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ORIGINAL PAPER

Production of bioactive phenolic acids and furanocoumarins in in vitro cultures of *Ruta graveolens* L. and *Ruta graveolens* ssp. *divaricata* (Tenore) Gams. under different light conditions

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Abstract Extracts from the biomass of *Ruta graveolens* and *Ruta graveolens* ssp. *divaricata* cultured in vitro under different light conditions (far-red, red and blue light, UV-A irradiation, in darkness and white light) were tested for the amounts of free phenolic acids and cinnamic acid (twelve compounds) as well as furanocoumarins and umbelliferone (seven compounds) using HPLC methods. Total amounts of the investigated groups of compounds in the cultures of both plants increased from 2.6 to 6.7 times, depending on light quality, and the maximum values reached were 106.50 and 1,276.74 mg 100 g^{-1} DW (in *R. graveolens*), and 106.97 and 262.54 mg 100 g^{-1} DW (in the subspecies), respectively. Both white light and blue light were equally beneficial for the total production of phenolic acids in cultures of both plants, whereas the total production of furanocoumarins was clearly better stimulated by blue light in *R. graveolens* and by darkness in the subspecies (i.e. the amounts were respectively 1.44 and 1.7 times higher than in the biomass cultivated under white light). The amounts of individual compounds in both plant cultures increased from about 2.2 to 26.3 times depending on light quality. The following bioactive compounds were obtained in quantities which are of interest from a practical perspective: in *R. graveolens* culture—protocatechuic acid (45 mg 100 g^{-1} DW), isopimpinellin (about 500 mg 100 g^{-1} DW) and bergapten (about 270 mg 100 g^{-1} DW), and in the subspecies culture: *p*-coumaric acid (70 mg 100 g^{-1} DW) and isopimpinellin (about 210 mg 100 g^{-1} DW).

Keywords *Rutaceae* · Common rue · Common rue subspecies · Secondary metabolites · HPLC analysis · Monochromatic light · White light · Darkness · Psoralens · Cinnamic acid

Abbreviations

BA	N^6-Benzyladenine
DW	Dry weight
HPLC	High-pressure liquid chromatography
L-S	Linsmaier and Skoog
NAA	α-Naphthaleneacetic acid

Introduction

Phenolic acids are a group of plant metabolites highly valued in phytotherapy and cosmetology. These compounds exhibit numerous biological activities, the most important of which are their immunostimulating, antioxidant and anticancer properties (Abdel-Wahab et al. 2003; De Graff et al. 2003; Ekiert and Czygan 2007; Nakamura et al. 2001; Sroka and Cisowski 2003). Linear furanocoumarins have an established position in therapy as antiproliferative and photosensitizing agents, and some of them are calcium and potassium channel blockers (Bohuslavizki et al. 1994; Ekiert and Czygan 2007). Our earlier studies on phenolic acid accumulation in in vitro cultures of *Ruta graveolens* and *Ruta graveolens* ssp. *divaricata* focused on the effect of the concentration of plant growth regulators in the Linsmaier-Skoog (L-S)

Dedicated memory to Professor Dr. Franz-Christian Czygan, Head of Department of Pharmaceutical Biology, Würzburg University, Honorary Doctor of I. Kant University, Kaliningrad, dead on 16th January 2012.

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medium (Ekiert et al. 2008, 2009; Piekoszewska et al. 2008). The studies on linear furanocoumarins concentrated on the effect of culture type and on the dynamics of accumulation in culture growth cycles (Ekiert et al. 2001, 2005; Ekiert and Czygan 2005).

Apart from light intensity and photoperiod, the quality of light is one of the known ambient factors influencing metabolite accumulation in plant in vitro cultures (Ramawat and Mathur 2007). The majority of studies on the effect of light quality on the production of secondary metabolites have been concerned with other groups of compounds, e.g. alkaloids, anthocyanins glycosides and flavonoids (Ramawat and Mathur 2007). Also, recently the influence of light quality on the accumulation of other groups of plant pigments such as naphthoquinones and betacyanins has been studied (Zhang et al. 2010; Zhao et al. 2010). A few papers have dealt with the effect of light quality on the accumulation of phenolic acids, e.g. chlorogenic acid accumulation in *Haplopappus sp.* and *Populus sp.* cultures (Ramawat and Mathur 2007).

