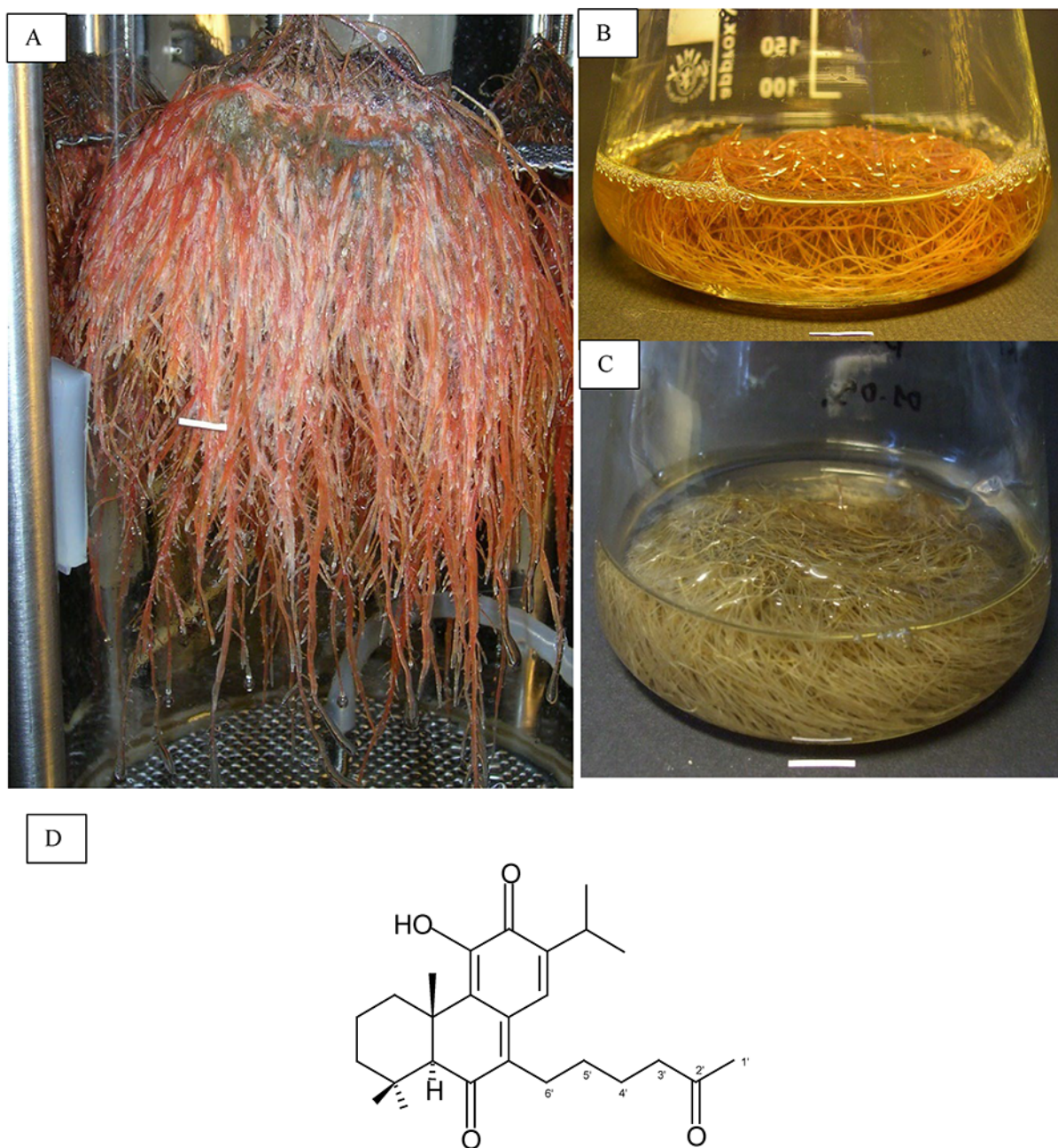
*Scutellaria baicalensis* hairy roots obtained by genetic transformation with *Rhizobium rhizogenes* LBA 9402 produced up to 10% DW major flavones (baicalein, wogonin, oroxylin A, and their glycosides) when cultured in an optimized nutrient medium supplemented with 5%–7% sucrose (Stojakowska & Malarz, 2000).

A detailed analysis of flavone aglycones synthesized in the culture revealed the presence of additional compounds such as chrysin, norwogonin, 5,7,2'-trihydroxyflavone, 5,2'-dihydroxy-6,7,8-trimethoxyflavone, and 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone (Stojakowska et al., 2001). The results have been cited in several Korean patents aimed at inventing compositions to prevent and treat enteroviral diseases.

#### 2.17. Diterpenoids in *Salvia sclarea* Transformed Root Cultures

*Salvia sclarea* L. is an aromatic herbaceous plant belonging to the Lamiaceae family that grows naturally in the Mediterranean basin, Southern Europe, and Iran. This species is used commercially in many countries for medicinal and cosmetic purposes. *Salvia sclarea* plant essential oil has been described in the *European Pharmacopoeia* (*Salviae sclareae aetheroleum*).

Under in vitro conditions, *Salvia sclarea* shoots have been transformed with *Rhizobium rhizogenes* LBA 9402 strain containing the pRi 1855 plasmid.



**Figure 19** (A) Thirty-day-old *Salvia sclarea* transformed roots after elicitation with 125  $\mu\text{M}$  methyl jasmonate (MJ) for 7 days in the nutrient-sprinkle bioreactor. (B) *Salvia sclarea* and (C) *S. austriaca* hairy roots (bar: 1 cm). (D) 7-2'-Oxohexyl-taxodione structure.

After bacteria elimination, the clary sage hairy roots were cultured in 300-mL Erlenmeyer flasks containing 80 mL half-strength macro- and microelement liquid sterile growth regulator-free Gamborg medium (1/2 B5) (Gamborg et al., 1968) supplemented with 3% sucrose. Genetic transformation in *sclarea* hairy roots has been confirmed using molecular methods (Kuźma et al., 2006). Hairy root cultures were maintained in a rotary shaker. Roots were cultivated in two lines: light and dark. The biomass of the hairy roots grown in light was  $72.8 \pm 2.9$  and  $6.75 \pm 0.25$  g/L fresh and dry, respectively. Light promoted both biomass and diterpenoid production in *S. sclarea* hairy roots (Kuźma et al., 2006, 2008, 2009), therefore MJ elicitation of *S. sclarea* hairy roots in light was studied by adding 62.5–1,250  $\mu\text{M}$  MJ on day 23. The 7-day treatment of the *Salvia sclarea* hairy roots with 125  $\mu\text{M}$  MJ was the best condition for biomass and secondary metabolite production, particularly in bioreactors (Kuźma et al., 2009) (Figure 19B).



Four diterpenoids, salvipisone, ferruginol, aethiopinone, and 1-oxoaethiopinone, have been isolated from *S. sclarea* (Kuźma et al., 2006). The further separations isolated ursolic acid, oleanolic acid, 2 $\alpha$ ,3 $\alpha$ -dihydroxy-urs-12-en-28-oic acid, 2 $\alpha$ ,3 $\alpha$ ,24-trihydroxy-urs-12-en-28-oic acid,  $\beta$ -sitosterol, stigmaterol, and campesterol (Kuźma et al., 2006). The dark- and light-grown roots biosynthesize the same secondary metabolites, but quantitative HPLC analysis has reported that light promotes diterpenoid biosynthesis (Kuźma et al., 2006).

The antibacterial properties of the diterpenoids isolated from *S. sclarea* hairy roots were tested. Salvipisone had the highest antibacterial activity, possible due to bacterial adhesion and biofilm formation inhibition (Kuźma et al., 2007).

Diterpenoids isolated from *S. sclarea* hairy roots were also tested for activity against *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) strains. Salvipisone and aethiopinone exhibit strong synergistic actions with antibiotics (Walenska et al., 2007). The cytotoxic properties of the diterpenoids isolated from clary sage hairy roots have also been tested against NALM-6, HL-60, and HL-60 ADR cells (Różalski et al., 2006).

## 2.18. Diterpenoids in Transformed *Salvia austriaca* Root Cultures

*Salvia austriaca* is an herbaceous plant native to Russia and Eastern Europe (Clebsch & Barner, 2003). The roots of this species can accumulate abietane-type diterpenoids (Nagy et al., 1999). After infecting in vitro cultivated *S. austriaca* shoots with *Rhizobium rhizogenes* A4 strain, genetically modified roots were obtained (Kuźma et al., 2011), which were maintained in half-strength Gamborg (1/2 B5) liquid medium supplemented with 3% sucrose in light (Gamborg et al., 1968) (Figure 19C). The fresh and dry biomass of the 30-day-old transformed root culture increased by 11- and 9-fold, respectively (Kuźma et al., 2011). The influence of culture medium type (and the half-strength of micronutrient variants) on growth and diterpene biosynthesis has been estimated (Kuźma et al., 2017). Biomass and diterpene production in shake flask culture has been examined using SH, Gamborg (B5) and woody plant (WPM) media, with both full and half-strength macro- and micronutrient concentrations supplemented with 3% sucrose.

*N*-hexane *S. austriaca* hairy root extract has been chromatographically separated (Kuźma et al., 2011) and three abietane-type diterpenoids have been identified, including taxodione, 15-deoxy-fuerstione, and royleanone, for the first time. Subsequently, taxodone and 7-2'-oxohexyl-taxodione has been isolated (Figure 19D) (Kuźma, Wysokińska, Różalski, Krajewska, & Kisiel, 2012). The last compound appears to be a novel compound previously unknown in the plant kingdom.

The cytotoxicity of taxodione, taxodone, 15-deoxyfuerstione, and 7-2'-oxohexyl-taxodione against HL-60, NALM-6, WM-115, and healthy HUVEC has been tested (Kuźma, Wysokińska, Różalski, Krajewska, & Kisiel, 2012). 7-2'-Oxohexyl-taxodione, an unusual diterpenoid, exhibits tenfold stronger cytotoxic activity than the highly active taxodione against all tested neoplastic cell lines. Moreover, taxodione and 7-2'-oxohexyl-taxodione exhibits seven- and eightfold lower cytotoxicity, respectively, against healthy cells (HUVEC) than that against neoplastic cells (Kuźma, Wysokińska, Różalski, Krajewska, & Kisiel, 2012). The antibacterial and antifungal actions of taxodione and 7-2'-oxohexyl-taxodione against the reference strains *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, and the clinical strains *Staphylococcus aureus* and *Enterococcus* spp. have also been examined. 7-2'-Oxohexyl-taxodione demonstrated strong activity against gram-positive bacteria, particularly *Staphylococcus aureus* (MRSA). This diterpenoid exhibits 4 times stronger activity than taxodione (Kuźma, Wysokińska, Różalski, Budzyńska, et al., 2012).

