Provided for non-commercial research and educational use only.
Not for reproduction or distribution or commercial use.

This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

http://www.elsevier.com/locate/permissionusematerial

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Enzyme and Microbial Technology 39 (2006) 1386–1391
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Induction of secondary metabolite production in transformed callus of *Ammi majus* L. grown after electromagnetic treatment of the culture medium

Aleksandra Królicka [a,*], Rafał Kartanowicz [b], Stanisław Adrian Wosiński [c], Anna Szpitter [a], Marian Kamiński [b], Ewa Łojkowska [a]

[a] *Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Poland*
[b] *Gdańsk University of Technology, Chemical Faculty, Analytical Chemistry Department, Poland*
[c] *Poznań University of Technology, Polymer Processing Division, Institute of Materials Technology, Poland*

Received 14 December 2005; received in revised form 7 March 2006; accepted 14 March 2006

Abstract

High-performance liquid chromatography analysis of crude chloroform and methanol extracts as well as methanol preparations subjected to enzymatic hydrolysis indicated two-fold higher accumulation of umbelliferone in transformed *Ammi majus* L. callus grown on medium exposed to ADR-4® (energy stimulator plate) in comparison to the control cultures. Elicitation with ADR-4® induced also two times higher accumulation of bergapten. Three different techniques: Soxhlet extraction, ultrasonication and accelerated solvent extraction (ASE) applied for the extraction of coumarins, furanocoumarins and corresponding glycosides from transformed callus of *A. majus* elicited by ADR-4® plates were compared. ASE is shown to be the most efficient extraction method.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Accelerated solvent extraction (ASE); ADR-4® plates; *Ammi majus*; Bergapten; Magnetic field; Umbelliferone

Author's personal copy

1. Introduction

Ammi majus L. (*Apiaceae*) is one of the natural sources of linear coumarins and furanocoumarins [1]. Due to the photosensitizing activity of these compounds, the fruit of *A. majus* has been used in the Mediterranean region in the treatment of leucoderma, psoriasis and vitiligo [2]. The majority of the photosensibilising remedies used now in dermatology [e.g. Meladinine® (Promedica, France), Psoraderm 5 (Expanscience Laboratories, France), Oxsoralen® (Gerot Pharmazeutica, Austria) and Geralen® (Gerot Pharmaceutica, Austria)] are produced on the basis of natural sources of *A. majus*. Great interest in the therapeutic use of coumarins has led to a series of studies dealing with their production in in vitro cultures of different Apiaceae plants [1,2].

Until now, a wide variety of elicitors have been employed in order to enhance the production of coumarins and furanocoumarins in in vitro cultures of plants from Apiaceae family [1,3–7]. Preliminary work in which ADR-4® plates modifying the intermolecular structure of water molecules were used as an elicitor indicate induction of coumarins and furanocoumarins production in *A. majus* culture [8]. ADR-4® is a special type of magnetizer, which consists of magnets generating specific gradients of magnetic field and a dielectric ceramics [http://www.adr.com.pl/].

Several methods for determination of coumarins and furanocoumarins in plant extracts have been reported previously. The most common techniques base on normal and reverse phase high performance liquid chromatography [9–12], capillary electrochromatography [13,14], capillary electrophoresis [15], gas chromatography combined with mass spectrometry [16] and one or two dimensional thin layer chromatography [11,17].

The goal of the presented research was to study elicitation of pharmacologically important secondary metabolites in transformed *A. majus* callus cultures grown on medium exposed

* Corresponding author at: Department of Biotechnology, University of Gdańsk, 24 Kładki Street, 80-822 Gdańsk, Poland. Tel.: +48 58 3202248; fax: +48 58 3012807.
E-mail address: krolicka@biotech.univ.gda.pl (A. Królicka).

0141-0229/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2006.03.042

to ADR-4® plates. The additional objective was to compare the effectiveness of three methods of coumarins and furanocoumarins extraction from dried *A. majus* callus culture.