Our preliminary studies indicated a marked effect of monochromatic light on the accumulation of both phenolic acids and linear furanocoumarins in in vitro cultures of *R. graveolens* and its subspecies (Ekiert and Czygan 2007; Ekiert and Gomółka 1999).

The aim of our present experiments was to examine the effect of light with different wavelengths (far-red, red and blue, and UV-A irradiation), darkness and white light on the accumulation of phenolic acids and cinnamic acid, one of the parent compounds of these metabolites (twelve compounds), and linear furanocoumarins and their biogenetic precursor—umbelliferone (seven compounds) in the biomass from *R. graveolens* and its subspecies *R. graveolens* ssp. *divaricata* in vitro cultures and to propose the best conditions for the production of these two groups of bioactive metabolites.

The effects of light quality on the accumulation of phenolic acids and linear furanocoumarins in in vitro cultures of *R. graveolens* and *R. graveolens* ssp. *divaricata* have so far not been investigated in other research centers. However, the current literature contains several reports describing the significance of other factors, such as elicitors for the production of coumarins, including linear furanocoumarins in *R. graveolens* in vitro cultures (Orlita et al. 2008a, b).

Materials and methods

Plant material

The shoot culture of *Ruta graveolens* L. was initiated in our Department of Pharmaceutical Botany from hypocotyl segments of sterile seedlings—for details see Ekiert and Czygan (2007).

The shoot-differentiating callus culture of *Ruta graveolens* ssp. *divaricata* (Tenore) Gams. was established in the Institute for Biosciences of Würzburg University (Germany)—for details see Ekiert and Czygan (2007).

Experimental stationary liquid shoot cultures of *R. graveolens* were maintained on two variants of Linsmaier and Skoog medium (L-S) (1965) with different concentrations of plant growth regulators; variant I: 2 mg l^{-1} α-naphthaleneacetic acid (NAA) and 2 mg l^{-1} N^6-benzyladenine (BA), variant II: 3 mg l^{-1} NAA and 1 mg l^{-1} BA, while the stationary shoot-differentiating liquid callus cultures of *R. graveolens* ssp. *divaricata* were maintained on a variant of L-S medium containing 2 mg l^{-1} NAA and 2 mg l^{-1} BA. The cultures were grown under different light conditions, at 25 ± 2 °C. They were subcultured every 4 weeks (three series).

Light conditions

In vitro cultures of both plants were maintained under light of different spectra: far-red light (770–800 nm)—60 W incandescent light with standard filter no. 405 orange + standard filter no. 420, Compact Light B.V. Amsterdam, red light (647–770 nm)—Philips lamp TLD 36 W, blue light (450–492 nm)—Philips lamp TLD 36 W, UV-A irradiation (360–450 nm)—Philips lamp TLD 36 W, darkness and white light (390–760 nm)—Philips lamp TLD 36 W. Cultures grown under white light served as the control.

Extraction

The biomass from in vitro cultures (1.0 g) collected after 4-week growth cycles (three series) was subjected to extraction twice with boiling methanol (50 ml) under a reflux condenser for 3 h. The extracts were combined, condensed and evaporated to dryness. The residue was quantitatively dissolved in 5 ml of methanol and analyzed by HPLC.

HPLC analysis

In the methanolic extracts of the biomass, eleven phenolic acids and cinnamic acid as one group, and six linear furanocoumarins and umbelliferone as another group were quantified using RP-HPLC methods according to Ellnain-Wojtaszek and Zgórka (1999) with our modifications and those of Ekiert and Gomółka (1999), respectively.

Separation of both groups of metabolites was performed using a LiChrospher 100 RP-18 (4 mm × 20 cm) analytical column. The separation conditions for phenolic acids

were as follows: solvent system composed of methanol: 0.5 % acetic acid—gradient elution, flow rate: 1 ml min^{-1}, UV detector: $\lambda = 254$ nm, standards: caffeic, chlorogenic, cinnamic, protocatechuic, rosmarinic, salicylic, sinapic and syringic acids from Sigma, and p-coumaric, ferulic, p-hydroxybenzoic and vanillic acids from Fluka.