## 2.19. Sesquiterpenoids and Phenolics in *Cichorium intybus* and *Lactuca* spp. Hairy Roots and Undifferentiated Cultures

Lactucin, lactucopicrin, and 8-deoxylactucin are bitter-tasting sesquiterpene lactone (SL) guaianolides, demonstrating several biological activities both in vivo and in

vitro (European Medicines Agency, 2013; Gromek et al., 1992). These compounds are secondary metabolites of wild growing and cultivated numerous *Lactuca* and *Cichorium* species and are accumulated mainly in the plant roots. Similar to many other SLs, 8-deoxylactucin and lactucopicrin demonstrate anti-inflammatory activity. Moreover, these compounds inhibit  $\alpha$ -glucosidase function (Fan et al., 2017). The analgesic activity and sedative property of lactucopicrin are similar to those of ibuprofen as shown in a spontaneous locomotor activity test in mice (Wesołowska et al., 2006). This result was similar to that observed for active *Lactuca virosa* preparations containing 8-deoxylactucin (Gromek et al., 1992).

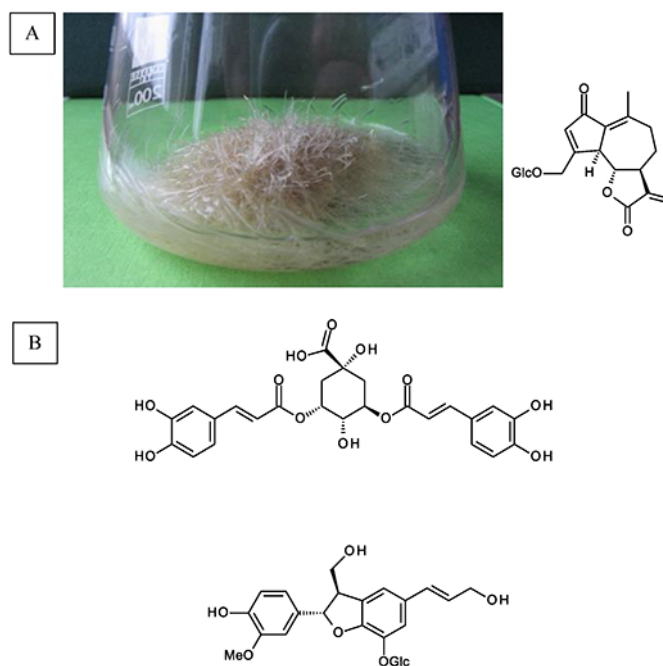
Lettuce and chicory are closely related taxonomically and produce similar specialized metabolites, including SLs, coumarins, flavonoids, and hydroxycinnamates. Hydroxycinnamates include biologically active caffeic acid derivatives, such as 5-*O*-caffeoylquinic acid (5-CQA) and 3,5-di-*O*-caffeoylquinic acid (3,5-DCQA), which are potent radical scavengers, lipid peroxidation inhibitors (Ohnishi et al., 1994; Olmos et al., 2008), and neuroprotective agents (Kwon et al., 2010). 3,5-DCQA exerts hepatoprotective effects (Hao et al., 2012) and is active against HIV-1 replication and HIV integrase (Robinson et al., 1996). Numerous pharmacological activities of chlorogenic acid (5-CQA), one of the most ubiquitous metabolites of Asteraceae, have been recently reviewed by Naveed et al. (2018).

Both plants have a long history of medicinal use except as leafy vegetables. *Cichorium intybus* L. roots and their preparations are used in Europe as traditional herbal medicinal products for the relief of symptoms of mild digestive disorders and temporary appetite loss (European Medicines Agency, 2013). *Lactuca virosa* L. and *Lactuca serriola* L. (a wild garden lettuce predecessor) are milky sap (latex) sources. In its condensed form, the latex (known as “lactucarium” or “lettuce opium”) was used as a sedative, analgesic, and antitussive remedy by the middle of the twentieth century (Stojakowska et al., 2000).

*Rhizobium rhizogenes*-transformed *L. virosa* and *C. intybus* roots were obtained using *Rhizobium rhizogenes* strain LBA 9402.

*Lactuca virosa* hairy roots produce three structural SLs, lactucin-like guaianolides (8-deoxylactucin, jacquinelin, crepidiaside B, and picriside A), zaluzanin-like guaianolides (macrocliniside A, ixerin F, and scorzoside), and germacranolide (lactuside A; Kisiel et al., 1995). Quantitative analysis of lactucin-like SLs in hairy root biomass demonstrated that 8-deoxylactucin glucoside (crepidiaside A, up to 0.72% DW), jacquinelin glucoside (crepidiaside B, 0.22% DW), and lactucopicrin (0.15% DW) are the major sesquiterpenes in the studied plant material (Stojakowska et al., 2012). Their contents are much higher than those in *L. virosa* plants (Tamaki et al., 1995). Moreover, 5-CQA (1.1% DW) and 3,5-DCQA (2.6% DW) are the major phenolic constituents of the examined hairy roots and responsible for their potent free-radical scavenging activity. The antiradical activity of the root extract is higher than that of the leaves of the intact plants. The contents of the two caffeic acid derivatives in hairy roots are much higher than those in undifferentiated *L. virosa* in vitro cultures and the parent plant (Stojakowska et al., 2012). Undifferentiated *L. virosa* tissue cultures did not produce lactucin-like guaianolides. Their major constituents are neolignans (formerly not found in *L. virosa*) and caffeic acid derivatives (Stojakowska & Kisiel, 2000; Stojakowska et al., 2012). It is worth noting that Kisiel et al. (1995) first described SL isolation from hairy roots. This study has also been cited by a US patent aimed at treating hepatitis C virus infection with SLs.

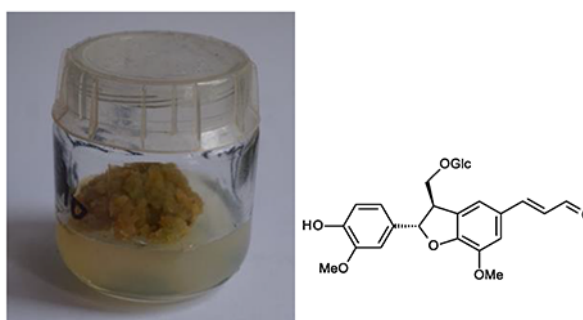
*Cichorium intybus* hairy roots of (Figure 20A), in contrast to undifferentiated *C. intybus* cultures (Malarz et al., 2005), produce SLs characteristic of the parent plant, including 8-deoxylactucin, lactucopicrin, crepidiaside B, sonchuside A, and ixeriside D (Malarz et al., 2002). To some extent, exogenous MJ stimulates SL production (Malarz et al., 2007). Further studies on the metabolism of the culture isolated a new natural compound, neolignan [(7*S*,8*R*)-3'-demethyl-dehydrodiconiferyl alcohol-3'-*O*- $\beta$ -glucopyranoside] (Malarz, Stojakowska, Szneler, & Kisiel, 2013) and identified 3,5-DCQA and 8-deoxylactucin glucoside (5.5% and 1.4%, respectively, calculated on a DW basis) (Figure 20B) as major constituents in long-term *C. intybus* hairy root cultures (Malarz, Stojakowska, & Kisiel, 2013). Ferioli and D'Antuono (2012) calculated that SL content in two



**Figure 20** (A) *Rhizobium rhizogenes* LBA 9402-transformed *Cichorium intybus* L. root culture. (B) Chemical structures of 3,5-di-O-caffeoylquinic acid and (7S,8R)-3'-demethyl-dehydrodiconiferyl alcohol-3'-O-β-glucopyranoside.

chicory cultivars, Catalogna and Head Radicchio, is maximum 0.22% over dry mass. The radical scavenging activities of *C. intybus* hairy root extracts are far superior to those of both *C. intybus* root and aerial part extracts due to caffeic acid derivative (5-CQA and 3,5-DCQA) accumulation in the transformed chicory roots (Malarz, Stojakowska, & Kisiel, 2013).

Total reducing capacity of *L. aculeata* Boiss. and Kotschy callus tissue is 26.4 mg GAeq/g DW, which is similar to that of *C. intybus* calli (24.5 mg GAeq/g DW). Chromatographic analysis of a hydroalcoholic *L. aculeata* undifferentiated tissue extract have demonstrated that mono- and di-caffeoylquinic acids substantially contribute to the antioxidative activity of cultured cells. The major hydroxycinnamate constituent of the examined plant material (1,5-DCQA) reached 1% DW. Several lignans, formerly known as *Lactuca* spp. constituents, have been isolated from callus tissue (Stojakowska & Malarz, 2017). Compounds such as (+)-5-methoxybalanophonin, syringaresinol, (+)-buddlenol A, (+)-balanophonin-9-O-β-glucopyranoside (Figure 21), (–)-dihydrodehydrodiconiferyl alcohol-4-O-β-glucopyranoside, and (–)-dihydrodehydrodiconiferyl alcohol-9-O-β-glucopyranoside are active in several



**Figure 21** Chemical structure of (+)-balanophonin-9-O-β-glucopyranoside.

in vitro assays as anti-inflammatory and mild cytotoxic agents (Jiao et al., 2011; Woo et al., 2016; Zálešák et al., 2019; Zhu et al., 2015).

