BIOLUMINESCENCE: IS IT POSSIBLE FOR A PLANT?

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An extensive collection of plants gathered in the European part of Russia was screened for a substrate of fungal luciferase. This work was inspired by the recently discovered mechanism of bioluminescence in higher fungi and the structural similarity of fungal luciferin with some plant metabolites. Of all studied leaf extracts obtained from 200 different plants, bioluminescent activity was discovered in 10 species. Each of these species contained a plurality of active compounds. All the luminescent substrates were not identical to fungal luciferin (3-hydroxyhispidin) and were chemically unstable, rendering the attempt to isolate individual compounds for further structural characterization yet unsuccessful. This study is the first step towards engineering a self-luminescent plant based on a fungal enzyme-substrate bioluminescent system.

Keywords: fungal bioluminescence, engineered luminescent plants, luciferins, plant methabolites

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ВОЗМОЖНА ЛИ БИОЛЮМИНЕСЦЕНЦИЯ У РАСТЕНИЙ?

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На основе открытого недавно механизма биолюминесценции высших грибов и сходства структуры люциферина грибов и некоторых метаболитов растений поставлена задача поиска растений, содержащих субстрат(ы) реакции грибной люминесценции. В результате скрининга коллекции растений европейской части России обнаружено 10 видов, экстракты листьев которых проявляют биолюминесцентную активность. Установлено, что изученные виды растений синтезируют не одно, а множество активных соединений. Все люминесцентные субстраты, содержащиеся в растениях, не идентичны грибному люциферину (3-гидроксигиспидину) и химически нестабильны, что препятствует выделению индивидуальных соединений. Данное исследование можно считать первым шагом в создании автономно люминесцентного растения на базе фермент-субстратной системы высших грибов.

Ключевые слова: биолюминесценция грибов, люминесцентные биоинженерные растения, люциферины, метаболиты растений

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Bioluminescence (BL) is the emission of visible light by living organisms. The phenomenon was demonstrated *in vitro* more than a century ago by Raphaël Dubois [1]. He mixed “cold” and “hot” extracts obtained from the light organs of the beetle *Pyrophorus noctilucus*. The extract prepared with cold water comprised a heat-labile enzyme luciferase and the hot water extract contained a heat-stable luciferin. Therefore, light emission from the mixture of the two extracts was the result of a substrate-enzyme reaction. Bioluminescence as a term was first used by Harvey [2]. In all known BL systems oxygen is required for the reaction producing the oxidized luciferin. Relaxation of oxyluciferin from the excited to ground state is accompanied by light emission.

BL is widely spread among animal and fungi kingdoms, but not a one luminescent plant is known in nature [3]. The first attempt for a glowing plant engineering was undertaken more

than 30 years ago [4]. Based on the well-studied BL system of the firefly, the bioengineered plant *Nicotiana tabacum* was obtained by inserting the luciferase gene. When mixing an extract of the plant with a solution of luciferin and ATP, or when immersing the intact plant in the same solution, BL light was detected. An image of the luminous plant was demonstrated by the exposure on an X-ray film. Later some more attempts were performed [5, 6] and even a project on the creation of a luminous plant was announced [7], but no fundamentally new achievements were obtained. Our recent investigation on BL of higher fungi allowed us to believe in the possibility of more successful results using the features of this enzyme-substrate system.

BL systems are very specific within animal taxa whereas higher fungi share a uniform BL mechanism [8]. Fungal BL was considered as a two-step process first in 1961 [9]. A luciferin precursor is reduced by an NAD(P)H-dependent enzyme to a true luciferin and then luciferin is oxidized by air under luciferase catalysis to produce visible light at 520–530 nm [10]. After multiple unsuccessful attempts [11, 12] luciferin precursor was isolated from the fruiting bodies of nonluminous fungus *Pholiota squarrosa* and recognized as hispidin (6-(3,4-dihydroxylstyryl)-4-hydroxy-2-pyrone) [13] — a well-known fungal and plant secondary metabolite [14] (Table 1). Further, hispidin was enzymatically converted into luciferin whose structure has been established as 3-hydroxyhispidin [13] (Fig. 1).

In the literature, there is a number of reports on isolation of hispidin and its derivatives from plants, including *Alpinia zerumbet* [15–17], *Pistacia atlantica* [18], *Peganum harmala* [19], *Pteris ensiformis Burm* [20], *Cassia alata* [21], *Rheum tataricum* [22] and others (Table 1). The wide occurrence of hispidin among fungal and plant species, along with the existence of similar compounds among plant metabolites have logically led us to an idea of challenging the possibility of engineering the self-luminescent plant by introducing a luciferase gene in a plant capable of biosynthesizing its substrate. Like other plant metabolites, hispidin derivatives are in the focus of modern investigations due to possible pharmaceutical applications. Hispidin and its derivatives are characterized by many bioactivities such as antioxidant, anti-cancer, anti-obesity and are regarded as potential leads for drug development [16–19].

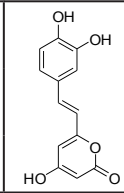
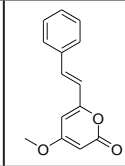
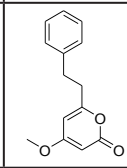
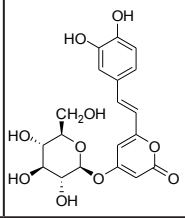
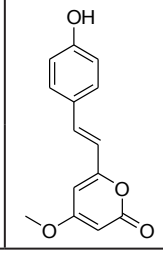
The purpose of this work was screening for the substrate(s) of fungal BL reaction in the extracts from the range of plants occurring in the European part of Russia and isolation of active compound(s).