The separation conditions for coumarins were different: the solvent system contained methanol: water—gradient elution, flow rate: 1 ml min^{-1}, detector UV: $\lambda = 310$ nm, standards: bergapten, imperatorin, psoralen, umbelliferone, xanthotoxin from Roth, and isopimpinellin, marmesin from Institute of Pharmacology, Polish Academy of Sciences, Kraków (Poland).

Results

Increases in biomass in the cultures of both plants

Ruta graveolens shoot cultures were maintained on two variants of L-S medium (variant I: 2 mg l^{-1} NAA and 2 mg l^{-1} BA, variant II: 3 mg l^{-1} NAA and 1 mg l^{-1} BA) chosen as "productive media" and as good "growth media" based on the experiments concerning the influence of growth regulators on the accumulation of phenolic acids (Ekiert et al. 2009). As shown by our earlier studies on the accumulation of linear furanocoumarins in *R. graveolens* cultures, variant I was beneficial for the accumulation of this group of compounds and good for increasing the biomass (Ekiert 2004). The changes in the dry biomass of the shoots cultivated under different light conditions on the two variants of L-S medium were different; namely it increased 1.80–3.64 times and 1.93–3.77 times, respectively, within a 4-week growth cycle. The greatest increases were observed in the shoots growing under blue light, while extremely low growth in biomass occurred under far-red light. The L-S medium containing 2 mg l^{-1} NAA and 2 mg l^{-1} BA was selected in our earlier experiments as a "productive medium" for the accumulation of both furanocoumarins and phenolic acids, and as a good "growth medium" for *R. graveolens* ssp. *divaricata* in vitro culture (Ekiert 2004; Piekoszewska et al. 2008). In the previous experiment, increases in dry biomass on this medium varied, depending on light conditions, from 2.75 times under far-red light to 6.35 times under blue light.

Accumulation of phenolic acids and cinnamic acid

Ruta graveolens cultures

Ruta graveolens shoots growing on variant I of L-S medium showed an ability to synthesize five phenolic acids, namely: ferulic, p-coumaric, protocatechuic, syringic, vanillic, and also cinnamic acid (Table 1). The tested extracts did not contain caffeic, chlorogenic, rosmarinic, salicylic, sinapic, or p-hydroxybenzoic acids. Both blue light and white light were beneficial for the total accumulation of the compounds determined. Their total amounts depended on light quality and ranged from 34.41 mg 100 g^{-1} DW (UV-A irradiation) to 83.16 mg 100 g^{-1} DW (blue light), and 88.03 mg 100 g^{-1} DW (white light). Three phenolic acids, namely: protocatechuic, p-coumaric and syringic, were the main metabolites that accumulated in the highest amounts under blue light and white light. The maximum amounts of the bioactive protocatechuic acid were about 26 and 32 mg 100 g^{-1} DW, whereas the respective values for p-coumaric and syringic acids were similar and reached about 17–20 mg 100 g^{-1} DW. The accumulation of cinnamic acid was facilitated by monochromatic light: either blue or red or far-red, and UV-A irradiation, with the amounts of 10–14 mg 100 g^{-1} DW. The maximum amounts of vanillic and ferulic acids did not exceed 9.50 and 4.50 mg 100 g^{-1} DW, respectively.

The extracts from the biomass of *R. graveolens* cultivated on variant II of L-S medium contained cinnamic acid and a composition of phenolic acids identical to that of the biomass cultivated on variant I of that medium (Table 1). The highest total amounts of these compounds were also observed in the extracts from the biomass growing under blue light and white light (91.16 and 106.50 mg 100 g^{-1} DW, respectively). These amounts were 1.15 times greater than in the biomass cultured on variant I of L-S medium. The lowest total amounts of the metabolites determined were observed under far-red light (23.63 mg 100 g^{-1} DW). Two compounds dominated quantitatively, namely protocatechuic acid and p-coumaric acid. Their maximum amounts were high and of a similar order of magnitude, about 45 mg 100 g^{-1} DW. The amounts of syringic, vanillic, ferulic and cinnamic acids were lower and did not exceed 12 mg 100 g^{-1} DW for syringic acid and 10 mg 100 g^{-1} DW for the other compounds. Accumulation of all the phenolic acids and cinnamic acid was facilitated by blue light and white light too, while for the accumulation of cinnamic acid red light and far-red light were beneficial.

