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Biotransformation of hydroquinone to arbutin in plant *in vitro* cultures - preliminary results

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Abstract

Cells from *Echinacea purpurea* (L.) Moench. (*Asteraceae*), *Exacum affine* Balf. f. (*Gentianaceae*), *Melittis melissophyllum* L. (*Lamiaceae*), *Ruta graveolens* L. and *Ruta graveolens ssp. divaricata* (Tenore) Gams. (*Rutaceae*) agitating cultures perform a biotransformation reaction on exogenously supplied hydroquinone into its β-D-glucoside – arbutin, product with valuable medicinal and cosmetic properties. The maximum content of arbutin (determined by HPLC) in the biomass from investigated cultures is 4.01; 3.44; 1.79; 2.48 and 5.07 g/100 g d.w., respectively. Nothing but *Ammi majus* L. (*Apiaceae*) cultures contain trace amounts of the product. Arbutin is accumulated in cells; it is occasionally found in media only in vestigial amounts. In most of the investigated cultures the efficiency of the biotransformation process is about 60 %.

List of abbreviations: BAP – 6-benzylaminopurine; biomass- shoots, differentiated callus, undifferentiated callus or cell aggregates from suspension; d. w. – dry weight; GA_3 – gibberellic acid; HPLC – high performance liquid chromatography; LS – medium of Linsmaier and Skoog, 1965; MS – medium of Murashige and Skoog, 1962; NAA – α-naphthaleneacetic acid; PLC – preparative layer chromatography; s.m. – sucha masa; TLC – thin layer chromatography

Introduction

Plant cell and tissue cultures are capable of performing various specific biotransformation reactions on exogenously supplied compounds (Wysokińska and Chmiel 1995, 2001). Up till now a whole range of reactions has been observed including, for example, estrification, oxidation, reduction, hydroxylation and glucosylation. The formation of glucosyl conjugates is of special interest because many groups of secondary metabolites are accumulated as glucosides in plant cells. Several plant cell and tissue cultures have been shown to be capable of converting exogenous compounds such as phenolics, steroids, cardenolids, flavonoids and anthraquinones to the corresponding glucosides unknown for adequate plants growing in the open air (*e.g.* Tabata *et al.* 1988, Stöckigt *et al.* 1995).

Arbutin is the O-β-D-monoglucoside of hydroquinone. Glucosylation of hydroquinone is catalyzed by a glucosyltransferase that requires uridine diphosphate glucose as its high energy donor of glucose (Fig. 1). Arbutin is a natural secondary metabolite occurring in leaves of some species of the family *Ericaceae, Rosaceae, Saxifragaceae, Rubiaceae, Fabaceae as* well as in other taxa. It is of greatest importance as an active principle of

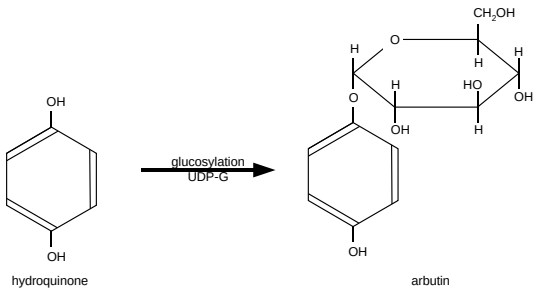

Fig. 1. Biotransformation of hydroquinone to arbutin.

Arctostaphylos uva-ursi (L.) Sprengel (*Ericaceae*) – which amounts up to 12 % – and *Vaccinium vitis idaea* L. (*Ericaceae)* – up to 7 % (Wichtl 1997, Kohlmünzer 1998, Stammwitz 1998). Leaves of both species are mentioned as herbal drugs in the 5th edition of the Polish Pharmacopoeia (1999) and also in its newest 6th edition (2002). *A. uva-ursi* is also described in the newest European Pharmacopoeia (2002) and in numerous other national pharmacopoeias (Newall *et al*. 1996, Wichtl 1997). Natural sources of arbutin containing plants – used in therapeutics – decrease. In Poland, *A. uva-ursi* grows only in a few localities and it is under strict protection (Piękoś-Mirkowa and Mirek 2003). From the pharmacological point of view, arbutin has attracted much interest for two main therapeutical applications. As it exhibits an antibacterial effect, arbutin containing plant extracts is widely used in traditional and modern medicine because of its urethal disinfectant activity. Arbutin is also known to be an efficient inhibitor of melanin biosynthesis in human skin, without having any apparent side effects (Akiu *et al*. 1988). This compound is used by the Japanese cosmetic company, Shiseido, as a lightener. Furthermore, drugs containing arbutin are used in traditional Chinese medicine in cough and chronic bronchitis therapy. Strong antitussive effect of arbutin was confirmed under experimental conditions (Strapková *et al*. 1991).