2. Materials and methods

2.1. Chemicals

Standards of umbelliferone, bergapten, psolaren and xanthotoxin were purchased from Carl Roth (Karlsruhe, Germany). One mg of each compound was dissolved in methanol (5 ml).

Petrol ether and chloroform (POCh, Gliwice, Poland) were of analytical-reagent grade. All other reagents [methanol (MeOH), ethanol (ETOH), tetrahydrofuran (THF)] were of chromatographic grade (Merck, Darmstadt, Germany). Bidistilled or deionised water provided by Milli-Q water purification system was used (Millipore, Warsaw, Poland).

2.2. In vitro cultures of A. majus

Callus culture was induced from 4-week-old hairy root culture obtained after transformation of *A. majus* plantlets by *Agrobacterium rhizogenes* strain A4 [16]. The efficient growth of transformed callus was obtained on Murashige and Skoog medium (MS) [18] containing 2.5 mg/l α-naphthaleneacetic acid (NAA), 1.0 mg/l 6-benzylaminopurine (BAP), 3% sucrose and solidified with 0.75% agar, pH 5.8 (adjusted prior to autoclaving). The callus cultures were grown at a temperature of 20–22 °C under white fluorescent light with a 16 h photoperiod (White cool fluorescent light, Philips, TLD 58W/84o, 20 μmol m^{-2} s^{-1}) for 4 weeks.

2.3. Induction of secondary metabolites production using ADR-4® plates

Immediately after autoclaving culture vessels containing still liquid MS media were exposed to the influence of ADR-4® plates [19] for 15 min by being whirled round over the magnetizer. Control cultures were grown on MS medium not treated with ADR-4® after autoclaving.

Both control callus as well as callus growing on MS medium exposed to ADR-4® plates were grown in phytotron at a temperature of 20–22 °C under white fluorescent light with a 16 h photoperiod (20 μmol m^{-2} s^{-1}) for 4 weeks. Then callus was collected, dried at 50 °C and extractions of the secondary metabolites were performed.

2.4. Analytical methods

2.4.1. Extraction techniques

2.4.1.1. Soxhlet extraction. Three grams of dried and powdered in a mortar callus samples were placed in a 500 ml Soxhlet glass thimble. The extraction was carried out using in turn petroleum ether (at the solvent boiling point 50–60 °C, for 10 h), chloroform (at the solvent boiling point 65–75 °C, for 12–16 h) and methanol (at the solvent boiling point 80–90 °C, for 12–18 h). Extracts obtained with each solvent were collected separately. Chloroform extracts contained free forms of coumarins and furanocoumarins but in methanol preparations glycosidic forms of these compounds were present. Crude chloroform and methanol extracts were evaporated in 50 °C and the residue was dissolved in 5 ml MeOH.

2.4.1.2. Ultrasonication extraction. The ultrasonication extraction was carried out on a Misonix incorporated XL-2020 Sonificator® Ultrasonic Liquid Processor (frequency 20 kHz, power output 150 W). The temperature inside the extraction solutions was kept below 5 °C by the use of an outer ice jacket. In pulsed mode, ultrasonic vibrations are being transmitted to the solution at a rate of 10 pulses for 5 s followed by a 5 s pause, for 30 min. Extraction of a 500 mg sample (dried and powdered in a mortar) was carried out either with EtOH or MeOH (total volume of 50 ml). After sonication ethanol and methanol extracts were centrifuged at 13,000 rpm (13,793 × *g*) and supernatant was evaporated at 50 °C and redissolved to obtain an equivalent of 3 g DW (dry weight) diluted in 5 ml MeOH.