The maximum total amounts of the metabolites in the extracts from the biomass cultured on variant II of L-S medium were greater in comparison with the biomass from variant I. The maximum amounts of therapeutically interesting compounds, i.e. protocatechuic acid (45.44 mg 100 g^{-1} DW—blue light) and p-coumaric acid (43.14 mg 100 g^{-1} DW—white light) were also higher. A more efficient accumulation of the examined metabolites on variant II of L-S medium confirmed our decision to test the two "productive media" selected in earlier studies (Ekiert et al. 2009).

Table 1 Amounts (mg 100 g^{-1} DW) of phenolic acids and cinnamic acid in extracts of *Ruta graveolens* shoots cultured under different light conditions

Metabolites	White light		Far-red light		Red light		Blue light		UV-A irradiation		Darkness	
	I	II	I	II	I	II	I	II	I	II	I	II
Cinnamic acid	5.04 ± 1.41	3.15 ± 0.57	11.76 ± 1.73	6.68 ± 1.27	13.64 ± 1.80	8.20 ± 0.80	9.60 ± 1.72	Trace	10.55 ± 1.41	Trace	5.59 ± 1.77	1.89 ± 0.09
p-Coumaric acid	19.70 ± 0.98	43.14 ± 0.59	5.83 ± 1.81	6.84 ± 0.43	5.67 ± 1.03	10.37 ± 1.79	17.57 ± 1.16	20.01 ± 1.35	5.63 ± 1.58	7.78 ± 1.75	6.99 ± 1.17	8.45 ± 0.17
Ferulic acid	4.21 ± 1.59	4.22 ± 0.56	3.10 ± 0.73	3.24 ± 0.68	4.34 ± 1.74	2.36 ± 0.06	3.29 ± 1.52	4.87 ± 0.29	1.34 ± 0.26	8.61 ± 0.66	1.94 ± 0.15	9.81 ± 0.12
Protocatechuic acid	32.00 ± 1.42	37.44 ± 1.45	12.18 ± 1.46	3.77 ± 0.22	9.45 ± 1.10	11.23 ± 0.38	26.43 ± 1.36	45.44 ± 0.42	9.53 ± 0.67	24.97 ± 0.28	13.26 ± 1.53	16.57 ± 1.63
Syringic acid	18.33 ± 1.51	9.93 ± 0.56	2.81 ± 0.93	1.38 ± 0.22	5.01 ± 1.91	1.69 ± 0.21	17.10 ± 1.83	11.62 ± 1.61	4.99 ± 1.55	2.70 ± 0.07	3.84 ± 0.85	1.51 ± 0.13
Vanillic acid	8.77 ± 1.71	8.62 ± 0.07	2.50 ± 0.53	1.73 ± 0.01	2.55 ± 0.22	1.87 ± 0.10	9.18 ± 1.48	2.50 ± 0.27	2.36 ± 0.81	2.09 ± 0.07	3.89 ± 0.56	2.50 ± 0.27
Total content	88.03 ± 8.62	106.50 ± 3.80	38.17 ± 7.19	23.63 ± 2.83	40.66 ± 7.80	35.73 ± 3.34	83.16 ± 9.07	91.16 ± 3.94	34.41 ± 6.28	46.15 ± 2.83	35.51 ± 6.03	40.74 ± 2.41

L-S medium variants: I—2 mg l^{-1} NAA and 2 mg l^{-1} BA, II—3 mg l^{-1} NAA and 1 mg l^{-1} BA