The difficulty with the supply of arbutin containing plant materials attempts to be solved by *via in vitro* methods. Jahodář *et al*. (1982) and Dušková *et al*. (1988) established *Arctostaphylos uva- ursi* cultures but cells did not synthetize arbutin. Furmanowa and Rapczewska (1993) suggest micropropagation of the Asiatic abounding with arbutin species- *Bergenia crassifolia* (L.) Fritsch (*Saxifragaceae*) as a quick and effective method, alternative to propagation from seeds. The arbutin content in *Bergenia* plants propagated *in vitro* is within the same range as in plants propagated by conventional methods. Biotransformation process performed by plant cells from *in vitro* cultures can be the next possibility. Although arbutin can be prepared chemically in a three step procedure, a one step bioconversion still seems to be of biotechnological interest. Many attempts were made to obtain arbutin by biotransformation of hydroquinone. Unfortunately, the efficiency of the process in *A. uva-ursi* cultures was very low (Dušková *et al*. 1994). *Bergenia crassifolia* cultures converted hydroquinone to arbutin with quite moderate product yield (Dušková *et al*. 1999). Numerous experiments were done on bioconversion of hydroquinone by other plant cell and tissue cultures mainly derived from plant species which did not synthetize arbutin as a natural secondary metabolite (Wysokińska and Chmiel 1995, Dušková *et al*. 1999). In most of cultures the product yields were relatively low. Arbutin content differs significantly *e.g*. from 0.43 g/100 g d.w. – for *Coronilla varia* L. cultures – to 7.4 g/100 g d.w. for *Datura meteloides* DC. ex Dun. cultures -Table 1 (Dušková *et al*. 1999). However, *Catharanthus roseus* (L.) G. Don cell cultures were proven to be able to produce up to 9 g of arbutin per liter cell suspension -Table 2 (Inomata *et al*. 1991, Yokoyama and Inomata 1998). In other experiment, *Rauvolfia serpentina* (L.) Benth. cell suspension converted hydroquinone with an optimum accumulation of 18 g arbutin per liter nutrition medium after 7 days of continuous substrate feeding (Lutterbach and Stöckigt 1992). That culture demonstrated the exceptional glucosylation potential of cells. It is one of the highest content of a natural product formed by a plant cell culture system.

We report here the results of our studies on the possibility of arbutin production by biotransformation of hydroquinone in *in vitro* cultures of *Ammi majus* L. (*Apiaceae*), *Echinacea purpurea* (L.) Moench. (*Asteraceae*), *Exacum affine* Balf. f. (*Gentianaceae*), *Melittis melissophyllum* L. (*Lamiaceae*), *Ruta graveolens* L. and *Ruta graveolens ssp. divaricata* (Tenore) Gams. (*Rutaceae*). Arbutin does

not occur as a natural secondary product in any of above mentioned species. This is the first information on the possibility of biotransformation of hydroquinone to arbutin in the *in vitro* cultures of these species. Two biotransformation experiments were carried out for each plant culture (series 1 and series 2) in order to determine the reproducibility of the procedure (exception – *A. majus* culture – only series 1). Our results were presented earlier at several scientific conferences (*e.g.* Skrzypczak-Pietraszek *et al*. 2002, Skrzypczak-Pietraszek and Piekoszewska 2002, Ekiert *et al*. 2003, Skrzypczak--Pietraszek *et al.* 2004).

Materials and methods

In vitro cultures

Tissue cultures were originally established from seedlings in the Department of Pharmaceutical zBotany, CMUJ in Kraków. Seeds were obtained from some European botanical gardens. *Ruta graveolens ssp. divaricata* culture is an exception – it was initiated in the Institute of Biosciences, University of Würzburg (Germany) from plants growing in the botanical garden of that university. The culture was delivered to our Department on a cooperation basis. All tissue cultures have been cultivated in the Department of Pharmaceutical Botany, CMUJ, for several years on two kinds of media:
– MS agar medium supplemented with 1 $mg \cdot l^{-1}$ BAP, 0.5 $mg \cdot l^{-1}$ NAA and 0.25 $mg \cdot l^{-1}$ GA_3 (*Echinacea purpurea* (L.) Moench., *Exacum affine* Balf. f. and *Melittis melissophyllum* L. cultures)
– LS medium supplemented with 2 $mg \cdot l^{-1}$ BAP and 2 $mg \cdot l^{-1}$ NAA (agar medium – *Ammi majus* L. culture, liquid medium– *Ruta graveolens* L. and *Ruta graveolens ssp. divaricata* (Tenore) Gams. stationary cultures)

