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Chemical profiling and effects of extracts from Laminaria japonica and Ulva prolifera on rooting and seedling development of common bean

César Freitas Ribeiro

cesarfreitasr@gmail.com

Universidade Federal de Santa Catarina

Geovanna de Oliveira Costa

Universidade Federal de Santa Catarina

Flávia Kauduinski Cardoso

Universidade Federal de Santa Catarina

Alessandro Antônio dos Santos

Universidade Federal de Santa Catarina

Mateus Brusco De Freitas

Universidade Federal de Santa Catarina

Louis Pergaud Sandjo

Universidade Federal de Santa Catarina

Marciel João Stadnik

Universidade Federal de Santa Catarina

Research Article

Keywords: Seaweeds, biostimulants, Laminaria japonica, Ulva prolifera, seed germination, seedling development

Posted Date: April 27th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4277123/v1

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Additional Declarations: No competing interests reported.

Abstract

Seaweed extracts contain a high content of diverse bioactive compounds that can stimulate growth and metabolism of plants. Thus, this study aimed to perform the chemical characterization of *Laminaria japonica* (LLE) and *Ulva prolifera* (ULE) liquid extracts and to assay their impact on seed germination and seedling development of common bean (*Phaseolus vulgaris* L.). Ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS) identified 10 and 17 chemical compounds in liquid extracts *L. japonica* and *U. prolifera*, respectively. Soaking common bean seeds with LLE or ULE increased the germination and seedling vigour. Both seaweed treatments also resulted in increased rooting, fresh weight, and length of roots and shoots in common bean seedlings. Soaking common bean seeds with LLE or ULE modified the protein, carbohydrate, and amino acid content of common bean roots and shoots, as well as the α-amylase activity in roots. Sugars (mannitol and sucrose), organic acids (malic acid), and amino acids found in both seaweed extracts may exert effects on common bean seeds and seedlings, particularly on root development. Additionally, gibberellin, which is also found in ULE, could play a role in these effects. These results accurately describe the biochemical compounds present in two seaweed biostimulants and their effects on common bean seeds and plants.

Introduction

Marine macroalgae are organisms widely utilized by humans for various purposes, including food, agriculture, cosmetic products, medicine, and textiles (Battacharyya et al. 2015; He et al. 2019; Ali et al. 2021). In agriculture, derivatives of seaweeds are utilized as mineral fertilizers, soil conditioners, and biostimulants, playing a crucial role in sustainable agriculture practices (Thorsen et al. 2010; Battacharyya et al. 2015; Ali et al. 2021; Gupta et al. 2022). Algae extracts have effects on plants due to several biochemical molecules present in their structure (Vera et al. 2011; Battacharyya et al. 2015; Wu et al. 2016; Gupta et al. 2022).

Seaweeds are divided into groups of red, brown, and green, comprising more than 10,000 species, which contributes to the presence of a high quantity of chemical compounds in their composition (Battacharyya et al. 2015; Ali et al. 2021). *Laminaria japonica* is a brown macroalgae widely found along the coast and is used as food for human consumption, herbal medicine, and agriculture (Kuwada et al. 2005; Thorsen et al. 2010; Li et al. 2022). *Ulva prolifera*, on the other hand, is a green macroalgae with similar uses (He et al. 2019). Both algae have biochemical molecules capable of influencing the metabolism of plants, including vitamins, minerals, sugars, proteins, amino acids, lipids, and phytohormones (Battacharyya et al. 2015; Ertani et al. 2018; Ali et al. 2021; Gupta et al. 2022). The effect of these molecules can occur individually or in synergy (Battacharyya et al. 2015; Gupta et al. 2022).

Seaweed extracts can exhibit a biostimulant effect not only on seed germination and seedling development but also on overall plant productivity (Castellanos-Barriga et al. 2017; Ali et al. 2021; Gupta et al. 2022). On seeds, biostimulants can induce "seed priming," which is a pre-sowing treatment that leads to improved germination rates and uniformity (Gupta et al. 2022). Biostimulants are substances or microorganisms of biological origin that, when applied to plants through root drench or foliar spray (or both), are intended to stimulate natural processes of efficient plant nutrient uptake and growth, and/or enhance tolerance to abiotic

and biotic stress (du Jardin 2015; Ali et al. 2021). In Brazil, two extracts derived from *Laminaria* and *Ulva* spp. have been commercialized as biofertilizers. However, to date, there has been no scientific evaluation to ascertain whether these products possess any biostimulant effects on plants.