Values are the means of three experiments ± SE

Ruta graveolens ssp. divaricata cultures

Extracts of *Ruta graveolens* ssp. *divaricata* biomass cultivated on variant I of L-S medium showed a different composition of phenolic acids in comparison with the biomass of *Ruta graveolens* (Table 2). Apart from protocatechuic and p-coumaric acids and cinnamic acid, they also contained caffeic and chlorogenic acids. Ferulic, rosmarinic, salicylic, sinapic, syringic, vanillic and p-hydroxybenzoic acids were not detected in the tested extracts. The total amounts of the main compounds were highest under blue light and white light. The total amounts of the above-mentioned metabolites varied considerably, from 15.92 mg 100 g^{-1} DW (far-red light) to 99.76 mg 100 g^{-1} DW (blue light) and 106.97 mg 100 g^{-1} DW (white light). The biomass of *R. graveolens* ssp. *divaricata* was characterized by a marked ability to accumulate one compound, p-coumaric acid, which confirmed the results of our earlier studies (Ekiert et al. 2008; Piekoszewska et al. 2008). The maximum amounts of this compound, interesting from a practical perspective, equal to 38.86 mg 100 g^{-1} DW (blue light) and 74.33 mg 100 g^{-1} DW (white light), indicate potential applicability of these results. The maximum amounts of the remaining phenolic acids did not exceed 10 mg 100 g^{-1} DW. Only cinnamic acid was accumulated in higher quantities; its maximum amount was 38.68 mg 100 g^{-1} DW (blue light).

Accumulation of linear furanocoumarins

Ruta graveolens cultures

Four of the seven coumarin compounds tested were found in the biomass extracts from *Ruta graveolens* cultured on variant I of L-S medium irrespective of the light conditions: bergapten, imperatorin, isopimpinellin and psoralen (Table 3). None of the extracts contained marmesin, umbelliferone or xanthotoxin. The total amounts of determined compounds were highest under blue light. They depended on light quality and ranged from 372.01 mg 100 g^{-1} DW (red light) to 1,276.74 mg 100 g^{-1} DW (blue light). The total amount of the compounds was also significant in the extracts from the biomass grown under white light (884.03 mg 100 g^{-1} DW), but 1.44 times lower than in that grown under blue light. The amounts of individual furanocoumarins fluctuated within a wide range, from several milligrams to about 0.5 g 100 g^{-1} DW. Their amounts increased from 2.20 to 6.10 times depending on light quality. Blue light was the most beneficial type of illumination for the accumulation of all the compounds determined. The maximum amounts of psoralen and isopimpinellin were very high, as high as about 500 mg 100 g^{-1} DW, the amounts of bergapten were also high, reaching about 270 mg 100 g^{-1} DW. The imperatorin

Table 2 Amounts (mg 100 g^{-1} DW) of phenolic acids and cinnamic acid in extracts of *Ruta graveolens* ssp. *divaricata* biomass cultured under different light conditions on the L-S medium variant containing 2 mg l^{-1} NAA and 2 mg l^{-1} BA

Metabolites	White light	Far-red light	Red light	Blue light	UV-A irradiation	Darkness
Caffeic acid	5.70 ± 0.05	3.91 ± 0.17	3.55 ± 0.07	5.95 ± 0.22	3.58 ± 0.10	3.90 ± 0.04
Chlorogenic acid	8.65 ± 0.53	Trace	3.05 ± 0.01	9.10 ± 0.20	3.90 ± 0.08	6.07 ± 0.15
Cinnamic acid	12.38 ± 0.87	2.25 ± 0.35	3.75 ± 0.36	38.68 ± 1.90	7.31 ± 0.38	5.32 ± 0.26
p-Coumaric acid	74.33 ± 1.15	6.40 ± 0.03	17.95 ± 2.98	38.86 ± 0.62	23.40 ± 1.07	8.32 ± 0.17
Protocatechuic acid	5.91 ± 0.60	3.37 ± 0.42	5.49 ± 0.31	7.17 ± 0.64	4.06 ± 0.29	3.86 ± 0.26
Total content	106.97 ± 3.2	15.92 ± 0.97	33.79 ± 3.73	99.76 ± 3.58	42.25 ± 1.92	27.45 ± 0.88

Values are the means of three experiments ± SE

content was much lower, equalling 14.02 mg 100 g^{-1} DW. The psoralen content was also the highest in the presence of white light (about 370 mg 100 g^{-1} DW).