Three types of *in vitro* cultures were used:
– callus cultures – *A. majus*, *E. purpurea*
– callus cultures with significant differentiation

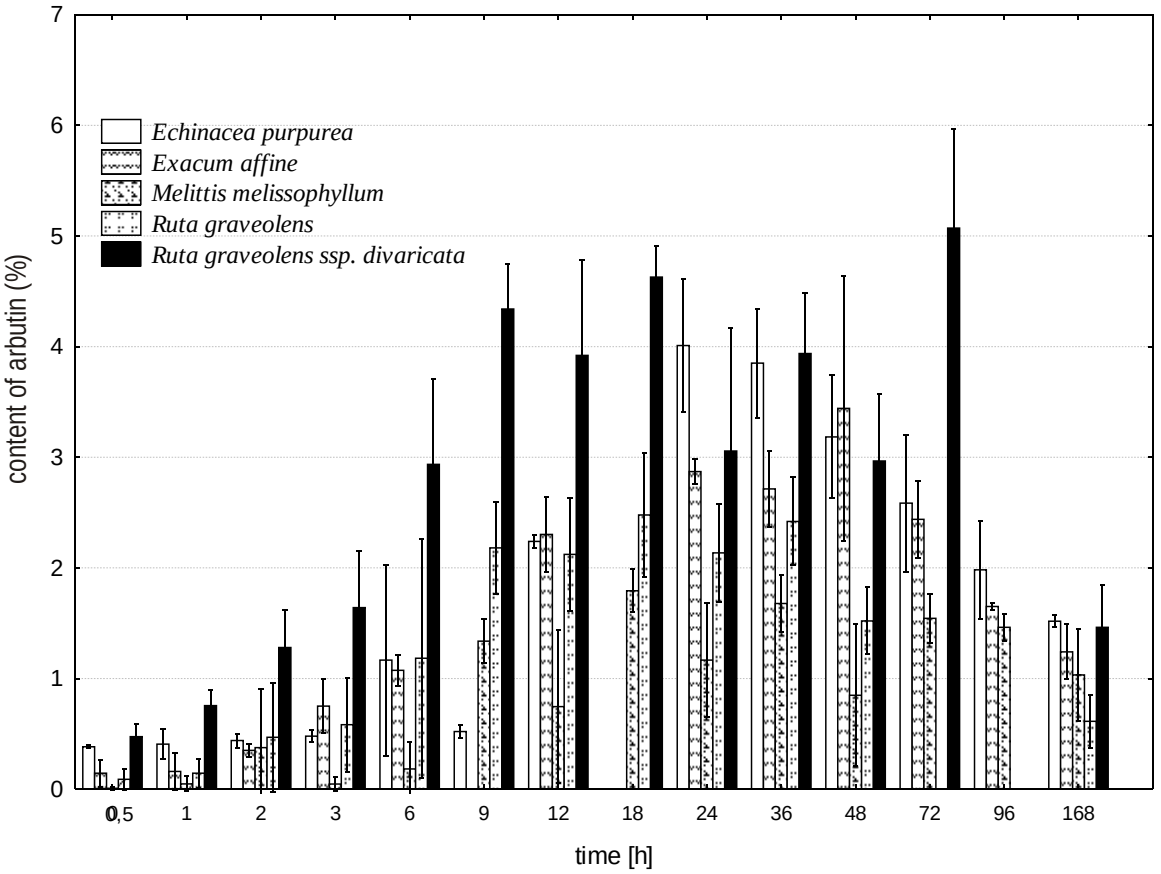

Fig. 2. Content of arbutin (%= g/100 g d.w.) in biomass from plant *in vitro* cultures; mean ± S. D. (n=3).

(shoot buds or little shoots) – *M. melissophyllum, R. graveolens ssp. divaricata*
– shoot cultures – *E. affine, R. graveolens*

All cultures were kept at a temperature of 25± 2 °C, under continuous artificial light (900 lx) and were transferred to a fresh medium every 5-7 weeks.