MATERIALS AND METHODS

Plant extracts

All plant samples were collected in Tver region of Russia in June 2015 (Table 1 in SI). Only leaves were collected. All samples were frozen and stored at –70°C, some of them were dried *in vacuo.*

Table 1. Plants containing hispidin and similar compounds

Compound name	Structure	Species	References
Hispidin		*Alpinia zerumbet*	[15–17]
		Pistacia atlantica	[18]
		Peganum harmala	[19]
5,6-Dehydrokawain		*Alpinia zerumbet*	[15–17]
Dihydro-5,6-dehydrokawain		*Alpinia zerumbet*	[15–17]
Hispidin 4-O-β-D-glucopyranoside		*Pteris ensiformis Burm*	[20]
Bisnoryangonin		*Cassia alata*	[21]
		Rheum tataricum	[22]

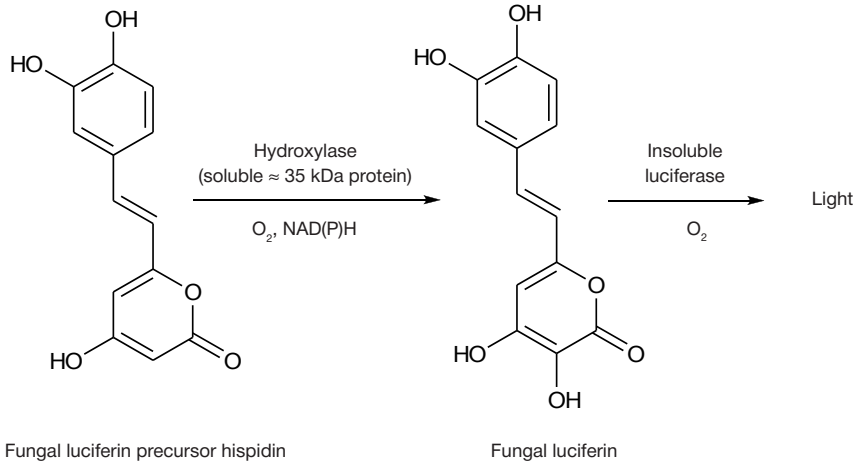


Fig 1. Fungal bioluminescence mechanism

Extraction of plant biomass is a well-designed technique [23]. Acetone was chosen for the initial screening of about 200 samples. Other solvents (methanol, ethanol, acetonitrile) were also tested for optimization of the extract conditions.

For preparation of a plant extract a piece of leave (30–500 mg) and 0.3–1.5 mL of a solvent were shaken in a 1.5 mL tube for 20 min on BioShake XP shaker at 1800 rpm at room temperature. The extract was centrifuged (5 °C, 10000 rcf on a 5424R Eppendorf centrifuge), and the solid residue was discarded. The extract could be further used for SPE or HPLC separation or, alternatively, dried in a vacuum centrifuge MiVac (SpScientific). For further operations dried residues were redissolved in an appropriate solvent and centrifuged. The solvent composition for the dried residue could be different from the solvent in the first extraction, in particular, a buffer solution was added to an organic solvent.

SPE technique with C18 pre-packed cartridges (500 mg, Phenomenex) was used: 0.5–1 mL of initial extract was eluted followed with 1 or 2 mL acetonitrile–water mixture 1 : 1, acetonitrile and acetone in this order.

Preparation of fungal luciferase is described in detail in our previous publication [13].

Bioluminescence assay

The bioluminescence assay comprised two components: fungal luciferase and plant organic extract. A diluted fungal enzymatic extract (3 µL) and a plant organic extract (3, 10 or 15 µl) were added to 100 µL of 0.2 M phosphate buffer, pH 7.5 with 1 mM DTT, in a test tube, immediately shaken and placed in a cuvette of a luminometer (Glomax 20/20, Promega). Luminescence was measured for 10 s with integration time 1 s (Table 2). Extracts from leaves, extracts of their dried residues of the first extracts, fractions after SPE or the fraction after HPLC were tested as organic extracts containing a potential substrate.

HPLC separations were performed on Nexera X2 (Shimadzu) instrument equipped with autosampler SIL-30AC, diode-array detector SPD-M20A and fraction collector FRC 10A. Columns of different types were applied (Table 3). Columns and detector were maintained at room temperature, autosampler temperature was 5°C. Mobile phase: buffer A — 0.02 M ammonium acetate pH 5.5, buffer B — acetonitrile or methanol or acetone (mobile phase composition and gradient see in Table 3). Solvents were of HPLC grade.

RESULTS

To discover a luciferin-like substrate in taxonomically diverse plants samples, the first task was a screening of the 200 species collection. Each plant extract was mixed with the fungal enzyme solution and luminescence was immediately measured. Totally, in 10 cases the luminescence (Table 4) was

Table 2. Samples for BL assay

Table/Figure	Sample preparation	Solvent/total volume, µL	Volume for assay, µL
Table 2	Frozen leaves 100 mg, extract	Acetone/300	3
Fig. 2	*P. natans* frozen leaves 100 mg, extract 300 µL in acetone dried and redissolved	AB solvent*/100	3
Fig. 3; 4, A, B blue	*R. nigrum* (A) and *B. pendula* (B) dried leaves 50 mg, extract	EtOH/1000 MeOH/1000	3 3
Fig. 3; 4, A, B red	SPE fractions of the same extracts	Acetone/1000	3
Fig. 3; 4, B	*B. pendula* leaves 100 mg (frozen) or 50 mg (dried), extract	MeOH/1000	10
Fig. 4	*B. pendula* HPLC fraction 500 µL dried and redissolved	MeOH/50	10
Fig. 5	*P. natans* HPLC fractions 1000 µL dried and redissolved	AB solvent/100	10
Fig. 6	*B. pendula* HPLC fraction of 1000 µL dried and redissolved	MeOH/50	10
Fig. 7	*P. natans* HPLC fractions 300 µL dried and redissolved	AB solvent/50	15
Fig. 8, A	*P. natans* HPLC 500 µL fractions	HPLC solvent/500	10
Fig. 8, B	HPLC fractions 500 µL	HPLC solvent/500	10

Note. * — AB binary solvent (A — acetone, B — 0.1 M NH_4Ac, pH 6.5).