## 2.20. Polyphenolic Compounds in *Agastache rugosa* and *Moluccella laevis* Shoot Cultures

Phenolic acids and flavonoids, two major phenolic compound classes, have been detected in *Agastache rugosa* (Fischer & C. A. Meyer) and *Moluccella laevis* L. in vitro shoot cultures. Light treatment [cool fluorescent lamps, white LED, and photosynthetic active radiation (PAR LED)] and culture conditions influenced the shoot chemical profile. Seven polyphenolic compounds have been identified in *Agastache rugosa* in vitro shoots using UPLC–DAD–qTOF–MS. RA is the predominant compound, accompanied by only few other phenolic constituents in significantly lower amounts. Plant growth regulator supplementation strongly decreased the RA content in microshoots cultured for 70–105 days than hormone-free media. The RA content increased after culturing for 140 days in hormone-enriched media. Interestingly, culture duration is the most influential factor for RA accumulation (Zielińska et al., 2019). A similar experimental approach was used for *M. laevis* (bells of Ireland) cell and organ cultures in vitro. Phytochemical analysis revealed the presence of caffeic acid, ferulic acid, and two conjugated caffeic acid derivatives (chlorogenic acid and RA) in shoots and two cyanidin glycosides (cyanidin 3-*O*-galactoside and cyanidin 3-*O*-malonylglucoside) in calli. The chlorogenic acid content is the maximum in shoots cultured on MS medium supplemented with 3 µM BA and 0.5 µM IAA under white and PAR LED light conditions (15.5–18.32 mg/100 g DW). It was the first report on the induction of *M. laevis* shoot and callus cultures using classical biotechnological methods and their specialized metabolite contents (Zielińska et al., 2020).

## 2.21. Volatile Organic Compounds in *Agastache rugosa* Shoot Cultures

Sixty-five volatile compounds were identified in *Agastache rugosa* shoot cultures using GC-MS. Phenylallyl volatiles, such as estragole, are almost completely absent in in vitro plant materials. Only the shoots cultured with thidiazuron and picloram emitted 0.7% *trans*-anethole, whereas at most 0.1% estragole or *trans*-anethole were detected. Monoterpenes are the most abundant class of volatiles. Shoots grown on culture media without hormone supplementation emitted a large proportion of pulegone (over 90%), whereas adding exogenous plant growth regulators (PGRs) increased monoterpene proportion (20%–50%; Zielińska et al., 2011).

To reveal factors other than the physical or chemical factors influencing in vitro *A. rugosa* volatile profile, shoots derived from mothers of different ages were examined under controlled conditions. In vitro shoots derived from the seeds of a 1-year-old mother plant emitted a relatively large proportion of volatile compounds that usually do not exceed 1% of the total headspace (sesquiterpenoids, aliphatic alcohols, esters, and ketones). In contrast, shoots obtained from 2- and 3-year-old mother plants abundantly produced the major phenylpropanoid estragole (over 90%). The sum of the five major monoterpene contents ( $\alpha$ -pinene, limonene, menthone, isomenthone, and pulegone) in these shoots did not exceed 31%. This was the first record of maternal parentage influence on the in vitro chemical profile of *A. rugosa* offspring plants (Zielińska et al., 2017).

## 2.22. Isoquinoline Alkaloids in *Chelidonium majus* In Vitro Cultures

Phytochemical analysis of *Chelidonium majus* shoots grown on eight different culture media and under two light regimes showed the presence of three isoquinoline alkaloid classes: benzophenanthridine, protoberberine, and protopine. The individual component contents mostly differ between in vitro shoot, root, and callus tissues (Figure 22). The protoberberine derivative, coptisine, is the most abundant compound in shoot cultures, followed by sanguinarine and chelerythrine in roots, while the callus is rich in protopine and sanguinarine. Interestingly, berberine has not been detected in callus cultures and minuscule amounts of





**Figure 22** *Chelidonium majus* rhizogenic callus tissue cultured on Murashige and Skoog (MS) solid medium supplemented with 1.0  $\mu\text{M}$  kinetin, 0.5  $\mu\text{M}$  1-naphthylacetic acid (NAA), and 2.5  $\mu\text{M}$  2,4-dichlorophenoxyacetic acid (2,4-D) under photosynthetic active radiation (PAR) LED light; scale: 1 mm.

coptisine have been detected. This is the first report of isoquinoline alkaloid production in in vitro *Ch. majus* cell and organ cultures (Zielinska et al., 2018).

### 3. Conclusions

Several plant biotechnological techniques described in this review have opened new perspectives in developing biotechnological protocols for bioactive compound production. Various in vitro culture systems have been developed for medicinal plants belonging to more than dozen botanical families, which produce morphological differentiation-independent family-specific bioactive secondary metabolites (Table 1). Using biotechnological methods such as elicitation, genetic transformation, in situ extraction or permeabilization, and culture media composition optimization allowed us to obtain active compounds in amounts equal to or higher than those in intact plants. Almost all plant species studied were susceptible to genetic transformation using different *Rhizobium rhizogenes* (*Agrobacterium rhizogenes*) strains as vectors for desired genes, which developed teratomas, including hairy roots, characterized by the high biosynthetic potential for pharmaceutical compound production. In vitro techniques are expected to help solve some problems associated with medicinal plant species productivity. In future, attention should be paid to large-scale plant tissue and organ cultivation in bioreactors to generate biomass for useful bioactive compound production. Practical applications should also be identified. The most significant advantage of the presented biotechnological approaches is the simultaneous natural environment biodiversity protection, including rare, endemic, or endangered plant species protection.

### References

- Alamgir, A. N. M. (2017). Cultivation of herbal drugs, biotechnology, and in vitro production of secondary metabolites, high-value medicinal plants, herbal wealth, and herbal trade. Therapeutic use of medicinal plants and their extracts. In *Therapeutic use of medicinal plants and their extracts* (Vol. 1, pp. 379–452). Springer. [https://doi.org/10.1007/978-3-319-63862-1\\_9](https://doi.org/10.1007/978-3-319-63862-1_9)
- Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., Conrad, L. J., Gelvin, S. B., Jackson, D. P., Kausch, A. P., Lemaux, P. G., Medford, J. I., Orozco-Cárdenas, M. L., Tricoli, D. M., Van Eck, J., Voytas, D. F., Walbot, V., Wang, K., Zhang, Z. J., & Stewart, C. N., Jr. (2016). Advancing crop transformation in the era of genome editing. *The Plant Cell*, 28, 1510–1520. <https://doi.org/10.1105/tpc.16.00196>
- Bethea, D., Fullmer, B., Syed, S., Seltzer, G., Tiano, J., Rischko, C., Gillespie, L., Brown, D., & Gasparro, F. P. (1999). Psoralen photobiology and photochemotherapy: 50 years of

- science and medicine. *Journal of Dermatological Science*, 19(2), 78–88.  
[https://doi.org/10.1016/s0923-1811\(98\)00064-4](https://doi.org/10.1016/s0923-1811(98)00064-4)
- Bohuslavizki, K. H., Hänsel, W., Kneip, A., Koppenhöfer, E., Niemöller, E., & Sanmann, K. (1994). Mode of action of psoralens, bezofurans, acridinons and coumarins on the ionic currents in intact myelinated nerve fibres and its significance in demyelinating diseases. *General Physiology and Biophysics*, 13(4), 309–328.
- Budzianowska, A., Kikowska, M., Małkiewicz, M., Karolak, I., & Budzianowski, J. (2019). Phenylethanoid glycosides in *Plantago media* L. organs obtained in in vitro cultures. *Acta Biologica Cracoviensia, Series Botanica*, 61(1), 75–86.  
<https://doi.org/10.24425/118060>
- Budzianowska, A., Skrzypczak, L., & Budzianowski, J. (2004). Phenylethanoid glucosides from in vitro propagated plants and callus cultures of *Plantago lanceolata* L. *Planta Medica*, 70(9), 834–840. <https://doi.org/10.1055/s-2004-827232>
- Budzianowski, J. (1995). Naphthoquinones of *Drosera spathulata* from in vitro cultures. *Phytochemistry*, 40(4), 1145–1148. [https://doi.org/10.1016/0031-9422\(95\)00313-V](https://doi.org/10.1016/0031-9422(95)00313-V)
- Budzianowski, J. (1996). Naphthohydroquinone glucosides of *Drosera rotundifolia* and *D. intermedia* from in vitro cultures. *Phytochemistry*, 42, 1145–1147.  
[https://doi.org/10.1016/0031-9422\(96\)00076-3](https://doi.org/10.1016/0031-9422(96)00076-3)
- Budzianowski, J. (1997). 2-Methylnaphthazarin 5-O-glucoside from the methanolic extracts of in vitro cultures of *Drosera* species. *Phytochemistry*, 44(1), 75–77.  
[https://doi.org/10.1016/S0031-9422\(96\)00520-1](https://doi.org/10.1016/S0031-9422(96)00520-1)
- Budzianowski, J. (2000). Naphthoquinone glucosides of *Drosera gigantea* from in vitro cultures. *Planta Medica*, 66(7), 667–669. <https://doi.org/10.1055/s-2000-8617>
- Budzianowski, J., Budzianowska, A., & Kromer, K. (2002). Naphthalene glucoside and other phenolics from shoot and callus cultures of *Drosophyllum lusitanicum*. *Phytochemistry*, 61(4), 421–425. [https://doi.org/10.1016/S0031-9422\(02\)00258-3](https://doi.org/10.1016/S0031-9422(02)00258-3)
- Budzianowski, J., Morozowska, M., & Wesołowska, M. (2005). Lipophilic flavones of *Primula veris* L. from field cultivation and in vitro culture. *Phytochemistry*, 66(9), 1033–1039.  
<https://doi.org/10.1016/j.phytochem.2005.03.024>
- Budzianowski, J., Skrzypczak, L., & Kukułczanka, K. (1993). Phenolic compounds of *Drosera intermedia* and *D. spathulata* from in vitro cultures. *Acta Horticulturae*, 330, 277–280.  
<https://doi.org/10.17660/ActaHortic.1993.330.36>
- Clebsch, B., & Barner, C. D. (2003). *Salvia austriaca* Jacquin. In B. Clebsch (Ed.), *The new book of salvias. Sages for every garden* (pp. 39–40). Timber Press.
- Debnath, M., Malik, C. P., & Bisen, P. S. (2006). Micropropagation: A tool for the production of high quality plant-based medicines. *Current Pharmaceutical Biotechnology*, 7, 33–49.  
<https://doi.org/10.2174/138920106775789638>
- Derda, M., Thiem, B., Budzianowski, J., Hadaś, E., Wojt, W. J., & Wojtkowiak-Giera, A. (2013). The evaluation of the amebicidal activity of *Eryngium planum* extracts. *Acta Poloniae Pharmaceuticae – Drug Research*, 70(6), 1027–1034.
- Ekiert, H. (1993). *Ammi majus* L. (Bishop's weed): In vitro culture and the production of coumarin compounds. In Y. P. S. Bajaj (Ed.), *Medicinal and aromatic plants IV* (pp. 1–17). Springer. [https://doi.org/10.1007/978-3-642-77004-3\\_1](https://doi.org/10.1007/978-3-642-77004-3_1)
- Ekiert, H. (2004). Accumulation of biologically active furanocoumarins within in vitro cultures of medicinal plants. In K. G. Ramawat (Ed.), *Biotechnology of medicinal plants. Vitalizer and therapeutic* (pp. 267–296). Science Publishers.
- Ekiert, H., Abou-Mandour, A. A., & Czygan, F. C. (2005). Accumulation of biologically active furanocoumarins in *Ruta graveolens* ssp. *divaricata* (Tenore) Gams in vitro culture. *Pharmazie*, 60(1), 66–68.
- Ekiert, H., Chołoniewska, M., & Gomółka, E. (2001). Accumulation of furanocoumarins in *Ruta graveolens* L. shoot culture. *Biotechnology Letters*, 23(7), 543–545.  
<https://doi.org/10.1023/A:1010386820799>
- Ekiert, H., & Czygan, F. C. (2005). Accumulation of biologically active furanocoumarins in agitated cultures of *Ruta graveolens* L. and *Ruta graveolens* ssp. *divaricata* (Tenore) Gams. *Pharmazie*, 60(8), 623–626.
- Ekiert, H., & Czygan, F. C. (2007). Secondary metabolites in in vitro cultures of *Ruta graveolens* L. and *Ruta graveolens* ssp. *divaricata* (Tenore) Gams. In K. G. Ramawat & J. M. Merillon (Eds.), *Biotechnology: Secondary metabolites. Plants and microbes* (pp. 445–482). Science Publishers. <https://doi.org/10.1201/b10756-17>
- Ekiert, H., & Gomółka, E. (1999). Effect of light on contents of coumarin compounds in shoots of *Ruta graveolens* L. cultivated in vitro. *Acta Societatis Botanicorum Poloniae*, 68(3), 197–200. <https://doi.org/10.5586/asbp.1999.026>
- Ekiert, H., & Gomółka, E. (2000a). Coumarin compounds in *Ammi majus* L. callus culture. *Pharmazie*, 55(9), 684–687.
- Ekiert, H., & Gomółka, E. (2000b). Furanocoumarins in *Pastinaca sativa* L. in vitro culture. *Pharmazie*, 55(8), 618–620.