The extracts from the biomass growing on the other test variant of L-S medium (variant II) showed the same composition of the metabolites (Table 3). Blue light was also more beneficial for the total amount of linear furanocoumarins than white light.

The total amounts of the compounds were generally lower than in the extracts from the biomass cultured on variant I of L-S medium. They were also dependent on light quality and varied considerably, from 164.22 mg 100 g^{-1} DW (far-red) to 993.75 mg 100 g^{-1} DW (blue light). A significant amount of the compounds was demonstrated to occur also in the extracts from the biomass growing under white light (724.85 mg 100 g^{-1} DW).

The increases in the amounts of individual linear furanocoumarins varied even more than those in the extracts from variant I of L-S medium, and ranged from 4.50 to 23.00 times, depending on light quality. Blue light was also the most effective illumination in stimulating the accumulation of the individual metabolites. Two compounds dominated quantitatively: psoralen and isopimpinellin. The maximum amount of psoralen, of about 500 mg 100 g^{-1} DW, was almost identical to that for variant I of L-S medium. Isopimpinellin was also accumulated in considerable amounts, but its maximum content of about 290 mg 100 g^{-1} DW was 1.69 times lower than on variant I of L-S medium. The accumulation of bergapten was also less efficient (max. 179.50 mg 100 g^{-1} DW). In contrast, the imperatorin content (23.00 mg 100 g^{-1} DW) was 1.64 times higher than on L-S medium variant I. Blue light was also the most effective in stimulating the accumulation of all four compounds. High isopimpinellin content of 288.16 mg 100 g^{-1} DW was also determined in the extracts of the biomass cultured in the dark.

Ruta graveolens ssp. divaricata cultures

Extracts of *Ruta graveolens* ssp. *divaricata* growing on L-S medium variant I were demonstrated to contain a qualitatively richer composition of linear furanocoumarins in comparison with *Ruta graveolens* biomass (Table 4). Five of the seven compounds tested were identified, namely bergapten, imperatorin, isopimpinellin, marmesin and xanthotoxin. The presence of psoralen and umbelliferone was not confirmed. In general, the highest total amount of linear furanocoumarins was produced in the dark. The total amounts of the metabolites were much lower than in the biomass from *R. graveolens* in vitro cultures and also fluctuated within a wide range depending on light quality, from 78.26 mg 100 g^{-1} DW (far-red light) to 262.54 mg 100 g^{-1} DW (darkness). The total amount of linear furanocoumarins in the extracts from the biomass growing under white light was relatively high and amounted to 172.74 mg 100 g^{-1} DW. Among the linear furanocoumarins determined, isopimpinellin dominated quantitatively. Its amount increased, depending on light quality, 7.76 times and reached a maximum value in the dark (206.39 mg 100 g^{-1} DW). The amounts of the remaining four metabolites increased from 3.20 to 26.30 times depending on light quality. Maximum amounts of other therapeutically important compounds were observed for xanthotoxin in the dark (21.37 mg 100 g^{-1} DW), and for bergapten and imperatorin under white light (about 37 and 20 mg 100 g^{-1} DW, respectively).

Discussion

Our studies demonstrated that blue light and white light were the most effective in stimulating the accumulation of free phenolic acids in extracts from the biomass of both *Ruta graveolens* and *Ruta graveolens* ssp. *divaricata* in vitro cultures. The total amounts of these compounds accumulated in the biomass of both plants cultured in vitro under the two types of illumination were comparable.

The blue light and white light were also shown to have a beneficial effect on the total accumulation of linear furanocoumarins in *R. graveolens* cultures. The amount of furanocoumarins obtained in the presence of blue light was 1.44 times higher than under white light.