Seed germination is the initial step towards successful seedling establishment and achieving optimal agronomic yield (Gupta et al. 2022). The utilization of sustainable products to enhance germination rates becomes compelling in the pursuit of achieving excellent agronomic productivity. Seeds soaked with *U. lactuca* extract exhibited enhanced seed germination rates and growth parameters in mung beans (*Vigna radiata*) (Hernández-Herrera et al. 2016; Castellanos-Barriga et al. 2017) and tomatoes (*Solanum lycopersicum*) (Hernández-Herrera et al. 2014, 2016). Despite some information about the chemical composition of the algae in these research studies, a more detailed study could provide deeper insights into the specific algal molecules that influence plant responses. Seed treatment with *L. digitata* also increased seed germination rates and rooting of crops and native plants (Thorsen et al. 2010). However, Thorsen and coauthors (2010) did not conduct a chemical characterization of the *L. digitata* extract and attributed its effects to the potential presence of phytohormones and betaines in the extract.

Seed germination is a complex process involving imbibition, activation of enzymes, hydrolysis of stored food reserves, rupture of the seed coat, and emergence of the radicle and plumule (Gupta et al. 2022). Seaweed biostimulants are capable of accelerating these processes, altering the metabolism of seeds and seedlings (Hernández-Herrera et al. 2014, 2016; Gupta et al. 2022). During seed germination and seedling development, α-amylase enzyme (EC 3.2.1.1) breaks down starch reserves to provide sugars and energy to the plant (Rayorath et al. 2008). In this way, nitrogen and amino acid metabolism (Engel et al. 2023) can also be modified due to stimulation by seaweed extract, thereby altering protein synthesis (Baud et al. 2002), as well as the translocation and signalling of phytohormones (Rayorath et al. 2008; Gupta et al. 2022). However, there is a need to identify the active compounds present in seaweed biostimulants, as well as to understand how they regulate the signalling cascade of phytohormones and plant metabolism (Gupta et al. 2022).

Algae possess significant potential for use in seeds and plants due to the high diversity of biochemical compounds present in their structure. This makes seaweed an intriguing resource for agriculture. However, there is still a lack of comprehensive understanding regarding which compounds can positively impact plant growth. Therefore, this study aimed to perform the chemical characterization of two liquid extracts, based on *L. japonica* and *U. prolifera*, and to assay their impact on seed germination and seedling development of common bean.

Materials and methods

Seaweed extracts

Laminaria japonica liquid extract (LLE) is a commercial product known as HidroExal® which has been used in Brazilian agriculture. On the other hand, *Ulva prolifera* (ULE) liquid extract is currently in the testing phase as a commercial product. Both of these products are marketed by ALAS Agricultura Sustentavel company (https://alasagri.com/).

Chromatographic separation and mass acquisition methods

Chromatographic analyses were performed on an *Acquity H-Class* UPLC-PDA system (Waters Co., USA) using a *UPLC BEH* C_{18} (50 x 2.1 mm i.d., 1.7 µm) column maintained at 40 ºC. The mass spectrometer *Xevo GS-2 QTof* used for detection is equipped with an electrospray ionization (ESI) source, a quadrupole orthogonal acceleration, a time-of-flight (QTOF) analyzer operating in both positive and negative modes with the mass range between 50-1500 Da and a scan time of 0.3 s.

The mobile phase system consisted of a gradient of 0.1% aqueous formic acid (pH 3.0) (A) and ACN (B) at a flow rate of 0.3 mL/min. The gradient was composed of 0–1 min 80% (A) and 20% (B); 1–12 min 10% (A) and 90% (B), 12–14 min 90% (B); 14–15 min 10% of (A) and 15–20 min: return to the initial conditions, flow ramp rate of 0.45 min^{-1}. The injection volume was 2 µL.

The instrument settings in the positive mode were a capillary voltage of 3.0 kV; sampling cone voltage of 40 V; source offset voltage of 80 V; desolvation temperature of 300 ºC; source temperature of 90 ºC; cone gas flow of 100 L/h and desolvation gas flow of 900 L/h. Nitrogen was used as nebulizer gas and argon as collision gas. MS and MS^E modes (in two scan functions) were acquired in centroid mode and monitored with a scan time of 1 s. The collision energy was 6 eV in function 1 and ramped from 25 to 35 eV in function 2. FDDA cycles included an m/z referring to MS1 above a threshold of 90000, these being the peaks selected as precursors for subsequent fragmentation. MS/MS spectra were recorded while the threshold remained above 30000 (Gao et al. 2020). To assure accurate mass values, data were corrected during the acquisition by an external reference (LockSprayTM) named leucine-enkephalin solution (1 ng/mL) at a flow rate of 20 µL/min. System control and data processing were performed with MassLynx 4.1 software (Waters Co., USA). All samples were prepared by dissolution of each extract in water to reach a concentration of 800 mg L^{-1} (0.8 µg L^{-1}).