- Ekiert, H., & Kisiel, W. (1997). Coumarins and alkaloids in shoot culture of *Ruta graveolens* L. *Acta Societatis Botanicorum Poloniae*, 66(3–4), 329–332. <https://doi.org/10.5586/asbp.1997.039>
- Ekiert, H., Turcza, K., Kwiecień, I., & Szopa, A. (2017). Od algologii do biotechnologii – 85 lat działalności Katedry i Zakładu Botaniki Farmaceutycznej w Krakowie. Część III, 1999–2016. Działalność naukowo-badawcza i dydaktyczna związana z biotechnologią roślin leczniczych [From algology to biotechnology – 85 years of activity of the Chair and Department of Pharmaceutical Botany in Krakow. Part III; 1999–2016. Scientific research and teaching related to the biotechnology of medicinal plants]. *Farmacja Polska*, 73(5), 285–302.
- European Medicines Agency. (2013). Cichorii intybi radix. <https://www.ema.europa.eu/en/medicines/herbal/cichorii-intybi-radix>
- Fan, H., Chen, J., Lv, H., Ao, X., Wu, Y., Ren, B., & Li, W. (2017). Isolation and identification of terpenoids from chicory roots and their inhibitory activities against yeast  $\alpha$ -glucosidase. *European Food Research and Technology*, 243, 1009–1017. <https://doi.org/10.1007/s00217-016-2810-1>
- Feroli, E., & D'Antuono, L. F. (2012). An update procedure for an effective and simultaneous extraction of sesquiterpene lactones and phenolics from chicory. *Food Chemistry*, 135, 243–250. <https://doi.org/10.1016/j.foodchem.2012.04.079>
- Ferreira, A., Rodrigues, M., Fortuna, A., Falcão, A., & Alves, G. (2016). Huperzine A from *Huperzia serrata*: A review of its sources, chemistry, pharmacology and toxicology. *Phytochemistry Reviews*, 15(1), 51–85. <https://doi.org/10.1007/s11101-014-9384-y>
- Fonseca-Santos, B., Antonio Corrêa, M., & Chorilli, M. (2015). Sustainability, natural and organic cosmetics: Consumer, products, efficacy, toxicological and regulatory considerations. *Brazilian Journal of Pharmaceutical Sciences*, 51(1), 17–26. <https://doi.org/10.1590/S1984-82502015000100002>
- Freeberg, J. A., & Wetmore, R. H. (1957). Gametophytes of *Lycopodium* as grown in vitro. *Phytomorphology*, 7, 204–217.
- Furmanowa, M., & Sykłowska-Baranek, K. (2000). Hairy root cultures of *Taxus × media* var. *Hicksii* Rehd. as a new source of paclitaxel and 10-deacetylbaccatin III. *Biotechnology Letters*, 22, 683–686. <https://doi.org/10.1023/A:1005683619355>
- Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*, 50, 151–158. [https://doi.org/10.1016/0014-4827\(68\)90403-5](https://doi.org/10.1016/0014-4827(68)90403-5)
- Graikou, K., Damianakos, H., Ganos, C., Sykłowska-Baranek, K., Małgorzata Jeziorek, M., Pietrosiuk, A., Christos Roussakis, C., & Chinou, I. (2021). Chemical profile and screening of bioactive metabolites of *Rindera graeca* (A. Dc.) Bois. & Heldr. (Boraginaceae) in vitro cultures. *Plants*, 10(5), Article 834. <https://doi.org/10.3390/plants10050834>
- Gromek, D., Kisiel, W., Kłodzińska, A., & Chojnacka-Wójcik, E. (1992). Biologically active preparations from *Lactuca virosa* L. *Phytotherapy Research*, 6, 285–287. <https://doi.org/10.1002/ptr.2650060514>
- Haberlandt, G. (1943). Culturversuche mit isolierten Pflanzenzellen [Cultivation experiments using isolated plant cells]. *Planta*, 33(4), 576–588. <https://doi.org/10.1007/BF01916543>
- Hao, B.-J., Wu, Y.-H., Wang, J.-G., Hu, S.-Q., Keil, D. J., Hu, H.-J., Lou, J.-D., & Zhao, Y. (2012). Hepatoprotective and antiviral properties of isochlorogenic acid A from *Laggera alata* against hepatitis B virus infection. *Journal of Ethnopharmacology*, 144, 190–194. <https://doi.org/10.1016/j.jep.2012.09.003>
- Härmälä, P., Vuorela, H., Hiltunen, R., Nyireddy, S., Törnquist, K., & Kaltia, S. (1992). Strategy for the isolation and identification of coumarins with calcium antagonistic properties from the roots of *Angelica archangelica*. *Phytochemical Analysis*, 3(1), 42–48. <https://doi.org/10.1002/pca.2800030108>
- He, G., He, G., Zhou, R., Pi, Z., Zhu, T., Jiang, L., & Xie, Y. (2016). Enhancement of cisplatin-induced colon cancer cells apoptosis by shikonin, a natural inducer of ROS in vitro and in vivo. *Biochemical and Biophysical Research Communications*, 469(4), 1075–1082. <https://doi.org/10.1016/j.bbrc.2015.12.100>
- Hehmann, M., Lukačín, R., Ekiert, H., & Matern, U. (2004). Furanocoumarin biosynthesis in *Ammi majus* L. Cloning of bergaptol O-methyltransferase. *European Journal of Biochemistry*, 271(5), 932–940. <https://doi.org/10.1111/j.1432-1033.2004.03995.x>
- Hussain, M. S., Fareed, S., Ansari, S., Rahman, M. A., Ahmad, I. Z., & Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences*, 4, 10–20. <https://doi.org/10.4103/0975-7406.92725>
- Jaferník, K., Szopa, A., Barnaś, M., Dziurka, M., & Ekiert, H. (2020). *Schisandra henryi* C. B. Clarke in vitro cultures: A promising tool for the production of lignans and phenolic compounds. *Plant Cell Tissue and Organ Culture*, 143, 45–60. <https://doi.org/10.1007/s11240-020-01895-2>