2.4.1.3. Accelerated solvent extraction (ASE). ASE also known as pressurized solvent extraction was carried out using an ASE 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA, USA). One gram of dried callus was mixed with approximately 12 g of quartz sand and transferred to the 11 ml stainless steel extraction cell. Cellulose filters were placed at the outlet of the extraction cell to prevent blocking up of the frit. Extraction was performed using chloroform followed by methanol. Extraction conditions were as follows: an extraction cell filled with solvent was heated-up to 100 °C for 5 min, the static extraction was performed for a period of 5 min under the pressure of 7 MPa; then 33% of the extraction cell volume was transferred to the collective vial. The static cycle was repeated three times. Finally, the extract was purged from the cell using nitrogen (180 s, 1 MPa). Chloroform and methanol extracts of 22 ml volume each were collected in separate vials. Chloroform and methanol extracts were evaporated and redissolved to obtain an equivalent of 3 g DW in 5 ml MeOH.

2.4.2. Enzymatic hydrolysis of coumarin and furanocoumarin glycosides

In order to obtain free forms of coumarins and furanocoumarins in methanol extract after Soxlet and ASE extraction 150 μl of methanol preparation extracted concentrated earlier to 5 ml was exposed to enzymatic hydrolysis by β-glucosidase (Fluka) according to the procedure described by Nguyen et al. [20]. Reaction mixture contained 150 μl of methanol extract and 0.5 mg of β-glucosidase dissolved in 150 μl of 0.1 M acetate buffer (pH 5). Reaction mixture was incubated at 37 °C for 24 h. Hydrolysis was stopped by adding 96% ethanol (1:1 v/v). Then, samples were evaporated at 50 °C and dissolved in 150 μl methanol (equivalent of 3 g DW per 5 ml).

2.4.3. Determination of coumarins and furanocoumarins—HPLC

A LaChrom (Merck–Hitachi, Darmstadt, Germany) chromatographic system, consisting of a L-7100 quaternary low pressure gradient pump, a L-7350 column thermostat equipped with a L-7350 cooling module, a L-7450A UV–vis DAD detector, and a RH-7725i injection valve with a sampling loop volume of 20 μl was used. A Lichrospher® RP-18e 5 μm (250 mm × 4.0 mm i.d.) (Merck, Darmstadt, Germany) column was employed.

All HPLC analyses were carried out at constant column temperature of 30 °C. The mobile phase flow rate was 1.5 ml/min. Separation and determination of the analyzed compounds was performed according to the previously published procedure [9].

3. Results and discussion

3.1. Comparison of methods for the extraction of coumarins and furanocoumarins from A. majus callus

In order to compare the efficiency of different extraction techniques the same batch of *A. majus* callus culture was used. In this study a two-stage extraction procedure when using ASE and Soxhlet apparatus was applied. This involved chloroform extraction in order to obtain free forms of coumarins and furanocoumarins followed by methanol extraction of their glycosides. Furanocoumarins are reported to be stored as glycosides within plant tissue [21]. Each extraction technique was optimised to achieve the highest yield (not published).

Because it was not possible to carry out chloroform extraction in sonificator due to rapid solvent evaporation, either MeOH or EtOH was used. This method yielded relatively small amounts of secondary metabolites from *A. majus* callus culture. In extracts obtained as a result of sonication only 50% of bergapten extracted by Soxhlet and only 25% of this compound gained with the use of ASE was determined. In addition, after application of sonication we were not able to detect umbelliferone, which in turn was present in extracts obtained using Soxhlet and ASE. Based on these findings, the later work was focused

Author's personal copy

Table 1
Comparison of the efficiency of coumarin and furanocoumarin extraction from transformed callus of *A. majus*

Metabolites	Coumarin and furanocoumarin content in transformed callus (μg/g DW)			
	Soxhlet extraction		ASE extraction	
	A	A + B	A	A + B
Umbelliferone	n.d.	0.5 ± 0.1	10.0 ± 0.5	25.0 ± 1.1
Bergapten	13.3 ± 0.7	23.3 ± 1.0	38.4 ± 1.2	51.7 ± 1.8

n.d.: not detected. (A) content of free umbelliferone or bergapten in chloroform extract; (B) content of umbelliferone or bergapten in hydrolysed MeOH extract. Experiment was performed in three replicates.

on comparison of coumarin and furanocoumarin recovery using Soxhlet extraction and ASE.