Table 3. HPLC conditions

Figure	Column	Mobile phase*, component B	Sample
5	Discovery C18 5 µm 4.6 × 150 mm	MeCN grad 30–90 % 0–5 min, 90 % 5–10 min, flow 1 mL/min	*P. natans* frozen leaves, 300 mg, in 1 mL acetone, extract 500 µL dried and redissolved in 130 µL of AB solvent, 100 µL on column
6	ZORBAX SB-C18 5 µm 9.4 × 150 mm	MeOH, grad 60–100 % 0–6 min, 100 % 6–45 min, flow 2 mL/min	*B. pendula* fresh leaves, 400 mg in 1500 µL MeOH; SPE fractionated, 800 µL of active fraction in MeCN on column
7	TSK ODS-120T 5 µm 4.6 × 250 mm	Acetone grad 40–90 % 0–7 min, 90 % 7–30 min, flow 1 mL/min	*P. natans* dried leaves, 50 mg in 500 AB solvent, 200 µL on column
8, A	Lichrosorb Diol 10 µm 4.6 × 250 mm	MeCN grad 90–80 % 0–7 min, flow 1 mL/min	*P. natans* frozen leaves, 70 mg in 200 µL acetone, 100 µL on column
8, B	Synergi Polar RP 80A 4 µm 2 × 150 mm	MeCN grad 70–95 % 0–5 min, 95 % 5–14 min, flow 0.7 mL/min	Fraction 3–3.5 min of the first chromatography concentrated to 100 µL

Note. * — Component A — 0.02 M NH_4Ac, pH 5.5.

Table 4. BL activity of plant extracts

Plant	Extract from frozen leaves, luminescence, rel. units	Dried and redissolved extract, total luminescence		
		Compared to initial extract, %	After exposing at 20 °C for 2 h compared to before exposure, %	After exposing at 0 °C for 2 h compared to before exposure, %
Andromeda polifolia	6 600	30	35	no data
Betula pendula	11 500	25	40	90
Chamardaphne calyculata	7 900	35	40	120
Potamugeton natans	290 000	25	6	150
Pyrola rotundifolia	1 500	55	70	130
Ribes nigrum	11 000	40	30	330
Ribes rubrum	8 500	35	20	140
Salix aurita	11 000	20	15	no data
Salix pentandra	10 000	30	15	60
Stachys sylvatica	11 000	20	25	no data

observed. For the initial screening we used only acetone for extraction. The composition of the solvents varied to achieve the maximum luminescence of the extracts with activity found. As a result, some quantitative, but not qualitative differences in BL values were observed. Drying the extract and redissolving of the residue in a smaller volume allowed to concentrate the sample and to increase the BL values. A buffer solution with a certain pH value was added to the organic solvent in some cases, the pH value being critical for the preservation of the extract activity. Thus, the following conditions were the best for frozen leaves of *P. natans*: acetone extraction from leaves material (first extraction) and with mixture 7 : 3 of acetone and aqueous acetate buffer pH 6.5 for redissolving the dried residue (Fig. 2).

The extracts of all active species were tested for stability. They were dried and dissolved again, then exposed for some hours at 0 °C or at room temperature. Measurements of BL activity after each operation demonstrated that all the substrates were unstable (Table 4). Loss of activity during the drying process was 50 % and more. The highest activity (observed for *P. natans* extract) decreased by an order after exposure at room temperature.

When exposing the extracts on ice evident increase of luminescence was observed during some hours. This effect was noticed for many species, and particularly a 10x increase was observed for *R. nigrum* extracts (Table 4, Fig. 3, A). The dependency of activity on the exposure time varied not only between species but also between initial extracts and SPE fractions of the extracts. The extracts from dried leaves of B. pendula seemed to be stable (Fig. 3, B), while the extracts from fresh and frozen leaves showed a significant growth of BL (Fig. 3, C). Furthermore, 10-s curves of the BL measurments for *B. pendula* extracts were often (but not always) of rising type instead of a usually observed declining type (Fig. 4).

Based on the highest value of initial luminescence, we chose *P. natans* as a first candidate to continue with HPLC separation of the BL substrate. Mobile phase conditions for HPLC separation of the extracts were optimized as follows: buffer A 0.02 M aqueous acetate buffer at pH 5.5, elution with gradient buffer B content up to 95 %. HPLC fractions were dried *in vacuo*, dissolved again with 20 times concentration and their luminescence activities were measured (Fig. 5). We observed two zones in the HPLC profile, which comprised active components, along with many components with different UV spectra, indicating that the extract contained more than one substrate and the components were not completely separated.

Looking for isolation of higher amounts of the unstable substrates we varied our technique and tried other species. Fig. 6 represents the results of these attempts. *B. pendula* was chosen as a source. Extract of fresh leaves (400 mg in 1500 µL of methanol, 1000 µL of extract taken) was fractionated on C18 cartridges. Activities of the fractions were measured and 800 µL of the most active fraction was loaded without drying onto a semi-preparative C18 column. Assaying BL activity of the fractions revealed many active zones on a 45 min chromatogram. It can be mentioned, that green color (absorption around 650 nm) always accompanied the activity. We could ascertain the presence of many unresolved peaks in the most active fractions at the beginning of the chromatograms, some active fractions at the end and the absence of correlation of major chromatographic peaks with activity. Second step separation of some fractions on the same column was performed, but activities disappeared due to chemical instability.

In another attempt we used acetone as an unusual mobile phase component. Acetone is not very suitable for UV detection in lower UV region (300 nm and less). However, we supposed that active BL substrates should probably absorb in the region above 300 nm, making possible the use of acetone. The chromatogram and the activity profile are shown on Fig. 7. The profile indicated the presence of several active components. The activities and the amounts of active substances were not high enough to perform the next chromatographic step.

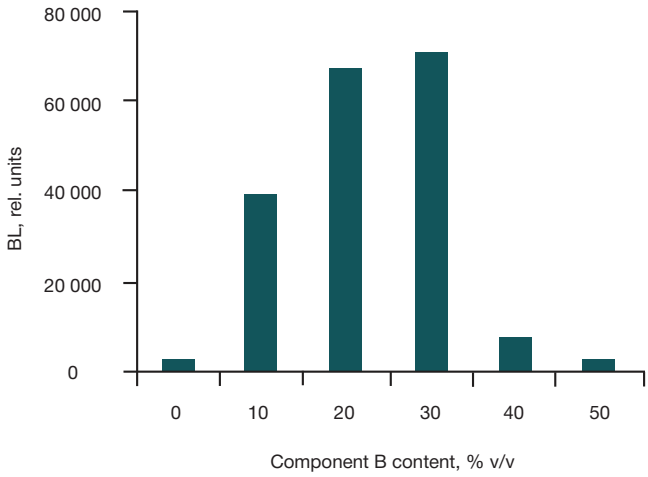


Fig. 2. Dependence of BL (rel. units) on component B content (% v/v) in binary solvent (A — acetone, B — 0.1 M NH_4Ac, pH 6.5) for redissoving of *P. natans* dried extract