Biotransformation experiments

Liquid agitating cultures were established from initial ones (inoculum – 4 g of fresh biomass per flask) and were maintained on a rotary shaker (Altel) at 140 rpm in 500 ml conical flasks containing 125 ml of MS (or LS) medium supplemented with the same growth regulators as for initial cultures. 14 days (or 19 days - for *E. purpurea* cultures) after inoculation a substrate (hydroquinone) dissolved in water (concentration: 1 mg·ml^{-1}) was administered aseptically through a membrane filter (Millex®, Millipore, 0.22 µm) to the culture. Additionally, 100 ml of fresh medium was added to each flask. Final concentration of hydroquinone was 25 mg per flask
(100 mg·l^{-1} of medium). In control cultures instead of hydroquinone solution fresh medium was added to the flask. The biomass and medium were harvested separately from two or three flasks after 0.5, 1, 2, 3, 6, 12, 24, 48, 168 hours (series 1; there were some changes in series 2- additional harvest for selected plant cultures after 9, 18, 36, 72 and 96 hours- see Fig. 2 and Fig. 3) after administration of substrate. Control cultures were harvested on 21st day (or 26th day for *E. purpurea* culture) after inoculation. Media (100 ml) were frozen and lyophilised. The biomass was dried at 35 °C and weighed.

All cultures were kept in the same temperature and light conditions as for initial cultures.

Extraction

0.2-1 g of dried biomass from each culture was milled and extracted twice with boiling methanol (2

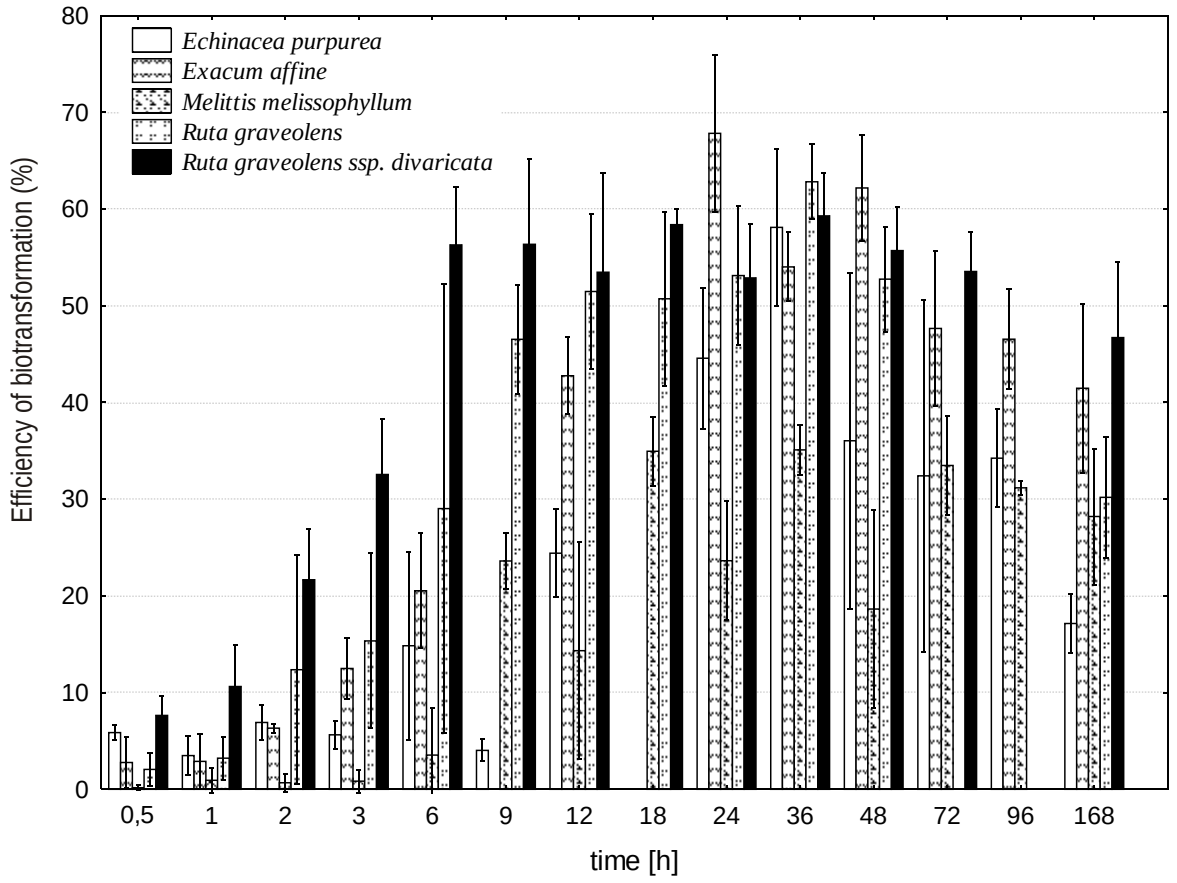

Fig. 3.

x 50 ml) for 4 hours. Methanol was evaporated to dryness and remains were dissolved in 10 ml of methanol. Lyophilised medium was also dissolved in 10 ml of methanol.