The highest yield of extraction was obtained with ASE. The total amount of secondary metabolites (free forms and glycosides) determined in ASE extracts is 2 times higher for bergapten and 50 times higher for umbelliferone than in respective Soxhlet extracts (Table 1). The explanation for this phenomenon could be the fact that umbelliferone is a more hydrophilic compound than bergapten (due to the presence of a hydroxy group) and it is partially insoluble in hydrophobic solvents used in Soxhlet extraction procedure (petroleum ether and chloroform). At the same time bergapten is more hydrophobic (carrying a methoxyl residue) and more soluble in these solvents under the same conditions. Having applied ASE we took an advantage of more efficient extraction conditions (temperature above solvent boiling point and elevated pressure) than those in Soxhlet extraction, which allowed us to obtain higher amounts of umbelliferone and bergapten regardless of the differences in their hydrophobicity. Also shorter extraction time decreases probability of degradation of these light-sensitive compounds. Waksmundzka-Hajnos et al. [22] compared different extraction methods applied for furanocoumarins extraction for *Pastinaca sativa* fruits and also found ASE to be the most efficient for bergapten isolation. The optimisation of ASE allowed the efficient extraction of analytes from *A. majus* callus and therefore this technique was used in further experiments. It was verified experimentally that the increase in number of static extraction cycles as well as application of higher extraction temperature (over 100 °C) and pressure elevation (above 7 MPa) did not improve the recovery of analyzed compounds (results not shown). ASE can be recommend as a useful and fully automated technique for isolation of analytes from plant tissues. This method enables better solvent penetration throughout the plant material. Another advantage of ASE in phytochemistry is better solubility of analytes in higher temperature as well as greater selectivity of the process carried out by successive extractions using solvents of different physico-chemical properties [23,24]. Moreover, the repeatability of this extraction procedure is very high—the relative standard deviation of the results obtained for four independent experiment was less than 10% for the entire analytical procedure.

3.2. *Effect of ADR-4® elicitation on growth of transformed callus*

Growth rates of control and elicited callus were significantly different. Transformed callus exhibited 20% higher increase in dry weight (DW) and fresh weight (FW) (Table 2) when grown on medium exposed to ADR-4® than when cultured on control medium (not treated with ADR-4®). The influence of magnetic field on growth of plant in vitro cultures was earlier shown by Pittman [25] and Germanà et al. [26]. In this case stimulation of callus growth from *Citrus clementina* anthers and pollen was observed after exposure to magnetic field at specific intensities. Muraji et al. [27] proved that alternating magnetic field at 10 Hz increased growth rate of primary roots in *Zea mays* seedlings. Also studies concerning effects of alternating magnetic fields on growth of *Raphanus sativus* were conducted [28,29]. Yaycili and Alikamanoglu [30] showed the positive effect of magnetic field on plant fresh weight, length, number of leaves and chlorophyll content in node explants of *Paulownia tomentosa* and *P. fortunei*.

3.3. *Effect of ADR-4® elicitation on coumarin and furanocoumarin accumulation*

A significant influence of ADR-4® elicitation on coumarin and furanocoumarin accumulation in transformed callus of *A.*

Table 2
Comparison of the effect of ADR-4® plate elicitation on coumarin and furanocoumarin accumulation in *A. majus* transformed callus after ASE extraction

Type of culture	Dry mass[a] (g)	Fresh weight[b] (g)	Umbelliferone[c] (μg/g DW)	Productivity of umbelliferone (μg/g DW/30 days)	Bergapten[c] (μg/g DW)	Productivity of bergapten (μg/g DW/30 days)
Transformed callus	1.83 ± 0.05	12.9 ± 1.1	25.0 ± 1.1	45.75 ± 1.5	51.7 ± 1.8	93.7 ± 2.7
Transformed callus + ADR-4®	2.2 ± 0.03	15.2 ± 1.9	45.9 ± 1.5	101 ± 3.0	91.7 ± 2.3	201.7 ± 5.9

[a] Dry mass (DM) of transformed callus from five culture vessels grown for 30 days on MS medium supplemented with 2.5 mg/l NAA, 1.0 mg/l BA, 3% sucrose and 0.75% agar; experiment was performed in three replicates.