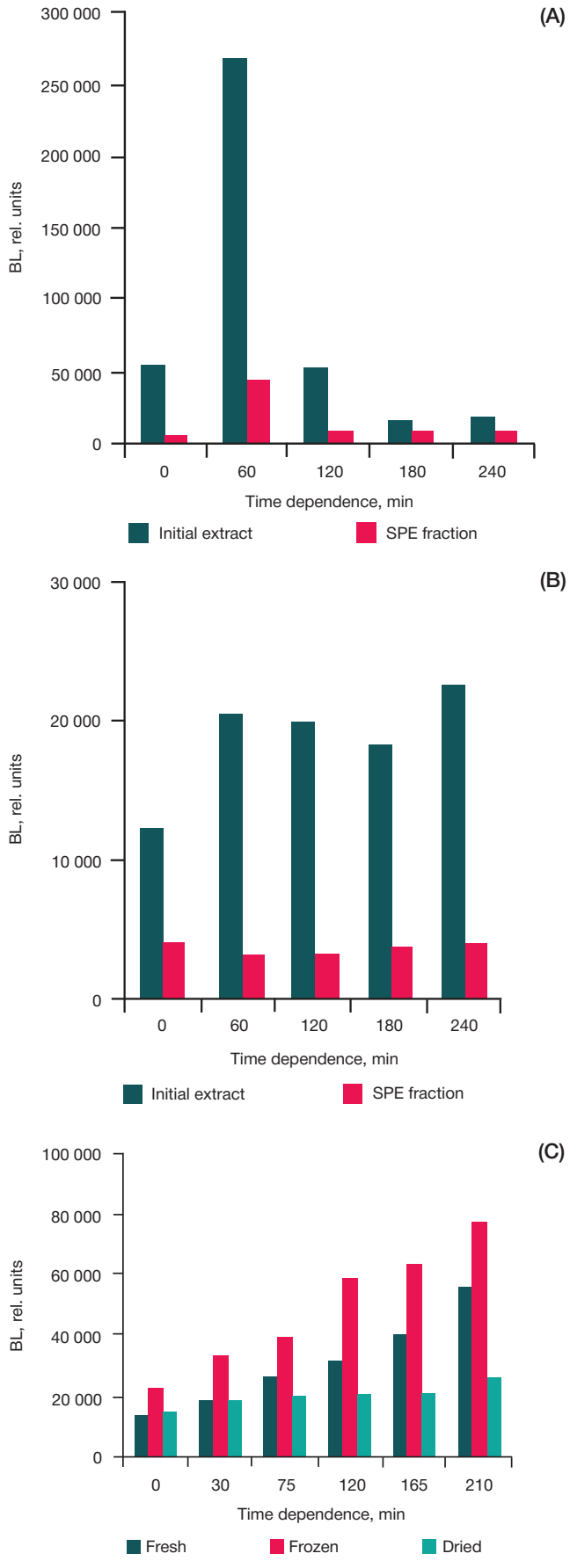


Fig. 3. Time dependence (min) of BL (rel. units) of leaves extracts and SPE fraction of the extracts (exposure at 0 °C): *R. nigrum* **(A)**; *B. pendula* **(B)**; extracts of different type material of *B. pendula* leaves **(C)**

In another attempt we applied faster separation of *P. natans* extracts on a polar column as a first step combined with separation of the most active fraction on a reverse phase column as a second step (Fig. 8). To prevent activity losses extracts and fractions were not dried before BL measurements. The most active 500 µL fraction (between 3 and 3.5 min) from the first column was concentrated to 100 µL and loaded onto the second column. We could reveal some active fractions after the second separation. Two peaks were distinguished on the cromatogram in the region of 11.5 and 12.0 min but separation was not complete and the activities were low.

We estimated the losses of the activity at each stage of the separation by BL measurement (Table 5). Total activity of the solutions was calculated for initial extracts and fractions after separations. These data showed that the losses occurred at each operation. As a result, only 0.2 % of the initial activity remained after final step. Thus, in all cases activity losses during separation did not allow us to isolate any individual substance for further characterization.

DISCUSSION

The study of 10 plants containing BL active compounds showed that our hypothesis about the presence of hispidin or 3-hydroxyhispidine in the leaves samples was wrong. In all cases NAD(P)H was unnecessary for the luminescence reaction, indicating similarity of the active compound to fungal luciferin, but not to hispidin. We applied the same chromatography conditions as those previously developed for isolation of the fungal luciferin [13]. However, activity profiling of HPLC fractions showed no activity and therefore the absence of 3-hydroxyhispidin.

The presence of many active fractions after chromatographic separation of the organic extracts indicates that each plant synthesizes not one but many substrates for the fungal luciferase. In addition, a variety of chromatographic conditions for separation of different plants extracts showed the diversity of their active compound sets. Anomalous increase of bioluminescent activity during exposure of organic extracts on ice suggests the possibility of chemical reactions resulting in decrease of some active compounds concentration and increase in the concentration of others. Unfortunately, no