Qualitative analysis (TLC)

Methanol solutions were qualitative analysed by TLC (plates- Merck, item No 1.05554) together with standard substances – arbutin (Sigma) and hydroquinone (POCh Gliwice) with the following solvent system: ethyl acetate–methanol–water (77 : 13 : 10, V/V/V). Detection reagents (spray): diasotized sulfanilic acid followed by 5 % KOH methanol solution. Colours of spots: arbutin- orange/ red; hydroquinone- grey.

Quantitative assay of arbutin and hydroquinone (HPLC)

Methanol solutions were analysed by an isocratic HPLC- system (Merck) with Purospher® RP-18e (250 x 4 mm; 5 µm) column eluted with methanol-water (1:9, V/V) at a flow rate of 1 ml/ min and UV detection at λ= 285 nm. Retention times: arbutin- 4.1 min; hydroquinone- 6.3 min.(Fig. 4). HPLC method- according to Štambergovŕ *et al.* (1985).

M. melissophyllum methanol extracts had to be purified before quantitative analysis. Ballast compounds were precipitated by basic lead acetate.

Quantitative determination of arbutin and hydroquinone was carried out using the external standard method. The amounts of these compounds were calculated from calibration curves, putting in relation the mean peak areas with standard concentrations.

Isolation and identification of arbutin

Methanol extracts from biomass were combined, concentrated to 40 ml. Ballast compounds were precipitated by basic lead acetate. Purified extracts were subjected to PLC on Silica gel plates (Merck, item No 1.05717) with solvent system ethyl acetate-methanol-water (77 : 13 : 10, V/V/V) and biotransformation product was eluted with methanol. Arbutin solution was concentrated and PLC was repeated with solvent system chloroform-methanol (3 : 1, V/V). Arbutin was eluted with chloroform- methanol mixture (1 : 1, V/V) and identified by ^{1}H NMR, HPLC and TLC. ^{1}H NMR spectrum of arbutin standard was prepared for comparison.

Results and discussion

All examined plant cultures converted hydroquinone to arbutin, but they showed different efficiency of the process (maximum from 35.1 % to 65.6 % – Table 3, Fig. 3). Biotransformation product was present in all methanol extracts from the

Table 1. Biotransformation of hydroquinone to arbutin in plant *in vitro* cultures (examples).

Plant *in vitro* cultures	Maximum content of arbutin (g/100g d.w.)	Authors
Agrostemma githago L.	+*	Pilgrim 1970
Bellis perennis L.	1.27 (24 h)**	Dušková *et al*. 1999
Bergenia crassifolia (L.) Fritsch	2.83 (12 h)	Dušková *et al*. 1999
Brassica oleracea L.	0.52 (168 h)	Dušková *et al*. 1999
Coronilla varia L.	0.43 (24 h)	Dušková *et al*. 1999
Datura ferox L.	+	Pilgrim 1970
Datura meteloides DC. ex Dun.	7.40 (168 h)	Dušková *et al*. 1999
Digitalis purpurea L.	+	Pilgrim 1970
Leonurus cardiaca (L.) Benth.	2.56 (12 h)	Dušková *et al*. 1999
Leuzea carthamoides DC.	2.38 (12h)	Dušková *et al*. 1999
Rheum palmatum L.	1.25 (12 h)	Dušková *et al*. 1999
Rhodiola rosea L.	3.44 (48 h)	Dušková *et al*. 1999

*presence of arbutin – qualitative determination
**time after administration of hydroquinone

Table 2. Maximum content of arbutin and maximum efficiency of optimised biotransformation process in plant *in vitro* cultures.

Plant *in vitro* cultures	Method of hydroquinone supply	Arbutin content (g/100g d.w.)	Arbutin content (g/l of medium)	Efficiency (%)	Authors
Catharanthus roseus (L.) G. Don.	continuous	45 (96 h)*	9.2 (96 h)*	98	Yokoyama and Inomata 1998
Datura innoxia Mill.	1 time	30-40 (24 h)	2.5 (24 h)	90-100	Suzuki *et al*. 1987
	repeated- 3 times	40-50 (72h)	7.1 (72h)	70	
Rauvolfia serpentina (L.) Benth.	continuous	23.7 (168h)	18 (168 h)	83	Lutterbach and Stöckigt 1992

* time after beginning of hydroquinone administration

biomass (Fig. 2) in the maximum content from 1.79 to 5.07 g/100 g d.w. (Table 3). Nothing but *Ammi majus* L. cultures contained only trace amounts of biotransformation product. Arbutin did not appear in the culture medium or was only in vestigial amounts. Hydroquinone was usually present in medium samples collected in the first hours after administration of substrate and later was not detected. Plant tissues did not contain hydroquinone (or trace amounts) and it was possible that all absorbed substrate was converted to arbutin.

