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Short communication

Quantification of plant growth biostimulants, phloroglucinol and eckol, in four commercial seaweed liquid fertilizers and some by-products

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ABSTRACT

Kelp species such as *Ecklonia maxima* are widely used as sources of liquid fertilizer for many economically important crops. Phloroglucinol and eckol are active biomolecules isolated from the brown seaweed *Ecklonia maxima*. They have shown root and growth promoting activity in some crop species. Currently there are many seaweed liquid products available in the markets that are sold as plant biostimulants. In some of these products auxins, cytokinins, polyamines, gibberellins, abscisic acid and brassinosteroids have been quantified and their role in plant growth and development has been well established. But, the presence and quantification of phloroglucinol and eckol in these products have not been determined yet. This would be useful for quality control of the products. In this study, four commercially available seaweed liquid fertilizers were analyzed for phloroglucinol and eckol content using High Performance Liquid Chromatography (HPLC). Additionally, a seaweed dry cake product ('Plant it' manufactured by Kelpak® from *Ecklonia maxima*) and Kelpak® cell wall paste by-product were also assessed for phloroglucinol and eckol content. The liquid seaweed products evaluated in this study were prepared from the seaweeds *Ascophyllum nodosum*, *Durvillaea potatorum* and *Ecklonia maxima*. HPLC analysis of these products showed that eckol content was greater than the known plant growth regulator phloroglucinol in three products. The amount of eckol detected in all the samples ranged from 96 to 860 μg L^{-1}. A higher quantity of both phloroglucinol and eckol was recorded for Kelpak® in comparison to the other products. The higher levels of these two plant growth promoting biomolecules in the Kelpak® samples can be attributed to the use of cold cellular-burst technology. More studies are necessary to determine the optimum levels of phloroglucinol and eckol required for plant growth in different liquid seaweed fertilizers.

1. Introduction

Seaweeds or marine macro algae are important renewable resources. Seaweed extracts promote plant growth and their application is easy and cost-effective [1]. Seaweed extracts are therefore studied extensively for their role in enhancing seed germination, seedling growth, plant growth, fruit set and fruit production. Alleviation of abiotic and biotic stresses and improving post-harvest shelf-life of fruit by application of seaweed extracts are also being thoroughly investigated [2].

Liquid seaweed concentrates (SWC) are the most commonly used commercial agricultural biostimulants. These are made by several processes such as stirring macerated seaweed in vats containing hot water; acidic or alkaline hydrolysis with or without steam; pressure burst techniques or the cell burst method [3]. Various bioactive compounds have been identified and quantified from various seaweeds and commercial SWCs. One such bioactive group of compounds is phlorotannins, the only group of tannins present in brown seaweeds (Phaeophyceae). They are polymers of phloroglucinol (1,3,5-trihydroxybenzene) and occur in relatively large amounts in seaweeds [4–5]. The relationship of phenolic substances to phloroglucinol in brown algae was first mentioned by Crato [6] and subsequently it was confirmed several times [7]. Brown seaweeds accumulate a variety of phloroglucinol-based polyphenols, as phlorotannins of low, intermediate and high molecular weights containing both phenyl and phenoxy units. The molecular weights of phlorotannins vary from 10 to 650 kDa, but commonly fall in the 10 to 100 kDa range [8].

The structural diversity and bioactivity of the phlorotannins are varied due to seasonality, habitat and nutrient availability [4]. With the exception of *Fucus vesiculosus* [9] and *Ecklonia cava* [10], the complexity of the phlorotannin composition in each species has meant that relatively few structures of these molecules have been successfully elucidated. Till now, only a small number of low molecular weight phlorotannins have been isolated and identified. This is attributed to the chemical properties of phlorotannins, which are similar with polymeric nature of production [11].

Among the brown seaweeds, the genera *Ecklonia*, *Eisenia* and *Ishige* are well studied for their phlorotannin content. The *Ecklonia* genus

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contains phloroglucinol (1,3,5-trihydroxybenzene) derivatives, which are eckol (a closed chain trimer of phloroglucinol), triphloroethol-A (an open chain trimer of phloroglucinol),6,6′-bieckol (a hexamer) and dieckol (a hexamer) [12]. Recently we reported a new phloroglucinol derivative, dibenzo [1,4]dioxine-2,4,7,9-tetraol from ethyl acetate fractions of *E. maxima* [12]. Although phlorotannins have been extensively studied for their beneficial health effects [13], there are not many reports on their role in agriculture. Recent research focused on establishing the role of phloroglucinol and eckol in seed germination, seedling and plant growth and overall productivity in crops [14]. According to Rengasamy et al. [14] these two phenolic compounds can significantly mobilize the biochemical pathways essential for crop productivity.