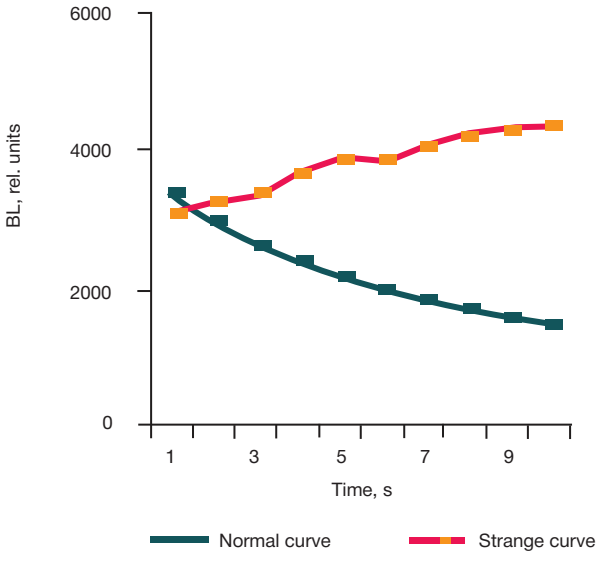
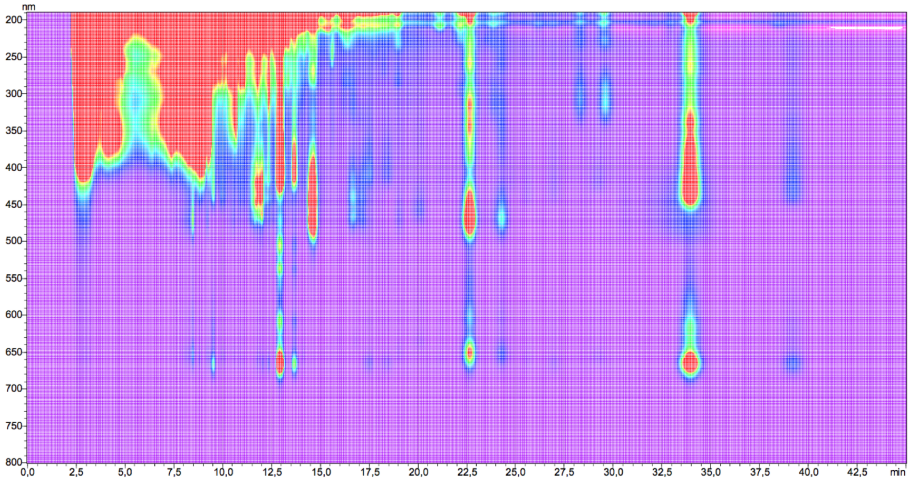


Fig. 4. Two types of 10 s BL assay curves of *B. pendula* HPLC fractions

Fig. 5. HPLC separation of *P. natans* extract: chromatogram (UV 430 nm, blue line), luminescence of fractions (red line) and mobile phase composition (light blue line). All other conditions see Tables 2 and 3

(A)



(B)

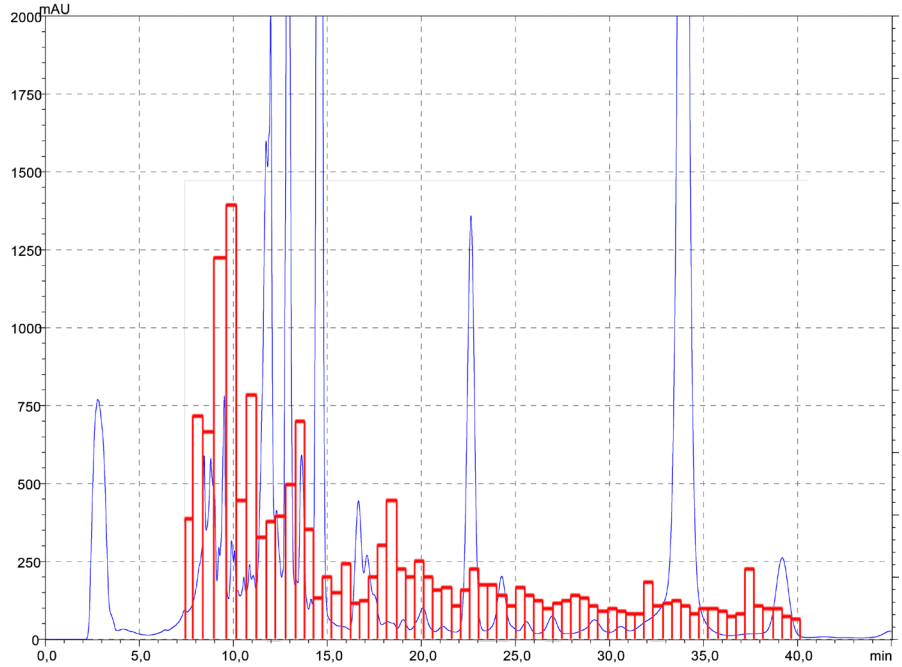


Fig. 6. HPLC separation and luminescence of *B. pendula* extract: **(A)** — UV profile; **(B)** — chromatogram (UV 430 nm, blue) and activity profile (red). All conditions see Tables 2 and 3