[b] Fresh weight (FW) of transformed callus from five culture vessels grown for 30 days on MS medium supplemented with 2.5 mg/l NAA, 1.0 mg/l BA, 3% sucrose and 0.75% agar; experiment was performed in three replicates.

[c] Content of free umbelliferone or bergapten in chloroform extract and content of umbelliferone or bergapten in hydrolysed MeOH extract.

Author's personal copy

majus was observed (Table 2). Among different *A. majus* culture types examined (elicited as well as non-elicited callus and hairy root cultures) [7,16], transformed callus grown on ADR-4®-exposed MS medium exhibited the highest umbelliferone content. Application of elicitors such as: BION® or autoclaved lysate of cell suspension of bacteria—*Enterobacter sakazaki* in hairy root cultures of *A. majus* in previous work allowed to obtain a maximal yield of this compound of 23 μg/g DW [7]. The use of ADR-4® for stimulation of secondary metabolites production resulted in an increase of umbelliferone production up to 45.9 μg/g DW in transformed callus (Table 2). The productivity of this compound rose as well and amounted to about 101 μg/30 days.

A significant influence of ADR-4® plate treatment on bergapten accumulation in callus was also noted (Table 2). Bergapten—a very important furanocoumarin is common in in vitro cultures of plants from *Rutaceae* family but it is present at relatively low levels [2,31]. From the point of view of application in dermathology this compound showed higher tolerability, with less side effects than xanthotoxin [32]. It was shown that callus and hairy root cultures of *A. majus* obtained after transformation with *A. rhizogenes* strain A4 did not contain bergapten [16]. Elicitation of the production of this compound was noted after application of autoclaved lysate of cell suspension of *E. sakazaki* as elicitor. However, the level of this furanocoumarin reached only 3 μg/g DW [7]. In transformed callus the level of bergapten was 17 fold higher, about 51.7 μg/g DW (Table 2). In addition, the use of ADR-4® plates stimulated accumulation of this furanocoumarin in transformed callus and increased its productivity about two times in comparison with callus grown on medium not treated with ADR-4®.

Numerous effects of magnetic as well as electromagnetic fields on growth and development of plants have been reported so far, including increased regeneration capability [30], delayed senescence, alterations in antioxidant enzyme levels [33] and stimulation of phosphoinositide breakdown [34]. Our results indicate that medium exposed to ADR-4®—a device which changes parameters of the surrounding electromagnetic field, can also significantly influence secondary metabolite production in callus of *A. majus*.

Medium modification by ADR-4® consists in action of specially formed magnetic field with ceramic dielectric on which these magnetic elements are fixed. Ceramic dielectric shows a strong, characteristic, low-frequency absorption of electric component of electromagnetic wave in the frequency range from 10 Hz to 10 kHz at a room temperature. The lower the frequency the stronger the absorption of this material. As a result, a considerable decrease in the electric field strength is visible when the ADR-4® plate is inserted between the alternating current (ac) field source and the object studied, i.e. the medium. Fig. 1 shows an example of electric field distribution without ADR-4® (A) and with the plate inserted between the field source and the measuring probe of an ESM-100 Maschek fieldmeter (B). The measurements were performed at the ac frequency of 10 and 100 Hz.