Identity of arbutin isolated from methanol extracts was confirmed by comparison its TLC and HPLC (Fig. 4) parameters and ^{1}H NMR spectrum with the same ones of standard compound.

The after-mentioned information on dynamics of biotransformation process and influence of hydroquinone on plant cells describe details connected with each plant culture (Fig. 2 and Fig. 3). Hydroquinone solutions can show harmful influence on plant cells. This problem is well known from literature (*e.g.* Suzuki *et al*. 1987, Yokoyama *et al*. 1990).

A. majus cultures

Traces of arbutin were found in collected cells and larger amounts – in the medium. Toxicity of hydroquinone was so strong that its addition not only stopped the growth of culture but also irreversible damaged the cells and liquid culture changed its colour from light green to brown.

E. purpurea cultures

Biotransformation product was found in callus tissue and it occurred in media only in trace amounts. The maximal yield of conversion was 58.10 % (after 36 h) and maximal content of arbutin – 4.01 g/100 g d.w. – was detected after 24 h. Culture growth was not equalized. Liquid culture formed suspension or quite big callus aggregates. Suspension culture was more sensitive on harmful influence of hydroquinone.

E. affine cultures

Table 3. Maximum content of arbutin and maximum efficiency of biotransformation process obtained for investigated cultures.

In vitro cultures	Arbutin content	Efficiency		
	g/100 g d.w.	time (h)*	%	time (h)*
Echinacea purpurea (L.) Moench.	4.01	24	58.1	36
Exacum affine Balf. f.	3.44	48	65.6	24
Melittis melissophyllum L.	1.79	18	35.1	18 and 36
Ruta graveolens L.	2.48	18	63.3	36
Ruta graveolens ssp. *divaricata* (Tenore) Gams.	5.07	72	59.7	36

* time after administration of hydroquinone

Amount of arbutin in plant tissue significantly grows at 12 h after administration of substrate and achieves its maximum at 48 h (3.44 g/100 g d.w.). The maximal efficiency of the process was observed at 24 h (65.58 %). Media contained trace amounts of arbutin. Hydroquinone did not influence shoot culture growth.

***M. melissophyllum* cultures**

Methanol extracts had to be purified before quantitative analysis because of numerous ballast compounds with the same HPLC retention time as arbutin. This procedure caused significant loss of biotransformation product. The maximal content of arbutin was 1.79 g/100 g d.w. (18h) and efficiency of biotransformation did not exceed 36 %. Arbutin was not detected in media. The presence of hydroquinone decreased the growth of cultures.

***R. graveolens and R. graveolens ssp. divaricata* cultures**

The maximal yield of conversion was observed at 36 h – 63.34 % (*R. graveolens*) and 59.70 % (*R. graveolens ssp. divaricata*). Amount of arbutin achieves its maximum at 18h (2.48 g/100 g d.w.) and at 72 h (5.07 g/100 g d.w.), respectively. Arbutin was not detected in media or found only in trace amounts. Hydroquinone had no harmful influence on the growth of both cultures.

High mean yield of biotransformation process (50-60 %) and mean arbutin content about 2.2 g/100 g d.w. (*R. graveolens*), about 2.7 g/100 g d.w. (*E. affine*) and about 4 g/100 g d.w. (*R. graveolens ssp. divaricata*) stay on the similar level for a long period of time (about 30 hours – *R. graveolens* and 60 hours – *E. affine, R. graveolens ssp. divaricata*).

In our investigation hydroquinone used in one concentration (100 $mg \cdot l^{-1}$ of medium) decreased the growth of *A. majus, E. purpurea, M. melissophyllum* cultures but its addition had no significant influence on *E. affine, R. graveolens, R. graveolens ssp. divaricata* cultures. Differentiated cultures – shoot cultures (*E. affine, R. graveolens*) or differentiating callus culture (*R. graveolens ssp. divaricata*)- seem to be more resistant to harmful influence of hydroquinone.