- Jeziorek, M., Damianakos, H., Kawiak, A., Laudy, A. E., Zakrzewska, K., Sykłowska-Baranek, K., Chinou, I., & Pietrosiuk, A. (2019). Bioactive rinderol and cynoglosol isolated from *Cynoglossum columnae* Ten. in vitro root culture. *Industrial Crops & Products*, 137, 446–452. <https://doi.org/10.1016/j.indcrop.2019.04.046>
- Jiao, W.-H., Gao, H., Zhao, F., He, F., Zhou, G.-X., & Yao, X.-S. (2011). A new neolignan and a new sesterterpenoid from the stems of *Picrasma quassioides* Bennet. *Chemistry & Biodiversity*, 8, 1163–1169.
- Karuppusamy, S. (2009). A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *Journal of Medicinal Plants Research*, 3(13), 1222–1239.
- Kawiak, A., Domachowska, A., Krolicka, A., Smolarska, M., & Lojkowska, E. (2019). 3-Chloroplumbagin induces cell death in breast cancer cells through MAPK-mediated Mcl-1 inhibition. *Frontiers in Pharmacology*, 10, Article 784. <https://doi.org/10.3389/fphar.2019.00784>
- Kawiak, A., Domachowska, A., & Lojkowska, E. (2019). Plumbagin increases paclitaxel-induced cell death and overcomes paclitaxel resistance in breast cancer cells through ERK-mediated apoptosis induction. *Journal of Natural Products*, 82, 878–885. <https://doi.org/10.1021/acs.jnatprod.8b00964>
- Kawiak, A., Krolicka, A., & Lojkowska, E. (2003). Direct regeneration of *Drosera* from leaf explants and shoot tips. *Plant Cell Tissue and Organ Culture*, 75, 175–178. <https://doi.org/10.1023/A:1025023800304>
- Kawiak, A., Królicka, A., & Lojkowska, E. (2011). In vitro cultures of *Drosera aliciae* as a source of a cytotoxic naphthoquinone: ramentaceone. *Biotechnology Letters*, 33, Article 2309. <https://doi.org/10.1007/s10529-011-0700-y>
- Kawiak, A., Piosik, J., Stasiłojc, G., Gwizdek-Wisniewska, A., Marczak, L., Stobiecki, M., & Lojkowska, E. (2007). Induction of apoptosis by plumbagin through reactive oxygen species-mediated inhibition of topoisomerase II. *Toxicology and Applied Pharmacology*, 223, 267–276. <https://doi.org/10.1016/j.taap.2007.05.018>
- Kawiak, A., Zawacka-Pankau, J., Wasilewska, A., Stasiłojc, G., Bigda, J., & Lojkowska, E. (2012). Induction of apoptosis in HL-60 cells through the ROS-mediated mitochondrial pathway by ramentaceone from *Drosera aliciae*. *Journal of Natural Products*, 75, 9–14. <https://doi.org/10.1021/np200247g>
- Kikowska, M. (2014). *Krajowe gatunki rodzaju Eryngium L. w kulturze in vitro – mikrorozmnażanie, kultury organów, ocena fitochemiczna i aktywność biologiczna* [In vitro cultures of Polish *Eryngium* L. species – Micropropagation, organ cultures, phytochemical investigation and biological activity] [Doctoral dissertation, Poznan University of Medical Sciences]. Digital Library of Wielkopolska. <https://www.wbc.poznan.pl/publication/411082>
- Kikowska, M., Budzianowski, J., Krawczyk, A., & Thiem, B. (2012). Accumulation of rosmarinic, chlorogenic and caffeic acid in in vitro of *Eryngium planum* L. *Acta Physiologiae Plantarum*, 34, 2425–2433. <https://doi.org/10.1007/s11738-012-1011-1>
- Kikowska, M., Długaszewska, J., Kubicka, M. M., Kędziora, I., Budzianowski, J., & Thiem, B. (2016). In vitro antimicrobial activity of extracts and their fractions from three *Eryngium* L. species. *Herba Polonica*, 62(2), 67–77. <https://doi.org/10.1515/hepo-2016-0012>
- Kikowska, M., Kędziora, I., Krawczyk, A., & Thiem, B. (2015). Methyl jasmonate, yeast extract and sucrose stimulate phenolic acid accumulation in *Eryngium planum* L. shoot cultures. *Acta Biochimica Polonica*, 62(2), 197–200. [https://doi.org/10.18388/abp.2014\\_880](https://doi.org/10.18388/abp.2014_880)
- Kikowska, M., Kowalczyk, M., Stochmal, A., & Thiem, B. (2019). Enhanced accumulation of triterpenoid saponins in in vitro plantlets and dedifferentiated cultures of *Eryngium planum* L.: A medicinal plant. *Horticulture, Environment, and Biotechnology*, 60, 147–154. <https://doi.org/10.1007/s13580-018-0103-2>
- Kikowska, M. A., Chmielewska, M., Włodarczyk, A., Studzińska-Sroka, E., Żuchowski, J., Stochmal, A., Kotwicka, M., & Thiem, B. (2018). Effect of pentacyclic triterpenoids-rich callus extract of *Chaenomeles japonica* (Thunb.) Lindl. ex Spach on viability, morphology, and proliferation of normal human skin fibroblasts. *Molecules*, 23, Article 3009. <https://doi.org/10.3390/molecules23113009>
- Kisiel, W., Stojakowska, A., Malarz, J., & Kohlmünzer, S. (1995). Sesquiterpene lactones in *Agrobacterium rhizogenes*-transformed hairy root culture of *Lactuca virosa*. *Phytochemistry*, 40, 1139–1140. [https://doi.org/10.1016/0031-9422\(95\)00433-8](https://doi.org/10.1016/0031-9422(95)00433-8)
- Kokotkiewicz, A., Luczkiewicz, M., Kowalski, W., Badura, A., Piekus, N., & Bucinski, A. (2013). Isoflavone production in *Cyclopia subternata* Vogel (honeybush) suspension cultures grown in shake flasks and stirred-tank bioreactor. *Applied Microbiology and Biotechnology*, 97, 8467–8477. <https://doi.org/10.1007/s00253-013-5099-z>



- Kokotkiewicz, A., Luczkiewicz, M., Pawłowska, J., Luczkiewicz, P., Sowinski, P., Witkowski, J., Bryl, E., & Bucinski, A. (2013). Isolation of xanthone and benzophenone derivatives from *Cyclopia genistoides* (L.) Vent. (honeybush) and their pro-apoptotic activity on synoviocytes from patients with rheumatoid arthritis. *Fitoterapia*, *90*, 199–208. <https://doi.org/10.1016/j.fitote.2013.07.020>
- Kokotkiewicz, A., Wnuk, M., Bucinski, A., & Luczkiewicz, M. (2009). In vitro cultures of *Cyclopia* plants (honeybush) as a source of bioactive xanthenes and flavanones. *Zeitschrift für Naturforschung C*, *64*, 533–540. <https://doi.org/10.1515/znc-2009-7-812>
- Kowalczyk, M., Masullo, M., Thiem, B., Piacente, S., Stochmal, A., & Oleszek, W. (2014). Three new triterpene saponins from roots of *Eryngium planum*. *Natural Product Research*, *28*(9), 653–660. <https://doi.org/10.1080/14786419.2014.895722>
- Królicka, A., Szpitter, A., Gilgenast, E., Romanik, G., Kaminski, M., & Lojkowska, E. (2008). Stimulation of antibacterial naphthoquinones and flavonoids accumulation in carnivorous plants by addition of elicitors. *Enzyme and Microbial Technology*, *42*, 216–221. <https://doi.org/10.1016/j.enzmictec.2007.09.011>
- Królicka, A., Szpitter, A., Maciag, M., Biskup, E., Gilgenast, E., Romanik, G., Kaminski, M., Wegrzyn, G., & Lojkowska, E. (2009). Antibacterial and antioxidant activity of the secondary metabolites from in vitro cultures of *Drosera aliciae*. *Biotechnology and Applied Biochemistry*, *53*(3), 175–184. <https://doi.org/10.1042/BA20080088>
- Królicka, A., Szpitter, A., Stawujak, K., Baranski, R., Gwizdek-Wisniewska, A., Skrzypczak, A., Kaminski, M., & Lojkowska, E. (2010). Teratomas of *Drosera capensis* var. *alba* as a source of naphthoquinone: ramentaceone. *Plant Cell Tissue and Organ Culture*, *103*, 285–292. <https://doi.org/10.1007/s11240-010-9778-5>
- Królicka, A., Staniszevska, I., Bielawski, K., Maliński, E., Szafranek, J., & Łojkowska, E. (2001). Establishment of hairy root cultures of *Ammi majus*. *Plant Science*, *160*, 259–264. [https://doi.org/10.1016/S0168-9452\(00\)00381-2](https://doi.org/10.1016/S0168-9452(00)00381-2)
- Krychowiak, M., Kawiak, A., Narajczyk, M., Borowik, A., & Królicka, A. (2018). Silver nanoparticles combined with naphthoquinones as an effective synergistic strategy against *Staphylococcus aureus*. *Frontiers in Pharmacology*, *9*, Article 816. <https://doi.org/10.3389/fphar.2018.00816>
- Krychowiak-Maśnicka, M., Krauze-Baranowska, M., Godlewska, S., Kaczyński, Z., Bielicka-Giełdoń, A., Grzegorzczak, N., Narajczyk, M., Frackowiak, J. E., & Królicka, A. (2021). Potential of silver nanoparticles in overcoming the intrinsic resistance of *Pseudomonas aeruginosa* to secondary metabolites from carnivorous plants. *International Journal of Molecular Sciences*, *22*, Article 4849. <https://doi.org/10.3390/ijms22094849>
- Kukulczanka, K., & Budzianowski, J. (2002). *Dionaea muscipula* Ellis (Venus fly-trap): In vitro culture and production of secondary metabolites. In T. Nagata & Y. Ebizuka (Eds.), *Medicinal and aromatic plants XII* (pp. 50–74). Springer. [https://doi.org/10.1007/978-3-662-08616-2\\_4](https://doi.org/10.1007/978-3-662-08616-2_4)
- Kuźma, Ł., Bruchajzer, E., & Wysokińska, H. (2008). Diterpenoid production in hairy root culture of *Salvia sclarea* L. *Zeitschrift für Naturforschung C*, *63*, 621–624. <https://doi.org/10.1515/znc-2008-7-827>
- Kuźma, Ł., Bruchajzer, E., & Wysokińska, H. (2009). Methyl jasmonate effect on diterpenoid accumulation in *Salvia sclarea* hairy root culture in shake flasks and sprinkle bioreactor. *Enzyme and Microbial Technology*, *44*, 406–410. <https://doi.org/10.1016/j.enzmictec.2009.01.005>
- Kuźma, Ł., Kaiser, M., & Wysokińska, H. (2017). The production and antiprotozoal activity of abietane diterpenes in *Salvia austriaca* hairy roots grown in shake flasks and bioreactor. *Preparative Biochemistry & Biotechnology*, *47*, 58–66. <https://doi.org/10.1080/10826068.2016.1168745>
- Kuźma, Ł., Kisiel, W., Królicka, A., & Wysokińska, H. (2011). Genetic transformation of *Salvia austriaca* by *Agrobacterium rhizogenes* and diterpenoid isolation. *Die Pharmazie*, *66*, 904–907. <https://doi.org/10.1691/ph.2011.1586>
- Kuźma, Ł., Różalski, M., Walencka, E., Różalska, B., & Wysokińska, H. (2007). Antimicrobial activity of diterpenoids from hairy roots of *Salvia sclarea* L.: Salvipisone as a potential anti-biofilm agent active against antibiotic resistant staphylococci. *Phytomedicine*, *14*, 31–35. <https://doi.org/10.1016/j.phymed.2005.10.008>
- Kuźma, Ł., Skrzypek, Z., & Wysokińska, H. (2006). Diterpenoids and triterpenoids in hairy roots of *Salvia sclarea*. *Plant Cell, Tissue and Organ Culture*, *84*, 171–179. <https://doi.org/10.1007/s11240-005-9018-6>
- Kuźma, Ł., Wysokińska, H., Różalski, M., Budzyńska, A., Więckowska-Szakiel, M., Sadowska, B., Paszkiewicz, M., Kisiel, W., & Różalska, B. (2012). Antimicrobial and anti-biofilm properties of new taxodione derivative from hairy roots of *Salvia austriaca*. *Phytomedicine*, *19*, 1285–1287. <https://doi.org/10.1016/j.phymed.2012.07.016>