However, the exact interaction of ADR-4® plate with the medium as well as the mode of action of so-formed modifications

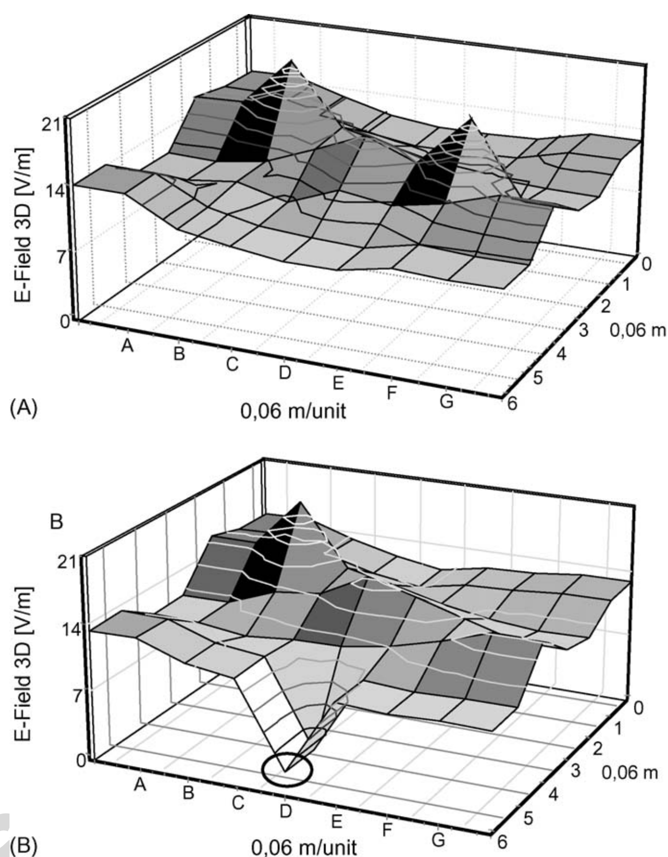

Fig. 1. Intensity of electric field in the area where MS medium for *A. majus* was prepared; the area without the presence of ADR-4® (A) and with ADR-4® placed in the C6 region (B).

of the latter on callus culture of *A. majus* are not known. We suppose that the elicitation effect might be due to ability of the device to change properties of water, which mediates some of the interactions between enzymes and substrates [35]. Water is an integral component of all living organisms. It does not only simply serve as an inert diluent but also takes part in biochemical reactions on the molecular level. Water molecules form clusters which compose a hydrogen-bounded network. It appears that water clustering can be affected by electric and magnetic fields which act by changing hydrogen bond strength. As a result, physical properties of water are altered [36,37].

It has also been proven that water treated either with magnetic or electromagnetic fields i.a. enhances efflux of calcium through biomembranes, changes colloid stability and influences the structure of model lysosomes. Many of the proposed hypotheses on influence of electromagnetic fields as well as magnetically conditioned water on organisms consider interaction with the cell membrane as the putative mechanism of changing cell signal transduction pathways [33,38]. As the observed properties of water persist minutes or hours after conditioning (probably due to the perturbation of the gas/liquid interface), the effect has been termed ‘magnetic memory of water’ [38]. In our experiments agar solidified medium exposed to ADR-4® retained its properties for weeks. It is widely accepted that in the gel state agar molecules form a three-dimensional network comprising of double helices and containing liquid water [39]. Such structur-

ing results in the reduction of water diffusion and stabilization of its presence, which could be the reason for a prolonged 'conditioned state' of the medium. Although the exact mechanism for 'storage of information' in water is not known our results suggest that agar solidification of ADR-4®-treated medium 'preserves' the effect exerted on water.

4. Conclusions

The economic importance of furanocoumarins, chemical synthesis of which is very expensive, has stimulated an interest in establishing conditions for production of these commonly in in vitro plant cultures. The present work indicates that biosynthesis of bergapten and umbelliferone in *A. majus* tissue can be induced by ADR-4® plates. These results allow to presume that the ADR-4® induces natural defence mechanisms in organisms towards a better balanced state. Most probably ADR-4® through its influence on water in callus culture of *A. majus* stimulates the plant's defence system that results in an increase of coumarin and furanocoumarin production. Moreover, it was shown that medium conditioning affects primary metabolism of *A. majus* callus by improving culture growth. In addition the presented results indicate that ASE is the most efficient method for extraction of coumarins and furanocoumarins.

Acknowledgement

This work was supported by Polish Committee for Scientific Research grant no. PBZ KBN 092/P05/2003.