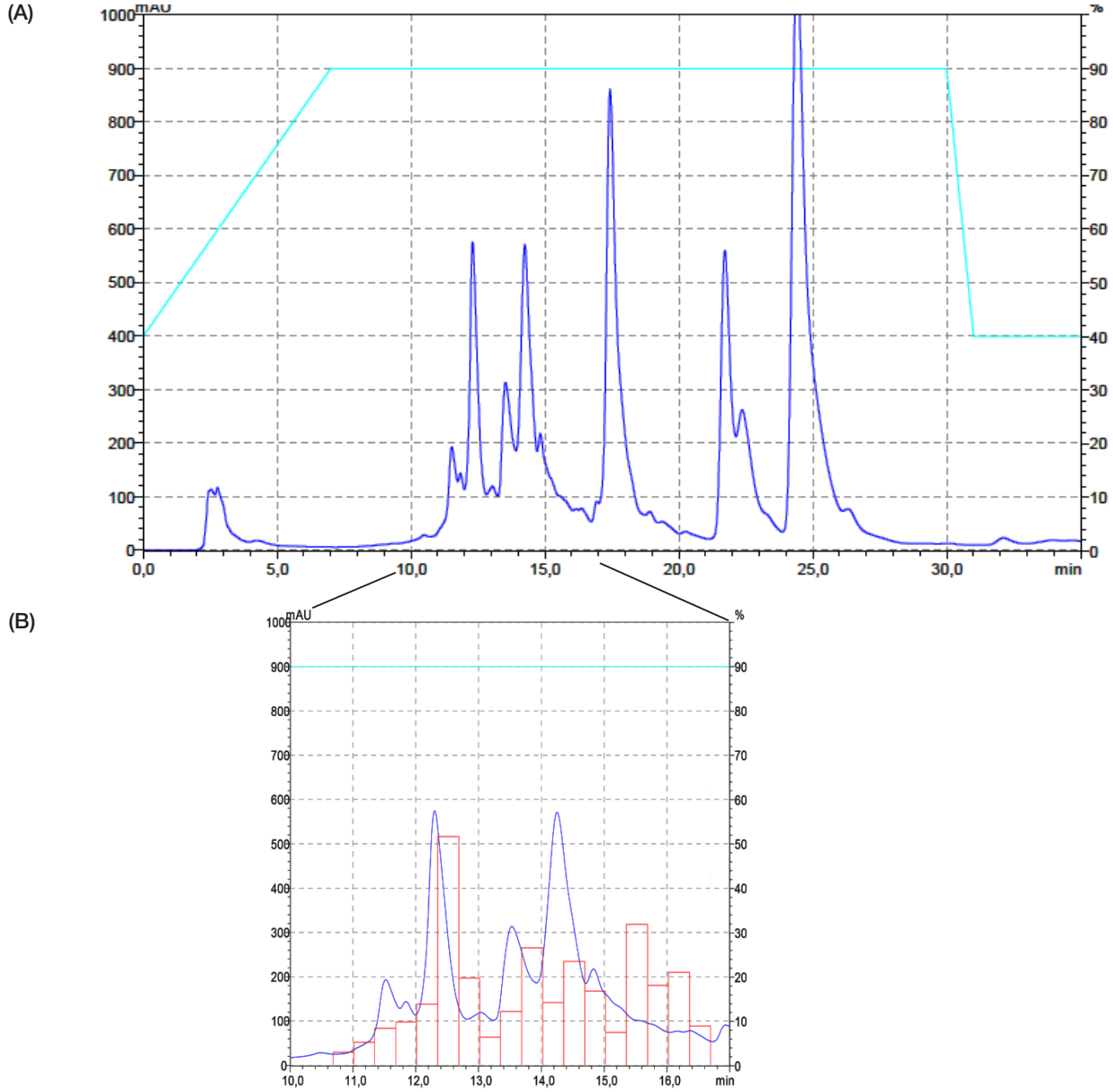


Fig. 7. HPLC separation of *P. natans* extract with acetone as a mobile phase component and luminescence profile: **(A)** — chromatogram (UV 430 nm, blue line) and mobile phase composition (light blue line), **(B)** — region of 10–17 min of the chromatogram (blue line), luminescence of fractions (red line). All conditions see Tables 2 and 3

stable substrates could be detected, as the luminescence of the extracts of all 10 plants decreased at room temperature.

We have applied many modifications for organic extracts preparation and their HPLC separation to satisfy the contradictory requirements of maximum recovery, maximum stability and selective isolation of the individual unstable compound(s) from complex mixtures. Some extracts were fractionated with SPE prior to HPLC, and the others were immediately loaded onto the chromatography column. We tried to dry the extracts for additional concentration, the same was applied to the solutions of the chromatographic fractions before measuring luminescence. Various organic solvents were used as mobile phase components for HPLC; two-dimensional chromatography was performed on columns with different polarity.

But ultimately, the stability of all the active compounds found was insufficient to achieve the substrate isolation in an amount sufficient to establish the compounds' structure and properties.

CONCLUSIONS

Bioluminescent plants do not exist in nature, making their creation by the means of synthetic biology a challenge. This research has revealed the ability of some plant components to react with fungal luciferase to produce luminescence. Thus, the present study may be considered as a first step to creation of self-luminescent plants. Within the species range covered in this work, all potential substrates were chemically unstable, making their isolation and structural characterization yet unsuccessful. However, the following conclusions may be considered in the future attempts: the luminescent luciferase substrates present in the plant biomass extracts are not identical to fungal luciferin (3-hydroxyhispidin); the species tested produce many different active substances. Probably, future work towards self-consistent bioluminescent plants should focus on finding the genes responsible of 3-hydroxyhispidin biosynthesis and expression of these genes in a transgenic plant together with fungal luciferase.