- Kuźma, L., Wysokińska, H., Różalski, M., Krajewska, U., & Kisiel, W. (2012). An unusual taxodione derivative from hairy roots of *Salvia austriaca*. *Fitoterapia*, 83, 770–773. <https://doi.org/10.1016/j.fitote.2012.03.006>
- Kwon, S.-H., Lee, H.-K., Kim, J.-A., Hong, S.-I., Kim, H.-C., Jo, T.-H., Park, Y.-I., Lee, C.-K., Kim, Y.-B., Lee, S.-Y., & Jang, C.-G. (2010). Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. *European Journal of Pharmacology*, 649, 210–217. <https://doi.org/10.1016/j.ejphar.2010.09.001>
- Luczkiewicz, M., & Kokotkiewicz, A. (2012). Elicitation and permeabilization affect the accumulation and storage profile of phytoestrogens in high productive suspension cultures of *Genista tinctoria*. *Acta Physiologiae Plantarum*, 34, 1–16. <https://doi.org/10.1007/s11738-011-0799-4>
- Luczkiewicz, M., Kokotkiewicz, A., & Glod, D. (2014). Plant growth regulators affect biosynthesis and accumulation profile of isoflavone phytoestrogens in high-productive in vitro cultures of *Genista tinctoria*. *Plant Cell Tissue and Organ Culture*, 118, 419–429. <https://doi.org/10.1007/s11240-014-0494-4>
- Luczkiewicz, M., & Glód, D. (2003). Callus cultures of *Genista* plants – In vitro material producing high amounts of isoflavones of phytoestrogenic activity. *Plant Science*, 165, 1101–1108. [https://doi.org/10.1016/S0168-9452\(03\)00305-4](https://doi.org/10.1016/S0168-9452(03)00305-4)
- Łuczkiwicz, M., & Glód, D. (2005). Morphogenesis-dependent accumulation of phytoestrogens in *Genista tinctoria* in vitro cultures. *Plant Science*, 168, 967–979. <https://doi.org/10.1016/j.plantsci.2004.11.008>
- Luczkiewicz, M., & Kokotkiewicz, A. (2005a). Co-cultures of shoots and hairy roots of *Genista tinctoria* L. for synthesis and biotransformation of large amounts of phytoestrogens. *Plant Science*, 169, 862–871. <https://doi.org/10.1016/j.plantsci.2005.06.005>
- Łuczkiwicz, M., & Kokotkiewicz, A. (2005b). *Genista tinctoria* hairy root cultures for selective production of isoliquiritigenin. *Zeitschrift für Naturforschung C*, 60, 867–875. <https://doi.org/10.1515/znc-2005-11-1209>
- Ma, X., & Gang, D. R. (2004). The *Lycopodium* alkaloids. *Natural Product Reports*, 21, 752–772. <https://doi.org/10.1039/b409720n>
- Makowski, W., Królicka, A., Nowicka, A., Zwyrtková, J., Tokarz, B., Pecinka, A., Banasiuk, R., & Tokarz, K. (2021). Transformed tissue of *Dionaea muscipula* J. Ellis as a source of biologically active phenolic compounds with bactericidal properties. *Applied Microbiology and Biotechnology*, 105, 1215–1226. <https://doi.org/10.1007/s00253-021-11101-8>
- Malarz, J., Stojakowska, A., & Kisiel, W. (2002). Sesquiterpene lactones in a hairy root culture of *Cichorium intybus*. *Zeitschrift für Naturforschung C*, 57, 994–997. <https://doi.org/10.1515/znc-2002-11-1207>
- Malarz, J., Stojakowska, A., & Kisiel, W. (2007). Effect of methyl jasmonate and salicylic acid on sesquiterpene lactone accumulation in hairy roots of *Cichorium intybus*. *Acta Physiologiae Plantarum*, 29, 127–132. <https://doi.org/10.1007/s11738-006-0016-z>
- Malarz, J., Stojakowska, A., & Kisiel, W. (2013). Long-term cultured hairy roots of chicory – A rich source of hydroxycinnamates and 8-deoxylactucin glucoside. *Applied Biochemistry and Biotechnology*, 171, 1589–1601. <https://doi.org/10.1007/s12010-013-0446-1>
- Malarz, J., Stojakowska, A., Szneler, E., & Kisiel, W. (2005). Furofuran lignans from a callus culture of *Cichorium intybus*. *Plant Cell Reports*, 24, 246–249. <https://doi.org/10.1007/s00299-005-0953-9>
- Malarz, J., Stojakowska, A., Szneler, E., & Kisiel, W. (2013). A new neolignane glucoside from hairy roots of *Cichorium intybus*. *Phytochemistry Letters*, 6, 59–61. <https://doi.org/10.1016/j.phytol.2012.10.011>
- Matern, U. (1999). Medicinal potential and biosynthesis of plant coumarins. In J. T. Romeo (Ed.), *Phytochemicals in human health protection, nutrition and plant defense* (pp. 161–183). Springer. [https://doi.org/10.1007/978-1-4615-4689-4\\_7](https://doi.org/10.1007/978-1-4615-4689-4_7)
- Michalak, A. M. (2021). *Kultury in vitro i in vivo roślin z gatunku Iris pseudacorus źródłem związków biologicznie czynnych* [In vitro and in vivo cultures of *Iris pseudacorus* plants as a source of biologically active compounds] [Unpublished doctoral dissertation]. University of Gdańsk.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant Physiology*, 15, 473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Muszyński, J. (1955). Alkaloidy i glikozydy flawonowe widłaków [Alkaloids and flavone glycosides of *Lycopodium* genus]. *Acta Societatis Botanicorum Poloniae*, 24(2), 237–244. <https://doi.org/10.5586/asbp.1955.014>
- Nagy, G., Günther, G., Máthé, I., Blunden, G., Yang, M., & Crabb, T. A. (1999). Diterpenoids from *Salvia glutinosa*, *S. austriaca*, *S. tomentosa* and *S. verticillata* roots. *Phytochemistry*, 52, 1105–1109. [https://doi.org/10.1016/S0031-9422\(99\)00343-X](https://doi.org/10.1016/S0031-9422(99)00343-X)

- Naveed, M., Hejazic, V., Abbasa, M., Kamboh, A. A., Khane, G. J., Shumzaid, M., Ahmad, F., Babazadeh, D., Xia, F. F., Modarresi-Ghazani, F., Li, W. H., & Zhou, X. H. (2018). Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomedicine & Pharmacotherapy*, 97, 67–74. <https://doi.org/10.1016/j.biopha.2017.10.064>
- Ohnishi, M., Morishita, H., Iwahashi, H., Toda, S., Shirataki, Y., Kimura, M., & Kido, R. (1994). Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. *Phytochemistry*, 36, 579–583. [https://doi.org/10.1016/S0031-9422\(00\)89778-2](https://doi.org/10.1016/S0031-9422(00)89778-2)
- Oksman-Caldentey, K.-M., & Inzé, D. (2004). Plant cell factories in the post-genomic era: New ways to produce designer secondary metabolites. *Trends in Plant Science*, 9(9), 433–440. <https://doi.org/10.1016/j.tplants.2004.07.006>
- Olmos, A., Giner, R. M., Recio, M. C., Rios, J. L., Gil-Benso, R., & Máñez, S. (2008). Interaction of dicaffeoylquinic derivatives with peroxynitrite and other reactive nitrogen species. *Archives of Biochemistry and Biophysics*, 475, 66–71. <https://doi.org/10.1016/j.abb.2008.04.012>
- Ożarowski, M., Thiem, B., Mikołajczak, P. L., Piasecka, A., Kachlicki, P., Szulc, M., Kaminska, E., Bogacz, A., Kujawski, R., Bartkowiak-Wieczorek, J., Kujawska, M., Jodynis-Libert, J., Budzianowski, J., Kędziora, I., Seremak-Mrozikiewicz, A., Czerny, B., & Bobkiewicz-Kozłowska, T. (2015). Improvement in long-term memory following chronic administration of *Eryngium planum* root extract in scopolamine model: Behavioral and molecular study. *Evidence-Based Complementary and Alternative Medicine*, 2015, Article 145140. <https://doi.org/10.1155/2015/145140>
- Pakulski, G., & Budzianowski, J. (1996a). Ellagic acid derivatives and naphthoquinones of *Dionaea muscipula* from in vitro cultures. *Phytochemistry*, 41(3), 775–778. [https://doi.org/10.1016/0031-9422\(96\)89675-0](https://doi.org/10.1016/0031-9422(96)89675-0)
- Pakulski, G., & Budzianowski, J. (1996b). Quercetin and kaempferol glycosides of *Dionaea muscipula* from in vitro cultures. *Planta Medica*, 62(1), 95–96. <https://doi.org/10.1055/s-2006-957824>
- Papageorgiou, V. P., Assimopoulou, A. N., & Ballis, A. C. (2008). Alkannins and shikonins: A new class of wound healing agents. *Current Medicinal Chemistry*, 15(30), 3248–3267. <https://doi.org/10.2174/092986708786848532>
- Papageorgiou, V. P., Assimopoulou, A. N., Couladouros, E. A., Hepworth, D., & Nicolaou, K. C. (1999). The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. *Angewandte Chemie International Edition*, 38, 270–300. <https://doi.org/10.1002/anie.199900000>
- Papageorgiou, V. P., Assimopoulou, A. N., Samanidou, V. F., & Papadopyannis, I. N. (2006). Recent advances in chemistry, biology and biotechnology of alkannins and shikonins. *Current Organic Chemistry*, 10(16), 2123–2142. <https://doi.org/10.2174/1385272066778742704>
- Pietrosiuk, A., Sykłowska-Baranek, K., Wiedenfeld, H., Wolinowska, R., Furmanowa, M., & Jaroszyk, E. (2006). The shikonin derivatives and pyrrolizidine alkaloids in hairy root cultures of *Lithospermum canescens* (Michx.) Lehm. *Plant Cell Reports*, 25, 1052–1058. <https://doi.org/10.1007/s00299-006-0161-2>
- Robinson, W. E., Jr., Reinecke, M. G., Abdel-Malek, S., Jia, Q., & Chow, S. A. (1996). Inhibitors of HIV-1 replication that inhibit HIV integrase. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 6326–6331. <https://doi.org/10.1073/pnas.93.13.6326>
- Różalski, M., Kuźma, Ł., Krajewska, U., & Wysokińska, H. (2006). Cytotoxic and proapoptotic activity of diterpenoids from in vitro cultivated *Salvia sclarea* roots. Studies on the leukemia cell lines. *Zeitschrift für Naturforschung C*, 61, 483–488. <https://doi.org/10.1515/znc-2006-7-804>
- Schlauer, J., Budzianowski, J., Kukulczanka, K., & Ratajczak, L. (1994). Acteoside and related phenylethanoid glycosides from *Byblis liniflora* Salisb. plants propagated in vitro culture and its systematic significance. *Acta Societatis Botanicorum Poloniae*, 73(1), 9–15. <https://doi.org/10.5586/asbp.2004.002>
- Sidwa-Gorycka, M., Krolicka, A., Kozyra, M., Głowniak, K., Bourgaud, F., & Łojkowska, E. (2003). Establishment of a co-culture of *Ammi majus* and *Ruta graveolens* for synthesis of furanocoumarins. *Plant Science*, 165, 1315–1319. [https://doi.org/10.1016/S0168-9452\(03\)00343-1](https://doi.org/10.1016/S0168-9452(03)00343-1)
- Sparzak, B., Krauze-Baranowska, M., Kawiak, A., & Sowiński, P. (2015). Cytotoxic lignan from the non-transformed root culture of *Phyllanthus amarus*. *Molecules*, 20, 7915–7924. <https://doi.org/10.3390/molecules20057915>
- Staniszewska, I., Królicka, A., Maliński, E., Łojkowska, E., & Szafranek, J. (2003). Elicitation of secondary metabolites in in vitro cultures of *Ammi majus* L. *Enzyme and Microbial Technology*, 33, 565–568. [https://doi.org/10.1016/S0141-0229\(03\)00180-7](https://doi.org/10.1016/S0141-0229(03)00180-7)

