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Simultaneous determination of nine phytohormones in seaweed and algae extracts by HPLC-PDA

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ABSTRACT

An RP-HPLC-PDA method for the simultaneous analysis of 9 compounds deriving from the phytohormones class was developed and optimized, namely indoleacetic acid (IAA), indolebutyric acid (IBA), phenyleacetic acid (PAA), naphtyleacetic acid (NAA), *trans*-zeatin (TZ), kinetin (KA), isopentenyladenine (IA), 6-benzylaminopurine (6-BA) and abscisic acid (ABA). Validation of the method was performed on the SFE-CO_2 extract made out of the mixture of Baltic algae. The regression coefficients for plant hormones were in the range from 0.997 to 0.999. The LOD and LOQ were on the levels from 0.05–0.29 and 0.15–0.88 mg/L, respectively. Developed method was used for the separation and determination plant hormones in extracts obtained by SFE-CO_2 (supercritical fluid extraction) made out of the mixture of Baltic algae, *Cladophora glomerata* and *Spirulina* sp. In the extract of Baltic seaweed 2 of tested compounds were present in the concentration of 154,45 ± 20,63 μg/g for TZ and 362,47 ± 13,00 μg/g for PAA, whereas in *Cladophora glomerata* extract contained PAA and IAA in the concentration of 229,30 ± 7,90 μg/g and 23,91 ± 0,80 μg/g, respectively (all values per g of extract). The differences in the hormones levels may occur due to the different scale of extract preparation (laboratory or industrial) and other factors like the place of algae collection, year of collection or the way of biomass preparation.

1. Introduction

Phytohormones are the main group of chemicals concluded in cyanobacteria, seaweeds and plants, which are responsible for various processes and can affect different hormonal pathways occurring in these organisms. These chemical compounds are usually divided into five classes: auxins, cytokinins, gibberellins, jasmonates and brassinosteroids. Other substances, which also exhibit hormone-like activity are ethylene, abcisic acid and salicylic acid. These compounds are responsible for, inter alia: cell division, differentiation and elongation, seeds germination, plant parts development, participation in the mobilization of nutrients flowering, fruiting and senescence, so all of the processes, which occur during the plant life cycle [1]. Phytohormones can effect on the plant body in various ways: additively, synergistically or antagonistically and dependently on their content [2]. Plant hormones cause the effects in very small concentrations and are commonly used as plant growth regulators (PGR's). The biomass of algae and seaweeds can trigger the plant growth and development, while being used in different forms: as unprocessed biomass, as compost or as extracts [3–7]. In the agricultural industry, products containing both natural like indoleacetic acid and synthetic phytohormones like NAA and 2,4-dichlorophencoxyacetic acid (2,4-D) are used. Among the structures presented on the Fig. 1 the auxins are constituted by IAA, IBA, PAA and NAA (synthetic), cytokinins by TZ, KA, IA and 6-BA and the structure of abcisic acid is also presented.

In many researches seaweed and algae extracts have been used as the plant growth stimulators of plants from different species and beneficial effects were often described [8–11]. The efficiency and effectiveness of products containing seaweed extracts is based mainly on the species of seaweed used for the production, but also on the content of other formulation components, like microelements, phenolic compounds or amino acids. Mainly brown seaweeds are being manufactured into PGRs, because of the high content of active substances and high availability throughout the year, nevertheless also red and green

Abbreviations: SFE-CO_2, supercritical fluid extraction with carbon dioxide; UAE, ultrasound assisted extraction; SPE, solid phase extraction; DLLME, dispersive liquid–liquid microextraction; HF-DLLLME, holow fiber liquid liquid microextraction; CE, capillary electrophoresis; PGR's, plant growth regulators; IAA, indoleacetic acid; IPA, indole-3-propionic acid; IBA, indolebutyric acid; PAA, phenyleacetic acid; NAA, naphtyleacetic acid; TZ, trans-zeatin; KA, kinetin; KR, kinetin riboside; IA, isopentenyladenine; 6-BA, 6-benzylaminopurine; ABA, abscisic acid; SA, salicylic acid; JA, jasmonic acid; 4-CA, 4-chlorophenoxyacetic acid; OPDA, oxophytodienoic acid; ACC, 1-aminocyclopropane carboxylic acid; 2,4-D, 2,4-dichlorophencoxyacetic acid