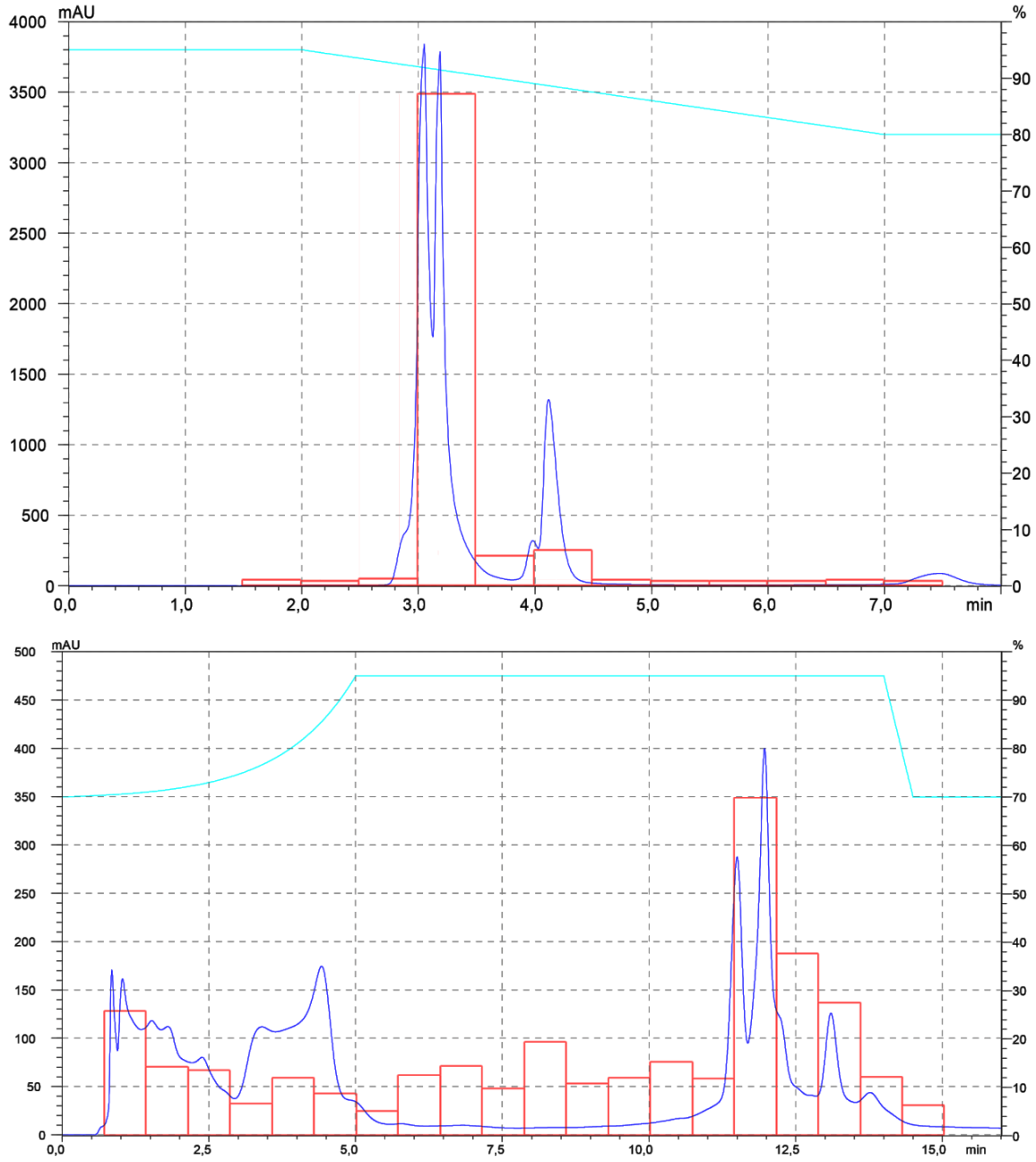


Fig. 8. Two dimensional HPLC separation of *P. natans* extract and luminescence profile: chromatograms (UV 430 nm, blue lines), activities profile (red lines) and mobile phase compositions (light blue line): **(A)** — diol column, **(B)** — RP column. All other conditions see in Tables 2 and 3

Table 5. Losses of activity in separation process (see Fig. 8)

Stage	Activity *a*, rel. units	V_1/V_2*	Total activity *A***, rel. units	Total activity compared to the previous step, %	Total activity compared to initial, %
Extraction	280 000	100/1	28 000 000	100	100
Chromatography #1	52 000	500/10	260 000	9	9
Effect of fractionation	45 000	500/10	230 000	87	8
Drying	27 000	100/3	890 000	39	3
Chromatography #2	6 500	500/10	33 000	37	1
Effect of fractionation	1 200	500/10	59 000	18	0,2

Note. * — V_1 — total solution volume; V_2 — solution volume in measurement; ** — $A = a \cdot V_1/V_2$.

References

1. Dubois R. Fonction photogenique des pyrophores. C R Seances Soc Biol Fil. 1885; 37: 559–62. French.
2. Harvey EN. A History of Luminescence from the Earliest Times Until 1900. Philadelphia, USA: American Philosophical Society; 1957.
3. Shimomura O. Bioluminescence: Chemical Principles and Methods. Singapore: World Scientific Publishing; 2006.
4. Ow DW, DE Wet JR, Helinski DR, Howell SH, Wood KV, Deluca M. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science. 1986 Nov 14; 234 (4778): 856–9.
5. Koncz C, Olsson O, Langridge WHR, Schell J, Szalay AA. Expression and assembly of functional bacterial luciferase in plants. Proc Natl Acad Sci U S A. 1987 Jan; 84 (1): 131–5.
6. Barnes WM. Variable patterns of expression of luciferase in transgenic tobacco leaves. Proc Natl Acad Sci U S A. 1990 Dec; 87 (23): 9183–7.
7. Evans A. Glowing Plants: Natural Lighting with no Electricity. Kickstarter campaign [Internet]. [cited 2017 Apr 3]; [about 20 screens]. Available from: https://www.kickstarter.com/projects/antonyevans/glowing-plants-natural-lighting-with-no-electricit
8. Oliveira AG, Desjardin DE, Perry BA, Stevani CV. Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages. Photochem Photobiol Sci. 2012 May; 11 (5): 848–52.
9. Airth RL. Characteristics of cell-free fungal bioluminescence. In: McElroy WD, Glass B, editors. Light and Life. Baltimore, Md.: Johns Hopkins Press; 1961. p. 262–73.
10. Airth RL, Foerster GE. The isolation of catalytic components required for cell-free fungal bioluminescence. Arch Biochem Biophys. 1962 Jun; 97: 567–73.
11. Isobe M, Uyakul D, Goto T. Lampteromyces bioluminescence. II. Lampteroflavin, a light emitter in the luminous mushroom Lampteromyces japonicus. Tetrahedron Lett. 1988; 44: 1169–72.
12. Hayashi S, Fukushima R, Wada N. Extraction and purification of a luminiferous substance from the luminous mushroom Mycena chlorophos. Biophysics (Nagoya-shi). 2012 Jul 6; 8: 111–4.
13. Purtov KV, Petushkov VN, Baranov MS, Mineev KS, Rodionova NS, Kaskova ZM, et al. The Chemical Basis of Fungal Bioluminescence. Angew Chem Int Ed Engl. 2015 Jul 6; 54 (28): 8124–8.
14. Lee IK, Yun BS. Styrylpyrone-class compounds from medicinal fungi Phellinus and Inonotus spp., and their medicinal importance. J Antibiot (Tokyo). 2011 May; 64 (5): 349–59.
15. Tu PTB, Tawata S. Anti-Obesity Effects of Hispidin and Alpinia zerumbet Bioactives in 3T3-L1 Adipocytes. Molecules. 2014 Oct 15; 19 (10): 16656–71.
16. Be Tu PT, Chompoo J, Tawata S. Hispidin and related herbal compounds from Alpinia zerumbet inhibit both PAK1-dependent melanogenesis in melanocytes and reactive oxygen species (ROS) production in adipocytes. Drug Discov Ther. 2015 Jun; 9 (3): 197–204.
17. Nguyen BC, Taira N, Tawata S. Several herbal compounds in Okinawa plants directly inhibit the oncogenic/aging kinase PAK1. Drug Discov Ther. 2014 Dec; 8 (6): 238–44.
18. Yousfi M, Djeridane A, Bombarda I; Chahrazed-Hamia, Duhem B, Gaydou EM. Isolation and characterization of a new hispolone derivative from antioxidant extracts of Pistacia atlantica. Phytother Res. 2009 Sep; 23 (9): 1237–42.
19. Benarous K, Bombarda I, Iriepa I, Moraleda I, Gaetan H, Linani A, et al. Harmaline and hispidin from Peganum harmala and Inonotus hispidus with binding affinity to Candida rugosa lipase: In silico and in vitro studies. Bioorg Chem. 2015 Oct; 62: 1–7.
20. Wei HA, Lian TW, Tu YC, Hong JT, Kou MC, Wu MJ. Inhibition of low-density lipoprotein oxidation and oxidative burst in polymorphonuclear neutrophils by caffeic acid and hispidin derivatives isolated from sword brake fern (Pteris ensiformis Burm.). J Agric Food Chem. 2007 Dec 26; 55 (26): 10579–84.
21. Samappito S, Page J, Schmidt J, De-Eknamkul W, Kutchan TM. Molecular characterization of root-specific chalcone synthases from Cassia alata. Planta. 2002 Nov; 216 (1): 64–71.
22. Samappito S, Page JE, Schmidt J, De-Eknamkul W, Kutchan TM. Aromatic and pyrone polyketides synthesized by a stilbene synthase from Rheum tataricum. Phytochemistry. 2003 Feb; 62 (3): 313–23.
23. Sarker SD, Nahar L, editors. Natural Products Isolation. Methods in Molecular Biology, vol. 864. New York: Springer Science+Business Media; 2012.