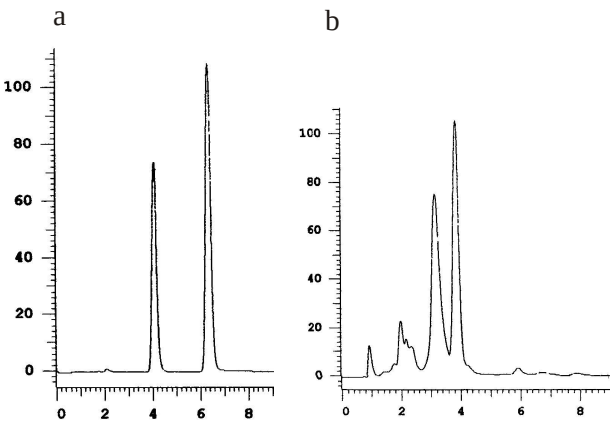

Fig. 4. HPLC chromatogram of a) standard substances – retention times: arbutin- 4.1 min; hydroquinone- 6.3 min, and b) exemplary *Melittis melissophyllum* callus extract.

We applied similar biotransformation conditions (hydroquinone concentration in media and time of the biomass harvesting) as Dušková *et al.* (1999) and we obtained similar results of the dynamics of the biotransformation process. Arbutin content grows rapidly in the first hours after hydroquinone application, achieves its maximum usually at 18-48 hours and significantly lowers after 7 days.

Some authors made different attempts to increase and optimise the biotransformation process. Dušková *et al.* (1994) mentioned the influence of auxins on arbutin production. Increasing NAA or IAA content influence larger amounts of arbutin in *in vitro* cultures of *Datura meteloides* or *Datura innoxia* (respectively). Continous or repeated addition of hydroquinone (Table 2) increase the efficiency of biotransformation process, reduce the harmful influence of hydroquinone and enable to employ higher concentrations of the substrate.

Our results compared to literature data are average, similar or higher than those obtained by other authors (Table 1 and Table 3) but the arbutin content is much lower than detected in *Catharanthus roseus*, *Datura innoxia* or *Rauvolfia serpentina* cultures – the best ones – with optimised biotransformation process (Table 2).

This is the first news about the possibility of biotransformation: hydroquinone to arbutin in the *in vitro* cultures of *E. purpurea, E. affine, M. melisso-*

phyllum, R. graveolens and R. graveolens ssp. divaricata. Our results show that *E. affine, R. graveolens* and *R. graveolens ssp. divaricata* cultures seems to be quite promising objects for further investigation on optimisation of biotransformation process.

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References

Akiu S., Suzuki Y., Fujinuma Y., Asahara T., Fukuda M. 1988. Inhibitory effect of arbutin on melanogenesis: biochemical study in cultured B16 melanoma cells and effect on the UV-induced pigmentation in human skin. Proc. Jpn. Soc. Invest. Dermatol. 12: 138-139.

Dušková J., Dušek J., Jahodář L. 1999. Zur Biotransformation von Hydrochinon zu Arbutin in den In Vitro-Kulturen. Herba Polonica 1: 23-26.

Dušková J., Jahodář L., Dušek J. 1994. Neue Möglichkeiten der Produktion von Arbutin durch Gewebekulturen. Pharmazie 49: 624.

Dušková J., Sovová M., Dušek J., Jahodář L. 1988. The effect of ionizing irradiation on the tissue culture of *Arctostaphylos uva- ursi* (L.) Sprengel. Pharmazie 43: 518-519.

Ekiert H., Krystian E., Szewczyk A. 2003. Conversion of hydroquinone to arbutin in shoot – differentiating callus culture of *Ruta graveolens ssp. divaricata* (Tenore) Gams. Polish-Austrian-German-Hungarian-Italian Joint Meeting on Medicinal Chemistry, Kraków, P-223.

European Pharmacopoeia IV. 2002. Council of Europe, Strasbourg: 694-695.

Farmakopea Polska V. 1999. P.T.Farm., Warszawa, Vol. 5: 505-507, 516-518.

Farmakopea Polska VI. 2002. P.T.Farm., Warszawa: 903-904, 908-909.

Furmanowa M., Rapczewska L. 1993. *Bergenia crassifolia* (L.) Fritsch (Bergenia): Micropropagation and arbutin contents. In: Biotechnology in Agriculture and Forestry. Vol. 21 Medicinal and Aromatic Plants IV. Bajaj Y.P.S. (ed.) Springer- Verlag, Berlin, Heidelberg: 18- 33.

Inomata S., Yokoyama M., Seto S., Yanagi M. 1991. High-level production of arbutin from hydroquinone in suspension cultures of *Catharanthus roseus* plant cells. Appl. Microbiol. Biotechnol. 36: 315-319.