Table 3 Amounts (mg 100 g^{-1} DW) of furanocoumarins in extracts of *Ruta graveolens* shoots cultured under different light conditions

Metabolites	White light		Far-red light		Red light		Blue light		UV-A irradiation		Darkness	
	I	II	I	II	I	II	I	II	I	II	I	II
Bergapten	267.19 ± 5.22	147.61 ± 6.26	114.13 ± 4.49	39.85 ± 1.19	49.64 ± 3.83	64.54 ± 2.35	266.91 ± 2.38	179.50 ± 6.64	149.70 ± 3.76	102.40 ± 4.26	102.10 ± 1.09	95.22 ± 2.62
Imperatorin	10.70 ± 0.28	12.74 ± 0.89	2.36 ± 0.16	1.00 ± 0.48	2.31 ± 0.52	2.59 ± 0.25	14.02 ± 0.76	23.00 ± 0.38	8.49 ± 0.67	3.72 ± 0.21	7.34 ± 0.02	3.88 ± 1.20
Isopimpinellin	235.55 ± 2.25	233.33 ± 2.87	247.78 ± 8.48	61.22 ± 5.75	224.40 ± 3.38	169.81 ± 3.78	489.12 ± 3.27	288.73 ± 6.10	284.99 ± 3.16	238.52 ± 4.47	269.28 ± 1.10	288.16 ± 5.94
Psoralen	370.59 ± 4.48	331.17 ± 2.77	183.37 ± 8.55	62.16 ± 4.24	95.66 ± 1.30	66.81 ± 2.21	506.68 ± 4.89	502.53 ± 2.31	204.01 ± 6.53	193.58 ± 1.37	126.43 ± 3.67	131.94 ± 0.49
Total content	884.03 ± 12.23	724.85 ± 12.79	547.64 ± 21.68	164.22 ± 11.66	372.01 ± 9.03	303.76 ± 8.59	1,276.74 ± 11.30	993.75 ± 15.43	647.18 ± 14.12	538.22 ± 10.31	505.15 ± 5.88	519.21 ± 10.25

L-S medium variants: I—2 mg l^{-1} NAA and 2 mg l^{-1} BA, II—3 mg l^{-1} NAA and 1 mg l^{-1} BA

Values are the means of three experiments ± SE

All the other lighting conditions were much less advantageous for the accumulation of the majority of the metabolites under study. Only cinnamic acid was accumulated more efficiently under red light and far-red light.

The beneficial effect of white light and blue light on the accumulation of plant metabolites is known. White light has been demonstrated to produce a beneficial effect on the accumulation of alkaloids in *R. graveolens* cultures and of flavonoids in *Citrus aurantium* cultures, as well as on the production of cardiac glycosides by *Digitalis lanata* or indole alkaloids and anthocyanin glycosides in *Catharanthus roseus* cultures (Ramawat and Mathur 2007). White light also stimulates the accumulation of betacyanins in callus cultures of *Suaeda salsa* (Zhao et al. 2010).

Blue light facilitates the accumulation of anthocyanins glycosides in *Haplopappus gracilis* and *Populus* sp. cultures. However, red light has been found beneficial for the accumulation of chlorogenic acid in in vitro culture of *Haplopappus gracilis* (Ramawat and Mathur 2007).

In contrast, the maximum accumulation of linear furanocoumarins in *R. graveolens* ssp. *divaricata* cultures occurred in the dark. This result is not surprising. Our earlier studies on furanocoumarin accumulation in *Ammi majus* cultures proved that the absence of light stimulated the accumulation of bergapten (Ekiert 1988, 1993). Studies of different groups of alkaloids, conducted in other research centers, have demonstrated a disadvantageous effect of white light on the accumulation of caffeine in *Camellia sinensis* cultures, nicotine in *Nicotiana tabacum* cultures and tropane alkaloids in *Hyoscyamus muticus* cultures (Aly et al. 2010). Recently, it has been demonstrated that white light inhibits the biosynthesis of naphthoquinone pigments, shikonin and its derivatives in cell cultures of *Lithospermum erythrorhizon* (Zhang et al. 2010).

The effect of light quality on morphogenesis in in vitro cultures is widely known. The influence of light quality on the induction and expression of embryogenesis in in vitro cultures of *Agave tequilana* var. *Azul* (Rodríguez-Sahagún et al. 2011) and *Campanula punctata* var. *rubriflora* (Sivanesan et al. 2011) and the induction of protocorm-like bodies in in vitro cultures of orchids – *Dendrobium officinale* (Lin et al. 2011) and *Oncidium* sp. (Mengxi et al. 2011) represent good examples. Experiments concerning the influence of light quality on the accumulation of primary and secondary plant metabolites are rare. Thus, our present results are of great value from the point of view of basic research as they document the effect of light quality on the accumulation of bioactive secondary metabolites such as phenolic acids, cinnamic acid and linear furanocoumarins. The study also proves that the optimal light quality for the accumulation of different compounds should be chosen empirically.