Литература

1. Dubois R. Fonction photogenique des pyrophores. C R Seances Soc Biol Fil. 1885; 37: 559–62. French.
2. Harvey EN. A History of Luminescence from the Earliest Times Until 1900. Philadelphia, USA: American Philosophical Society; 1957.
3. Shimomura O. Bioluminescence: Chemical Principles and Methods. Singapore: World Scientific Publishing; 2006.
4. Ow DW, DE Wet JR, Helinski DR, Howell SH, Wood KV, Deluca M. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science. 1986 Nov 14; 234 (4778): 856–9.
5. Koncz C, Olsson O, Langridge WHR, Schell J, Szalay AA. Expression and assembly of functional bacterial luciferase in plants. Proc Natl Acad Sci U S A. 1987 Jan; 84 (1): 131–5.
6. Barnes WM. Variable patterns of expression of luciferase in transgenic tobacco leaves. Proc Natl Acad Sci U S A. 1990 Dec; 87 (23): 9183–7.
7. Evans A. Glowing Plants: Natural Lighting with no Electricity. Kickstarter campaign [Internet]. [cited 2017 Apr 3]; [about 20 screens]. Available from: https://www.kickstarter.com/projects/antonyevans/glowing-plants-natural-lighting-with-no-electricit
8. Oliveira AG, Desjardin DE, Perry BA, Stevani CV. Evidence that a single bioluminescent system is shared by all known bioluminescent fungal lineages. Photochem Photobiol Sci. 2012 May; 11 (5): 848–52.
9. Airth RL. Characteristics of cell-free fungal bioluminescence. In: McElroy WD, Glass B, editors. Light and Life. Baltimore, Md.: Johns Hopkins Press; 1961. p. 262–73.
10. Airth RL, Foerster GE. The isolation of catalytic components required for cell-free fungal bioluminescence. Arch Biochem Biophys. 1962 Jun; 97: 567–73.
11. Isobe M, Uyakul D, Goto T. Lampteromyces bioluminescence. II. Lampteroflavin, a light emitter in the luminous mushroom Lampteromyces japonicus. Tetrahedron Lett. 1988; 44: 1169–72.
12. Hayashi S, Fukushima R, Wada N. Extraction and purification of a luminiferous substance from the luminous mushroom Mycena chlorophos. Biophysics (Nagoya-shi). 2012 Jul 6; 8: 111–4.
13. Purtov KV, Petushkov VN, Baranov MS, Mineev KS, Rodionova NS, Kaskova ZM, et al. The Chemical Basis of Fungal Bioluminescence. Angew Chem Int Ed Engl. 2015 Jul 6; 54 (28): 8124–8.
14. Lee IK, Yun BS. Styrylpyrone-class compounds from medicinal fungi Phellinus and Inonotus spp., and their medicinal importance. J Antibiot (Tokyo). 2011 May; 64 (5): 349–59.
15. Tu PTB, Tawata S. Anti-Obesity Effects of Hispidin and Alpinia zerumbet Bioactives in 3T3-L1 Adipocytes. Molecules. 2014 Oct 15; 19 (10): 16656–71.
16. Be Tu PT, Chompoo J, Tawata S. Hispidin and related herbal compounds from Alpinia zerumbet inhibit both PAK1-dependent melanogenesis in melanocytes and reactive oxygen species (ROS) production in adipocytes. Drug Discov Ther. 2015 Jun; 9 (3): 197–204.

17. Nguyen BC, Taira N, Tawata S. Several herbal compounds in Okinawa plants directly inhibit the oncogenic/aging kinase PAK1. Drug Discov Ther. 2014 Dec; 8 (6): 238–44.
18. Yousfi M, Djeridane A, Bombarda I; Chahrazed-Hamia, Duhem B, Gaydou EM. Isolation and characterization of a new hispolone derivative from antioxidant extracts of Pistacia atlantica. Phytother Res. 2009 Sep; 23 (9): 1237–42.
19. Benarous K, Bombarda I, Iriepa I, Moraleda I, Gaetan H, Linani A, et al. Harmaline and hispidin from Peganum harmala and Inonotus hispidus with binding affinity to Candida rugosa lipase: In silico and in vitro studies. Bioorg Chem. 2015 Oct; 62: 1–7.
20. Wei HA, Lian TW, Tu YC, Hong JT, Kou MC, Wu MJ. Inhibition of low-density lipoprotein oxidation and oxidative burst in polymorphonuclear neutrophils by caffeic acid and hispidin derivatives isolated from sword brake fern (Pteris ensiformis Burm.). J Agric Food Chem. 2007 Dec 26; 55 (26): 10579–84.
21. Samappito S, Page J, Schmidt J, De-Eknamkul W, Kutchan TM. Molecular characterization of root-specific chalcone synthases from Cassia alata. Planta. 2002 Nov; 216 (1): 64–71.
22. Samappito S, Page JE, Schmidt J, De-Eknamkul W, Kutchan TM. Aromatic and pyrone polyketides synthesized by a stilbene synthase from Rheum tataricum. Phytochemistry. 2003 Feb; 62 (3): 313–23.
23. Sarker SD, Nahar L, editors. Natural Products Isolation. Methods in Molecular Biology, vol. 864. New York: Springer Science+Business Media; 2012.