Recently, the effect of phloroglucinol and its derivative eckol, which were isolated from the brown seaweed *Ecklonia maxima* were tested on maize growth and physiology [15]. The activity of the isolated eckol was compared with Kelpak®, commercially available phloroglucinol and the auxin indole-3-butyric acid (IBA). The isolated eckol from *E. maxima* stimulated maize root and shoot growth, number of seminal roots and biochemical activities of α-amylase significantly greater than Kelpak®, phloroglucinol and IBA. Furthermore, eckol showed auxin-like activity at 10^{-5} M in the mung bean rooting bioassay with increases in number of roots, shoot elongation and seedling weight [14]. In another experiment [15], maize kernels were soaked in eckol solution (10^{-6} M) for 18 h prior to planting and were compared with a water control and phloroglucinol solution (10^{-6} M). A number of physiological and biochemical parameters were evaluated after 60 days. Generally, a considerable difference in growth, enzyme activity and secondary metabolite content were observed between the treatments. The roots of eckol-soaked kernels showed higher levels of proteins (124 ± 0 μg g^{-1} Fresh Weight), total phenolic (7.0 ± 0.14 mg GAE g^{-1} Dry Weight) and iridoid glycosides (1.74 ± 0 mg HE g^{-1} Dry Weight) compared to the water control. The phloroglucinol treatment showed a significant increase in total phenolic and iridoid glycosides of maize roots. Eckol and phloroglucinol-soaked kernels showed enhanced α-amylase and malate dehydrogenase activities. Eckol treatment enhanced both growth and biochemical physiology of the maize cultivar used, possibly through synergistic effects with other plant growth hormones [15]. More recent findings show that eckol improves growth of cabbage plants and increases the myrosinase activity, which resists aphid attack through the accumulation of glucosinolate [16]. This result clearly indicates the dual beneficial role of the bioactive molecule eckol which is derived from *Ecklonia maxima*.

Currently, a number of seaweed products are available in the markets, which are sold as plant biostimulants to improve the growth and productivity of a variety of crops. The quantity of phlorotannins (phloroglucinol and eckol) present in these seaweed liquid products has not yet been determined. The aim of this study was therefore to quantify the amount of phloroglucinol and eckol present in some of the branded seaweed liquid products as well as in dried seaweed cake and in the cell wall paste by-product.

2. Materials and methods

2.1. Sample collection and preparation

Four commercially available seaweed liquid concentrates Afrikelp®, Basfoliar®, Kelpak® and Seasol® were purchased from the market. Seaweed cake marketed as 'Plant it', and a cell wall paste by-product were obtained from Kelp Products (Pty) Ltd., Simon's Town, South Africa. All liquid seaweed samples and residue were freeze-dried and used for phlorotannin extraction.

2.2. Reagents

All reagents used in this study were of analytical grade. MilliQ water was obtained using a millipore water system.

2.3. Extraction of phlorotannins

Phlorotannins from the liquid seaweed concentrates were extracted according to Chowdhury et al. [17]. Briefly, freeze-dried liquid seaweed concentrates (5 g); 'Plant it' (dry cakes) and fresh kelp residue were sonicated in methanol (20 mL) for 1 h and then chloroform (40 mL) was added. The mixture was shaken for 5 min and then vacuum filtered through Whatman No. 1 filter paper. After filtration, the mixture was partitioned into upper and lower layers by adding deionized water (15 mL). After shaking, the upper layer was collected and extracted with diethyl ether (30 mL). The extracts were dried completely in a fume hood and the crude phlorotannin residue was dissolved in HPLC grade 80% methanol. The crude extracts were filtered through a 0.22 μm solvent filter (Millipore Corporation, USA) before injecting into the HPLC column. A flow chart for the extraction of phlorotannins is illustrated in Fig. 1.

2.4. HPLC quantification of phloroglucinol and eckol

Phloroglucinol and eckol, which had previously been isolated and characterized from *Ecklonia maxima* were used as standards for quantification. Appropriate concentrations of crude phlorotannin extracts were dissolved in 80% methanol and injected into the HPLC column. To quantify phloroglucinol and eckol, HPLC (Thermo Scientific™ Dionex™ Corporation Sunnyvale, CA, USA) system included a reverse phase (Nucleosil 100-5, C18; Macherey–Nagel, Germany) column. HPLC elution was performed at a flow rate of 1.0 mL min^{-1} using a linear gradient of 10 to 100% methanol over 40 min. The phloroglucinol and eckol were detected by monitoring absorbance at 230 nm using a UV–Vis detector. A 20 μL injection loop was used and triplicate injections (10 μL) were measured. Phlorotannin content was determined by measuring the areas under the peaks of the chromatogram and comparing the values with a standard curve for phloroglucinol and eckol using Chromeleon™ Dionex software version 6.80 SR 11 Build 3161.

2.5. Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA) with GenStat® (17th edition, VSN International, Hemel Hempstead, United Kingdom) statistical package and Duncan's multiple range test was used to separate means ($P < 0.05$).

3. Results and discussion

Afrikelp®, Basfoliar® and Seasol® seaweed liquid samples showed a greater quantity of eckol compared to phloroglucinol content (Fig. 2). In the case of Kelpak® the result was opposite exhibiting a greater quantity of phloroglucinol than eckol. The highest quantities of phloroglucinol and eckol were detected in the Kelpak® samples, which were significantly different from the other samples (Fig. 2). For Basfoliar® product, phloroglucinol was below the detection threshold. Seasol® sample showed a lower quantity of phloroglucinol content compared to the samples of Afrikelp® and Kelpak®. The eckol content in Basfoliar® sample was detected below 100 μg L^{-1}.

The quality and nature of plant growth substances present in the commercial seaweed liquid fertilizer depends on four major factors i.e., 1) selection of species used as a raw material; 2) variations in the extraction procedure and conditions; 3) variations in the formulation protocol; and 4) chemical preservatives used to preserve the final commercial product. The variations of phloroglucinol and eckol content in different seaweed liquid samples might also be due the listed factors. Seasol®, a product of Seasol International Pty Ltd. is produced from the blend of seaweed *Durvillaea potatorum* and *Ascophyllum nodosum*, whereas the other three products evaluated are prepared from *Ecklonia maxima*. It is well documented that phloroglucinol and eckol are higher in order Laminariales compared to the Fucales [18–19]. Although