References

[1] Ekiert H. Medical plant biotechnology: the *Apiaceae* family as the example of rapid development. Pharmazie 2001;55:561–7.
[2] Hamerski D, Beier RC, Kneusel RE, Matern U, Himmelspach K. Accumulation of coumarins in elicitor-treated cell suspension cultures of *Ammi majus*. Phytochemistry 1990;4:1137–42.
[3] Miksch M, Boland W. Airborne methyl jasmonate stimulates the biosynthesis of furanocoumarins in the leaves of celery plants (*Apium graveolens*). Experientia 1996;52:739–43.
[4] Fawe A, Abou-Zaid M, Menzies JG, Belanger RR. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Phytopathology 1998;88:396–401.
[5] Katz VA, Thulke OU, Conrath U. A benzothiadiazole primes parsley cells for augmented elicitation of defence responses. Plant Physiol 1998;117:1333–9.
[6] Królicka A, Staniszewska I, Krauze-Baranowska M, Maliński E, Szafranek J, Turkiewicz M, et al. Identification of secondary metabolites in in vitro culture of *Ammi majus* treated with elicitors. Acta Hort 2001;560:255–8.
[7] Staniszewska I, Królicka A, Maliński E, Łojkowska E, Szafranek J. Elicitation of secondary metabolites in in vitro cultures of *Ammi majus* L. Enzyme Microb Technol 2003;33(5):565–8.
[8] Królicka A, Kamiński M, Wosiński SA, Łojkowska E. ADR-4® energy stimulator—new elicitor inducing secondary metabolites in in vitro culture of *Ammi majus*. Proceedings of the 50th Annual Congress of the Society for Medicinal Plant Research. Revista de Fitoterapia 2002;2(S1):332.
[9] Kamiński M, Kartanowicz R, Kamiński MM, Królicka A, Sidwa-Gorycka M, Łojkowska E, et al. HPLC-DAD in identification and quantification of selected coumarins in crude extracts from plant cultures of *Ammi majus* and *Ruta graveolens*. J Sep Sci 2003;26(14):1287–91.
[10] Nguyen C, Bourgaud F, Forlot P, Guckert A. Establishment of hairy root cultures of *Psoralea* species. Plant Cell Rep 1992;11:424–7.
[11] Hawryl MA, Soczewiński E, Dzido TH. Separation of coumarins from Archangelica officinalis in high-performance liquid chromatography and thin-layer chromatography systems. J Chromatogr A 2000;886: 75–81.
[12] Cappiello A, Famiglini G, Mongani F, Tirillini B. Analysis of coumarins by micro high-performance liquid chromatography–mass spectrometry with a particle beam interface. J Am Soc Mass Spec 1995;2: 132–9.
[13] Ganzera M, Sturm S, Stuppner H. HPLC-MS and MECC analysis of coumarins. Chromatographia 1997;46:197–203.
[14] Chen CT, Sheu SJ. Separation of coumarins by miceller electrokinetic capilary chromatography. J Chromatogr A 1995;710:323–9.
[15] Ochocka RJ, Rajzer D, Kowalski P, Lamparczyk H. Determination of coumarins from *Chrysanthemum segetum* L. by capillary electrophoresis. J Chromatogr A 1995;709:197–202.
[16] Królicka A, Staniszewska I, Bielawski K, Maliński E, Szafranek J, Łojkowska E. Establishment of hairy root cultures of *Ammi majus*. Plant Sci 2001;160:259–64.
[17] Bogucka-Kocka A. The analysis of furanocoumarins in fruits of *Heracleum sibiricum* L. Acta Pol Pharma 1999;56:399–401.
[18] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 1962;15:473–97.
[19] Wosiński SA, Sakowsky J. Liquid treatment device, ceramic material with liquid treatment properties, liquid treatment method, yield of reaction and/or chemical processes and/or enzymatic processes change method and course of biological reaction change method. Patent NR EP06461003.3 (2005).