Table 4 Amounts (mg 100 g^{-1} DW) of furanocoumarins in extracts of *Ruta graveolens* ssp. *divaricata* biomass cultured under different light conditions on the L-S medium variant containing 2 mg l^{-1} NAA and 2 mg l^{-1} BA

Metabolites	White light	Far-red light	Red light	Blue light	UV-A irradiation	Darkness
Bergapten	37.46 ± 4.02	8.41 ± 3.06	16.80 ± 1.25	8.54 ± 0.90	11.39 ± 0.17	26.63 ± 0.54
Imperatorin	19.76 ± 2.71	3.64 ± 1.90	8.79 ± 0.15	12.51 ± 1.16	4.27 ± 1.76	6.04 ± 1.44
Isopimpinellin	79.67 ± 3.44	63.65 ± 5.65	91.98 ± 6.17	26.60 ± 2.62	59.87 ± 3.58	206.39 ± 6.12
Marmesin	25.89 ± 3.10	1.43 ± 0.06	1.99 ± 0.06	29.49 ± 3.58	1.12 ± 0.05	2.11 ± 0.20
Xanthotoxin	9.95 ± 0.41	1.13 ± 1.09	2.38 ± 1.45	1.78 ± 1.88	3.19 ± 1.29	21.37 ± 1.30
Total content	172.74 ± 13.68	78.26 ± 11.76	121.93 ± 9.08	78.92 ± 10.14	79.84 ± 6.85	262.54 ± 9.60

Values are the means of three experiments ± SE

Some of the results obtained may have practical value. The most important findings include the demonstration that higher total amounts of biologically active linear furanocoumarins can be obtained from cultures maintained under blue light (*R. graveolens* cultures) or in the dark (*R. graveolens* ssp. *divaricata* cultures) than under white light. Such lighting conditions, namely continuous blue light or darkness, can be easily applied to in vitro cultures and the biomass of both plants cultivated in vitro under such conditions can be proposed as a rich, potential source of the therapeutically important metabolites investigated in our study.

The maximum total amounts of the phenolic acids confirmed in the biomass from the in vitro cultures of both plants were 1.60 times greater than (*R. graveolens* culture) or equal to (*R. graveolens* ssp. *divaricata*) their amounts in the above-ground parts of plants growing under natural conditions (Ekiert et al. 2009; Piekoszewska et al.—unpublished). The maximum total amounts of coumarins determined in *R. graveolens* cultures were 2.20 times greater than in the above-ground parts of plants growing in vivo (Ekiert and Gomółka 1999). Only the maximum total amount of coumarins in in vitro cultures of *R. graveolens* subspecies was lower than in the above-ground parts of plants growing in vivo (Ekiert et al. 2005).

The present data confirm that in vitro cultures of both plants are an abundant source of some therapeutically important compounds, namely: *R. graveolens* cultures—protocatechuic acid (45 mg 100 g^{-1} DW), bergapten (about 270 mg 100 g^{-1} DW) and isopimpinellin (about 500 mg 100 g^{-1} DW), and *R. graveolens* ssp. *divaricata* cultures—p-coumaric acid (70 mg 100 g^{-1} DW) and isopimpinellin (about 210 mg 100 g^{-1} DW). Higher amounts of the metabolites determined here can be obtained from cultures with a higher degree of organogenesis, i.e. *R. graveolens* shoot cultures. Our results confirm this known dependence in plant biotechnology (Charlwood et al. 1990).

Conclusions

The results obtained in the present study have shown that the production of free phenolic acids and cinnamic acid, and of linear furanocoumarins is dependent on light quality. White light and blue light stimulated the total production of phenolic acids in cultures of both test plants. The highest total amounts of furanocoumarins were found in *Ruta graveolens* culture under blue light and in the subspecies culture in the absence of light. The maximum amounts of some bioactive metabolites are of interest from a practical point of view.

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