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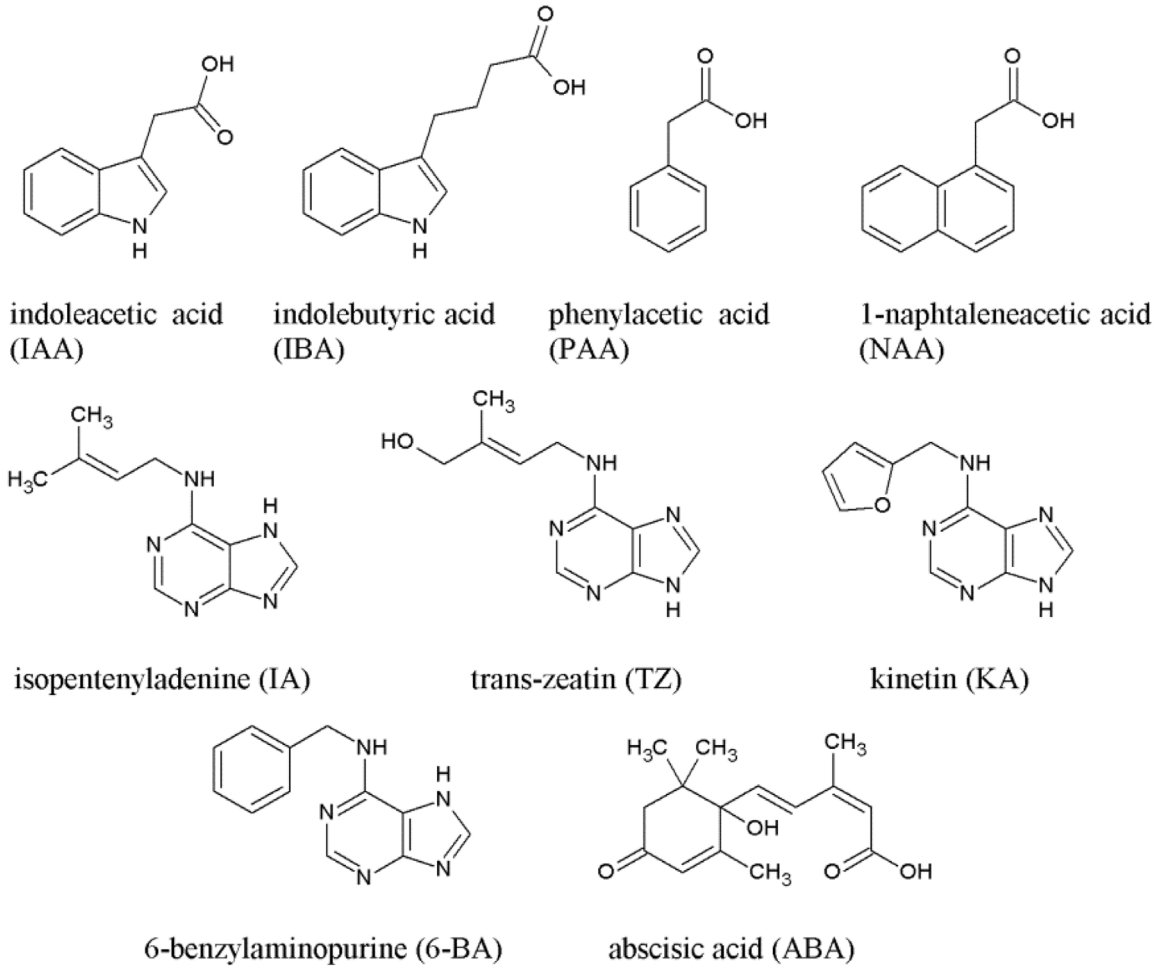

Fig. 1. Chemical structures of the plant hormones used in the HPLC method development (this study).

algae are used for this purpose. Among the species used as a substrate in the biofertilizer production, brown algae like *Ascophylum nodosum*, *Macrocystis pyrifera*, *Laminaria digitata*, *Durvillaea potatorum*, red algae *Kappaphycus alvarezii* or green algae from the genus *Ulva* are being mentioned [12]. Information about stimulating the growth of shoots, leaves, roots or increasing the pigments concentration or the yield of crops by seaweed biofertilizers can be find in the literature [13]. Seaweed extracts do not only stimulate the plant growth directly, but also improves the condition of the environment in which crops grow, for instance promote the growth of beneficial soil microorganisms, induce nodulation or improve moisture-holding capacity of the soil [14–17] or improve the resistance to abiotic stress factors like drought or salinity [18].

Several of the traditional (solvent extraction) and novel extraction techniques can be applied in order to extract the phytohormones from plant samples. Among novel method of extraction microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), pressurized liquid extraction (PLE), enzyme-assisted extraction (EAE) and super-critical fluid extraction (SFE) can be distinguished [19,20]. For the analytical purposes some of the most popular methods of sample purification or pre-concentration are being conducted, for example liquid–liquid extraction (LLE), different types of liquid micro-extraction (LME), solid phase extraction (SPE) or molecularly imprinted extraction (MIPE) [21,22].

SFE is one of the recent and considered as “green”, eco-friendly method of extraction, mainly due to the use of low pressure and temperature levels, low solvent consumption and non-toxicity. It is primarily used for the extraction of non-polar groups of compounds from various biological samples such as fatty acids or pigments. The extraction of polar components is possible, when the co-extractant is added to the reaction vessel. In the case of natural compounds from plants, ethanol or water is widely used for this purpose but this process requires the solvent removal after the extraction [23–26].

The mixture of compounds isolated from plants is usually very complex and the purification and separation steps are needed. However analytes, which are present in the sample in very low concentration may be lost during the clean-up procedures, which itself might be multistage, thus very time and money consuming. Therefore, the industrial production of seaweed and algae extracts should be designed in the way, which limits the necessity of its purification.

Plant hormones can induce positive growth effects on treated plants in very small concentrations. Therefore, it is very useful to check the content of these compounds in the substrates for the natural additives in fertilizer production, in order to estimate the accurate dose of final product, with which plants will be treated. Evaluation of the raw material may prevent the adverse effects, which might occur when the phytohormones content is too high [27]. For this purpose many analytical techniques are conducted and the concentration of active ingredients can be measured [28].

Analytical techniques used for the separation and identification of plant hormones in different samples have been developed and described in many papers. Among them gas chromatography (GC) [29–32], capillary electrophoresis (CE) [33–35] and liquid chromatography (LC) [36–42] combined with several types of detectors like ultraviolet (UV), fluorescence (FL) or mass spectrometer (MS).

The HPLC/LC technique is the most commonly applied one for the