[20] Nguyen C, Bouque V, Bourgaud F, Guckert A. Quantification of daidzein and furanocoumarin conjugates of *Psoralea cinerea* L (Leguminosae). Phytochem Anal 1997;8:27–31.
[21] Innocenti G, Cappelletti EM, Caporale G. Morphological and chemical characteristics of some Australian *Psoralea* species. Int J Drug Res 1984;22:97–109.
[22] Waksmundzka-Hajnos M, Petruczynik A, Dragan A, Wianowska D, Dawidowicz AL, Sowa I. Influence of the extraction mode on the yield of some furanocoumarins from *Pastinaca sativa* fruits. J Chromatogr B 2004;800:181–7.
[23] Ong ES, Woo SO, Yong YL. Pressurized liquid extraction of berberine and aristolochic acids in medicinal plants. J Chromatogr A 2000;313:57–64.
[24] Benthin B, Danz H, Hamburger M. Pressurized liquid extraction of medicinal plants. J Chromatogr A 1999;837:211–9.
[25] Pittman UJ. Magnetism and plant growth. Can J Plant Sci 1963;43:513–8.
[26] Germanà MA, Chiancone B, Melati MR, Firetto A. Preliminary results on the effect of magnetic fields on anther culture and pollen germination of *Citrus clementina* hort. Ex tan. Acta Hortic 2003;625:411–8.
[27] Muraji M, Asai T, Tatebe W. Primary root growth rate of *Zea mays* seedlings grown in an alternating magnetic field of different frequencies. Bioelectrochem Bioenergy 1998;44:271–3.
[28] Potts MD, Parkinson WC, Noodén LD. *Raphanus sativus* and electromagnetic fields. Bioelectrochem Bioenergy 1997;44:131–40.
[29] Smith SD, Mcleod BR, Liboff AR. Effects of CR-tuned 60 Hz magnetic fields on sprouting and early growth of *Raphanus sativus*. Bioelectrochem Bioenergy 1993;32:67–76.
[30] Yaycili O, Alikamanoglu S. The effect of magnetic field on *Paulownia* tissue culture. Plant Cell Tissue Org Cult 2005;83:109–14.
[31] Ekiert H, Gomółka E. Coumarin compounds in *Ammi majus* L. callus cultures. Pharmazie 2000;55:684–7.
[32] Berg M, Ros AM. Treatment of psoriasis with psolarens and UVA. A double-blind comparison of 8-MOP and 5-MOP. Life Sci 1993;53:355–8.
[33] Piacentini MP, Fraternale D, Piatti E, Ricci D, Vetrano F, Dacha M, et al. Senescence delay and change of antioxidant enzyme levels in *Cucumis sativus* L. etiolated seedlings by ELF magnetic fields. Plant Sci 2001; 161:45–53.
[34] Piacentini MP, Piatti E, Fraternale D, Ricci D, Albertini MC, Accorsi A. Phospholipase C-dependent phosphoinositide breakdown induced by ELF-EMF in *Peganum harmala* calli. Biochimie 2004;86:343–9.
[35] Wojtkowiak D, Górski S, Wosiński S. Das wasser entdecken. Naturheilpraxis 2002;9:1307–10.

Authors personal copy

[36] Inaba H, Saitou T, Tozaki K, Hayashi H. Effect of the magnetic field on the melting transition of H_2O and D_2O measured by a high resolution and supersensitive differential scanning calorimeter. J Appl Phys 2004;96:6127–32.
[37] Barteri M, Pala A, Rotella S. Structural and kinetic effects of mobile phone microwaves on acetylcholinesterase activity. Biophys Chem 2005;113(3):245–53.
[38] Colic M, Morse D. The elusive mechanism of the magnetic 'memory' of water. Colloid Surf A 1999;154(1/2):167–74.
[39] Labropoulos KC, Niesz DE, Danforth SC, Kevrekidis PG. Dynamic rheology of agar gels: theory and experiments. Part I. Development of a rheological model. Carbohydr Polym 2002;50:393–406.

Author's personal copy