- Stojakowska, A., & Kisiel, W. (2000). Neolignan glycosides from a cell suspension culture of *Lactuca virosa*. *Polish Journal of Chemistry*, 74, 153–155.
- Stojakowska, A., & Malarz, J. (2000). Flavonoid production in transformed root cultures of *Scutellaria baicalensis*. *Journal of Plant Physiology*, 156, 121–125. [https://doi.org/10.1016/S0176-1617\(00\)80282-5](https://doi.org/10.1016/S0176-1617(00)80282-5)
- Stojakowska, A., & Malarz, J. (2017). Bioactive phenolics from in vitro cultures of *Lactuca aculeata* Boiss. et Kotschy. *Phytochemistry Letters*, 19, 7–11. <https://doi.org/10.1016/j.phytol.2016.11.003>
- Stojakowska, A., Malarz, J., & Kisiel, W. (2000). *Lactuca virosa* L. (bitter lettuce): In vitro culture and production of sesquiterpene lactones. In Y. P. S. Bajaj (Ed.), *Medicinal and aromatic plants XI* (pp. 261–273). Springer. [https://doi.org/10.1007/978-3-662-08614-8\\_15](https://doi.org/10.1007/978-3-662-08614-8_15)
- Stojakowska, A., Malarz, J., & Kisiel, W. (2001). Flavonoid aglycones from transformed root culture of *Scutellaria baicalensis*. *Polish Journal of Chemistry*, 75, 1935–1937.
- Stojakowska, A., Malarz, J., Szewczyk, A., & Kisiel, W. (2012). Caffeic acid derivatives from a hairy root culture of *Lactuca virosa*. *Acta Physiologiae Plantarum*, 34, 291–298. <https://doi.org/10.1007/s11738-011-0827-4>
- Syklowska-Baranek, K., Pietrosiuk, A., Kokoszka, A., & Furmanowa, M. (2009). Enhancement of taxane production in hairy root culture of *Taxus × media* var. Hicksii. *Journal of Plant Physiology*, 166, 1950–1954. <https://doi.org/10.1016/j.jplph.2009.05.001>
- Syklowska-Baranek, K., Grech-Baran, M., Naliwajski, M. R., Bonfill, M., & Pietrosiuk, A. (2015). Paclitaxel production and PAL activity in hairy root cultures of *Taxus × media* var. Hicksii carrying a taxadiene synthase transgene elicited with nitric oxide and methyl jasmonate. *Acta Physiologiae Planarum*, 37, Article 2018. <https://doi.org/10.1007/s11738-015-1949-x>
- Syklowska-Baranek, K., Pietrosiuk, A., Kuźma, Ł., Chinou, I., Kongel, M., & Jeziorek, M. (2008). Establishment of *Rindera graeca* transgenic root culture as a source of shikonin derivatives. *Planta Medica*, 74, Article PG54. <https://doi.org/10.1055/s-0028-1084806>
- Syklowska-Baranek, K., Pietrosiuk, A., Szyszko, E., Graikou, K., Jeziorek, M., Kuźma, Ł., & Chinou, I. (2012). Phenolic compounds from in vitro cultures of *Rindera graeca* Boiss. & Heldr. *Planta Medica*, 78, Article PI342. <https://doi.org/10.1055/s-0032-1321029>
- Syklowska-Baranek, K., Pilarek, M., Bonfill, M., Kafel, K., & Pietrosiuk, A. (2015). Perfluorodecalin-supported system enhances taxane production in hairy root cultures of *Taxus × media* var. Hicksii carrying a taxadiene synthase transgene. *Plant Cell and Tissue Organ Culture*, 120, 1051–1059. <https://doi.org/10.1007/s11240-014-0659-1>
- Syklowska-Baranek, K., Rymaszewski, W., Gaweł, M., Rokicki, P., Pilarek, M., Grech-Baran, M., Hennig, J., & Pietrosiuk, A. (2019). Comparison of elicitor-based effects on metabolic responses of *Taxus × media* hairy roots in perfluorodecalin-supported two-phase culture system. *Plant Cell Reports*, 38, 85–99. <https://doi.org/10.1007/s00299-018-2351-0>
- Szopa, A., Barnaś, M., & Ekiert, H. (2019). Phytochemical studies and biological activity of three Chinese *Schisandra* species (*Schisandra sphenanthera*, *Schisandra henryi* and *Schisandra rubriflora*): Current findings and future applications. *Phytochemistry Reviews*, 18, 109–128. <https://doi.org/10.1007/s11101-018-9582-0>
- Szopa, A., Dziurka, M., Warzecha, A., Kubica, P., Klimek-Szczykutowicz, M., & Ekiert, H. (2018). Targeted lignan profiling and anti-inflammatory properties of *Schisandra rubriflora* and *Schisandra chinensis* extracts. *Molecules*, 23, Article 3103. <https://doi.org/10.3390/molecules23123103>
- Szopa, A., & Ekiert, H. (2011). Lignans in *Schisandra chinensis* in vitro cultures. *Pharmazie*, 66, 633–634.
- Szopa, A., & Ekiert, H. (2014). *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) in vitro cultures. In J. Govil & P. Ananda Kumar (Eds.), *Biotechnology and genetic engineering II* (pp. 405–434). Studium Press.
- Szopa, A., Ekiert, R., & Ekiert, H. (2017). Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) as a medicinal plant species: A review on the bioactive components, pharmacological properties, analytical and biotechnological studies. *Phytochemistry Reviews*, 16, 195–218. <https://doi.org/10.1007/s11101-016-9470-4>
- Szopa, A., Klimek, M., & Ekiert, H. (2016). Chinese magnolia vine (*Schisandra chinensis*) – Therapeutic and cosmetic importance. *Polish Journal of Cosmetology*, 19, 274–284.
- Szopa, A., Klimek-Szczykutowicz, M., Kokotkiewicz, A., Maślanka, A., Król, A., Luczkiewicz, M., & Ekiert, H. (2018). Phytochemical and biotechnological studies on *Schisandra chinensis* cultivar Sadova No. 1 – A high utility medicinal plant. *Applied Microbiology and Biotechnology*, 102, 5105–5120. <https://doi.org/10.1007/s00253-018-8981-x>
- Szopa, A., Kokotkiewicz, A., Klimek-Szczykutowicz, M., & Luczkiewicz, M. (2019). Different types of in vitro cultures of *Schisandra chinensis* and its cultivar (*S. chinensis* cv. Sadova): A rich potential source of specific lignans and phenolic compounds.