Jahodář L., Vondrová I., Leifertová I., Kolb I., 1982. Tissue culture of *Arctostaphylos uva- ursi*, examination of phenolic glycosides and isolation of oleanolic acid. Pharmazie 37: 509-511.

Kohlmünzer S. 1998. Farmakognozja. Wydawnictwo Lekarskie PZWL, Warszawa: 233-237.

Linsmaier E.M., Skoog F. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 100-127.

Lutterbach R., Stöckigt J. 1992. High yield formation of arbutin from hydroquinone by cell-suspension cultures of *Rauvolfia serpentina*. Helv. Chim. Acta 75: 2009-2011.

Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473- 497.

Newall C.A., Anderson L.A., Phillipson J.D. 1996. Herbal Medicines. A Guide for Health- care Professionals. The Pharmaceutical Press, London: 258-259.

Piękoś-Mirkowa H., Mirek Z. 2003. Flora Polski. Atlas Roślin Chronionych. Multico Oficyna Wydawnicza, Warszawa: 132-133.

Pilgrim H. 1970. Untersuchungen zur Glykosidbildung in pflanzlichen Gewebekulturen. Pharmazie 25: 568.

Skrzypczak-Pietraszek E., Piekoszewska A. 2002. Biotransformations in *in vitro* cultures of *Exacum affine* Balf. f. and *Melittis melissophyllum* L. International Symposium on Promotion of International Cooperation in Eastern and Southern Europe in the Field of Medicinal Biotechnology, Łódź, P-26.

Skrzypczak-Pietraszek E., Szewczyk A., Ekiert H. 2002. Optimisation of biotransformation conditions in *Ruta graveolens* L. *in vitro* cultures. 5 Ogólnopolska Konferencja – „Zastosowanie kultur *in vitro* w fizjologii roślin" Kraków, p. 68 (in Polish).

Skrzypczak-Pietraszek E., Szewczyk A., Piekoszewska A., Ekiert H. 2004. *In vitro* cultures of medicinal plants as a potential source of arbutin. Phytotherapie Kongress "Phytopharmaka und Phytotherapie 2004- Forschung und Praxis", Berlin, p 67.

Štambergovŕ A., Šupčikovŕ M., Leifertovŕ I. 1985. Hodnoceni fenolických lŕtek v *Arctostaphylos uva- ursi.* IV. Stanoveni arbutinu, metylarbutinu a hydrochinonu v listech metodou HPLC. Českoslov. Farm. 34: 179- 182.

Stammwitz U. 1998. Pflanzliche Harnwegsdesinfizienzien – heute noch aktuell?. Z. Phytoth. 19: 90-95

Stöckigt J., Obitz P., Falkenhagen H., Lutterbach R., Endress S. 1995. Natural products and enzymes from plant cell cultures. Plant Cell, Tissue and Organ Culture 43: 97-109

Strapková A., Jahodář L., Nosalová G. 1991. Antitussive effect of arbutin. Pharmazie 46: 611-612.

Suzuki T., Yoshioka T., Tabata M., Fujita Y. 1987. Potential of *Datura innoxia* cell suspension cultures for glucosylating hydroquinone. Plant Cell Reports 6: 275-278.

Tabata M., Umetani Y., Ooya M., Tanaka S. 1988. Glucosylation of phenolic compounds by plant cell cultures. Phytochemistry 27: 809-813.

Wichtl M. 1997. Teedrogen Und Phytopharmaka. Wissenschaftliche Verlagsgesellschaft mbH., Stuttgart: 599-602.

Wysokińska H., Chmiel A. 1995. Biotransformacje w kulturach komórek roślinnych. Część I – Reakcje biotransformacji. Biotechnologia 1: 114-130.

Wysokińska H., Chmiel A. 2001. Biotransformacje substancji chemicznych. In: Biotechnologia Roślin. Malepszy S. (ed.) PWN, Warszawa: 144-170.

Yokoyama M., Inomata S. 1998. *Catharanthus roseus* (Periwinkle): *In vitro* culture and high-level production of arbutin by biotransformation. In: Biotechnology in Agriculture and Forestry. Vol. 41 Medicinal and Aromatic Plants X. Bajaj Y.P.S. (ed.) Springer, Berlin, Heidelberg, New York: 67-80.

Yokoyama M., Inomata S., Seto S., Yanagi M. 1990. Effects of sugars on the glucosylation of exogenous hydroquinone by *Catharanthus roseus* cells in suspension culture. Plant Cell Physiol. 31: 551-555.

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