- In K. Ramawat, H. Ekiert, & S. Goyal (Eds.), *Plant cell and tissue differentiation and secondary metabolites* (pp. 1–28). Springer.  
[https://doi.org/10.1007/978-3-030-30185-9\\_10](https://doi.org/10.1007/978-3-030-30185-9_10)
- Szopa, A., Kokotkiewicz, A., Luczkiewicz, M., & Ekiert, H. (2017). *Schisandra lignans* production regulated by different bioreactor type. *Journal of Biotechnology*, 247, 11–17.  
<https://doi.org/10.1016/j.jbiotec.2017.02.007>
- Szopa, A., Kokotkiewicz, A., Marzec-Wróblewska, U., Bucinski, A., Luczkiewicz, M., & Ekiert, H. (2016). Accumulation of dibenzocyclooctadiene lignans in agar cultures and in stationary and agitated liquid cultures of *Schisandra chinensis* (Turcz.) Baill. *Applied Microbiology and Biotechnology*, 100, 3965–3977.  
<https://doi.org/10.1007/s00253-015-7230-9>
- Szypuła, W., Pietrosiuk, A., Suchocki, P., Olszowska, O., Furmanowa, M., & Kazimierska, O. (2005). Somatic embryogenesis and in vitro culture of *Huperzia selago* shoots as a potential source of huperzine A. *Plant Science*, 168, 1443–1452.  
<https://doi.org/10.1016/j.plantsci.2004.12.021>
- Szypuła, W. J., Kiss, A. K., Pietrosiuk, A., Świst, M., Danikiewicz, W., & Olszowska, O. (2011). Determination of huperzine A in *Huperzia selago* plants from wild population and obtained in in vitro culture by high performance liquid chromatography using a chaotropic mobile phase. *Acta Chromatographica*, 23(2), 339–352.  
<https://doi.org/10.1556/AChrom.23.2011.2.11>
- Szypuła, W. J., Mistrzak, P., & Olszowska, O. (2013). A new and fast method to obtain in vitro cultures of *Huperzia selago* (Huperziaceae) sporophytes, a club moss which is a source of Huperzine A. *Acta Societatis Botanicorum Poloniae*, 82, 313–320.  
<https://doi.org/10.5586/asbp.2013.034>
- Szypuła, W. J., & Pietrosiuk, A. (2021). Production of cholinesterase-inhibiting compounds in in vitro cultures of club mosses. In K. G. Ramawat, H. M. Ekiert, & S. Goyal (Eds.), *Plant cell and tissue differentiation and secondary metabolites* (pp. 921–960). Springer.  
[https://doi.org/10.1007/978-3-030-30185-9\\_30](https://doi.org/10.1007/978-3-030-30185-9_30)
- Szypuła, W. J., Wileńska, B., Misicka, A., & Pietrosiuk, A. (2020). Huperzine A and huperzine B production by prothallus cultures of *Huperzia selago* (L.) Bernh. ex Schrank et Mart. *Molecules*, 25, Article 3262. <https://doi.org/10.3390/molecules25143262u>
- Tamaki, H., Robinson, R. W., Anderson, J. L., & Stoewsand, G. S. (1995). Sesquiterpene lactones in virus-resistant lettuce. *Journal of Agricultural and Food Chemistry*, 43, 6–8.  
<https://doi.org/10.1021/jf00049a002>
- Thiem, B., Kikowska, M., Krawczyk, A., Więckowska, B., & Sliwińska, E. (2013). Phenolic acid and DNA contents of micropropagated *Eryngium planum* L. *Plant Cell and Tissue Organ Culture*, 114(2), 197–206. <https://doi.org/10.1007/s11240-013-0315-1>
- Thorpe, T. A. (2007). History of plant tissue culture. *Molecular Biotechnology*, 37(2), 169–180.  
<https://doi.org/10.1007/s12033-007-0031-3>
- Toton, E., Kedziora, I., Romaniuk-Drapala, A., Konieczna, N., Kaczmarek, M., Lisiak, N., Paszel-Jaworska, A., Rybska, A., Duszyńska, W., Budzianowski, J., Rybczyńska, M., & Rubis, B. (2020). Effect of 3-O-acetylaeuritic acid from in vitro-cultured *Drosera spatulata* on cancer cells survival and migration. *Pharmacological Reports*, 72(1), 166–178. <https://doi.org/10.1007/s43440-019-00008-x>
- Toton, E., Lisiak, N., Rubis, B., Budzianowski, J., Gruber, P., Hofmann, J., & Rybczyńska, M. (2012). The tetramethoxyflavone zapotin selectively activates protein kinase C epsilon, leading to its down-modulation accompanied by Bcl-2, c-Jun and c-Fos decrease. *European Journal of Pharmacology*, 682, 21–28.  
<https://doi.org/10.1016/j.ejphar.2012.02.020>
- Toton, E., Romaniuk, A., Budzianowski, J., Hofmann, J., & Rybczyńska, M. (2016). Zapotin (5,6,2',6'-tetramethoxyflavone) modulates the crosstalk between autophagy and apoptosis pathways in cancer cells with overexpressed constitutively active PKC. *Nutrition and Cancer*, 68(2), 290–304. <https://doi.org/10.1080/01635581.2016.1134595>
- Walenccka, E., Rozalska, S., Wysokinska, H., Rozalski, M., Kuzma, Ł., & Rozalska, B. (2007). Salvipisone and aethiopinone from *Salvia sclarea* hairy roots modulate staphylococcal antibiotic resistance and express anti-biofilm activity. *Planta Medica*, 73, 545–551.  
<https://doi.org/10.1055/s-2007-967179>
- Wang, Y., Zhou, Y., Jia, G., Han, B., Liu, J., Teng, Y., Lv, J., Song, Z., Li, Y., Ji, L., Pan, S., Jiang, H., & Sun, B. (2014). Shikonin suppresses tumor growth and synergizes with gemcitabine in a pancreatic cancer xenograft model: Involvement of NF-κB signaling pathway. *Biochemical Pharmacology*, 88(3), 322–333. <https://doi.org/10.1016/j.bcp.2014.01.041>
- Wesołowska, A., Nikiforuk, A., Michalska, K., Kisiel, W., & Chojnacka-Wójcik, E. (2006). Analgesic and sedative activities of lactucin and some lactucin-like guaianolides in mice. *Journal of Ethnopharmacology*, 107, 254–258.  
<https://doi.org/10.1016/j.jep.2006.03.003>

- Whittier, D. P., & Storchova, H. (2007). The gametophyte of *Huperzia selago* in culture. *American Fern Journal*, 97(3), 149–154. <https://doi.org/crphdd>
- Wilczańska-Barska, A., Krauze-Baranowska, M., Majdan, M., & Głód, D. (2011). Wild type root cultures of *Scutellaria barbata*. *BioTechnologia*, 92, 369–377. <https://doi.org/10.5114/bta.2011.46554>
- Wilczańska-Barska, A., Królicka, A., Głód, D., Majdan, M., Kawiak, A., & Krauze-Baranowska, M. (2012). Enhanced accumulation of secondary metabolites in hairy root cultures of *Scutellaria lateriflora* following elicitation. *Biotechnology Letters*, 34, 1757–1763. <https://doi.org/10.1007/s10529-012-0963-y>
- Wolf, P. (2016). Psoralen-ultraviolet A endures as one of the most powerful treatments in dermatology: Reinforcement of this “triple-product therapy” by the 2016 British guidelines. *British Journal of Dermatology*, 174, 11–14. <https://doi.org/10.1111/bjd.14341>
- Woo, K. W., Suh, W. S., Subedi, L., Kim, S. Y., Kim, A., & Lee, K. R. (2016). Bioactive lignan derivatives from the stems of *Firmiana simplex*. *Bioorganic and Medicinal Chemistry Letters*, 26, 730–733. <https://doi.org/10.1016/j.bmcl.2016.01.008>
- World Health Organization. (2007). *WHO monographs on selected medicinal plants* (Vol. 3). World Health Organization. <https://apps.who.int/iris/handle/10665/42052>
- Yared, J. A., & Tkaczuk, K. H. R. (2012). Update on taxane development: New analogs and new formulations. *Drug Design, Development and Therapy*, 6, 371–384. <https://doi.org/10.2147/DDDT.S28997>
- Zálešák, F., Bon, D. J.-Y. D., & Pospíšil, J. (2019). Lignans and noelignans: Plant secondary metabolites as a reservoir of biologically active substances. *Pharmacological Research*, 146, Article 104284. <https://doi.org/10.1016/j.phrs.2019.104284>
- Zhao, Q., Kretschmer, N., Bauer, R., & Efferth, T. (2015). Shikonin and its derivatives inhibit the epidermal growth factor receptor signaling and synergistically kill glioblastoma cells in combination with erlotinib. *International Journal of Cancer*, 137(6), 1446–1456. <https://doi.org/10.1002/ijc.29483>
- Zhu, J.-Y., Cheng, B., Zheng, Y.-J., Dong, Z., Lin, S.-L., Tang, G.-H., Gu, Q., & Yin, S. (2015). Enantiomeric neolignans and sesqueneolignans from *Jatropha integerrima* and their absolute configurations. *RSC Advances*, 5, 12202–12208. <https://doi.org/10.1039/c4ra15966g>
- Zielinska, S., Wójciak-Kosior, M. J., Płachno, B., Sowa, I., Włodarczyk, M., & Matkowski, A. (2018). Quaternary alkaloids in *Chelidonium majus* in vitro cultures. *Industrial Crops and Products*, 123, 17–24. <https://doi.org/10.1016/j.indcrop.2018.06.062>
- Zielińska, S., Dąbrowska, M., Kozłowska, W., Kalembe, D., Abel, R., Dryś, A., Szumny, A., & Matkowski, A. (2017). Ontogenetic and trans-generational variation of essential oil composition in *Agastache rugosa*. *Industrial Crops and Products*, 97, 612–619. <https://doi.org/10.1016/j.indcrop.2017.01.009>
- Zielińska, S., Dryś, A., Piątczak, E., Kolniak-Ostek, J., Podgórska, M., Oszmiański, J., & Matkowski, A. (2019). Effect of LED illumination and amino acid supplementation on phenolic compounds profile in *Agastache rugosa* in vitro cultures. *Phytochemistry Letters*, 31, 12–19. <https://doi.org/10.1016/j.phytol.2019.02.029>
- Zielińska, S., Piątczak, E., Kalembe, D., & Matkowski, A. (2011). Influence of plant growth regulators on volatiles produced by in vitro grown shoots of *Agastache rugosa* (Fisher & C. A. Meyer) O. Kuntze. *Plant Cell Tissue and Organ Culture*, 107, 161–169. <https://doi.org/10.1007/s11240-011-9954-2>
- Zielińska, S., Piątczak, E., Kozłowska, W., Bohater, A., Jezierska-Domaradzka, A., Kolniak-Ostek, J., & Matkowski, A. (2020). LED illumination and plant growth regulators' effects on growth and phenolic acids accumulation in *Moluccella laevis* L. in vitro cultures. *Acta Physiologia Plantarum*, 42, Article 72. <https://doi.org/10.1007/s11738-020-03060-w>