Non-targeted metabolomic data acquired with UPLC-ESI-QToF (Xevo G2-S QTof, Waters) proceeded to deconvolution and peak extraction by MS-DIAL software (4.9), ion table was exported as msp file to MS-FINDER for structure elucidation. Data collection was performed with 0.02 Da MS1 tolerance and 0.06 Da MS2 tolerance, isotope recognition for maximum charged number 1. Peak detection was applied with 10000 amplitudes for minimum peak height and 0.1 Da for mass slice width. Deconvolution parameters were set as follows: sigma window value of 0.5 and MS/MS abundance cutoff of 30 amplitudes. Alignment was performed considering retention time tolerance of 0.05 min and MS1 tolerance of 0.015 Da for all data obtained in data-dependent acquisition (DDA) mode. Features based on blank information were removed by a minimum 10-fold change between sample max over blank average peak height (Tsugawa et al. 2015).

Bioassay for seed and seedling assessments

Germitest bioassay was conducted according to Paulert et al. (2009) and methods of the Association of Official Seed Analysts (AOSA 2005) with some modifications. For this experiment, seeds of the common bean (*Phaseolus vulgaris* L.) cultivar Uirapuru (Ribeiro et al. 2023) were used. The seeds were initially surface-sterilized by immersing them in a 2.5% sodium hypochlorite (NaClO) solution for 5 minutes, followed by three rinses in sterile distilled water for 1 minute each. After surface sterilization, the seeds were then

soaked in LLE (at concentrations of 1, 2, and 4 mL/L), ULE (at concentrations of 1, 2, and 4 mL/L), or water (as a control) for 4 hours at 25°C, to allow for absorption of the solutions (333 µL per seed). Then, seeds were placed equidistantly (5 columns x 5 rows) on two moist sheets of filter paper (50 × 50 cm; Whatman no.1), covered with a paper sheet, rolled up and placed in a plastic bag. Subsequently, the plastic bags containing the seeds were transferred to a germination chamber maintained at a temperature of 25 ± 1°C with a humidity level of at least 95%, and kept there until the evaluation time. Four replications were performed for each treatment (100 seeds per replication).

Germination parameters of seed assessments

For evaluating germination parameters, germinated seeds (radicle longer than 2 mm) were counted daily over 9 days after soaking (das). The variables evaluated included germination percentage (GP) = (number of germinated seeds/total number of seeds)×100, germination index (GI) = Σ(Gt/Tt), in which Gt is the number of seeds germinated on day *t* and Tt is the number of days, mean germination time (MGT) = Σ(D×n)/Σn, which n is the number of seeds germinated on day D and D is the number of days counted from the beginning of the test, and seedling vigour index (SVI) = (seedling length (cm)×germination percentage (AOSA 2005; Hernández-Herrera et al. 2014). GP was measured in seeds soaked with concentrations of 1, 2, and 4 mL/L at 9 days, while the other variables were evaluated only at a concentration of 2 mL/L, in accordance with the product recommendation. Five seedlings were sampled from the central row of sheets of filter paper for bioassays related to root development, seedling growth, and biochemical analyses.

Seedling development assessment

The number of seedlings with secondary roots formed was determined by counting the plants with these root types longer than 2 mm. The length of primary and secondary roots, as well as the thickness of primary roots, were measured using a digital calliper. The thickness of the primary roots was measured up to 3 mm above the point where the first secondary root emerged. The number of secondary roots was determined by counting the secondary roots that were longer than 2 mm.

Fresh and dry weight of roots, shoots and seedlings were determined according to Hernández-Herrera et al. (2016). Plant parts were dried in an oven at 60°C for 72 hours to obtain dry weights. Lengths of roots, shoots, and seedlings were measured using a digital calliper.

Biochemical analyses of seedlings

Roots and shoots were collected at 6 and 9 das. The material was rapidly weighed, frozen in liquid nitrogen, and stored at -80°C until analysis. The frozen plant parts were grounded using a chilled mortar and pestle in liquid nitrogen and then homogenized with potassium phosphate buffer (200 mM, pH 7.0) at a ratio of 1:5 (w:v). The homogenate was centrifuged at 16,000 ×g for 10 min at 1°C, and the supernatant was collected (extract sample) and placed on ice for further analysis (Ribeiro et al. 2023). Biochemical analyses were determined spectrophotometrically using a microplate reader (SpectraMax Paradigm, Molecular Devices, USA).

The total protein content was determined according to Bradford (1976) by mixing 15 µL of the extracted sample with 185 µL of Bradford reagent (Sigma-Aldrich). Following this, the mixture was incubated for 15

minutes at 25°C. The absorbance was read at 595 nm, and the standard curve was obtained using bovine serum albumin (BSA) as the standard, and the results were expressed in mg g^{-1}.

Carbohydrate content was determined using the methods of Dubois et al (1956). A mixture of 100 µL of the sample and 2.5 µL of phenol reagent (80%) was prepared. Subsequently, 250 µL of sulfuric acid was added to the mixture and incubated for 10 minutes at 25°C. The absorbance was read at 490 nm, the standard curve was constructed using glucose as the standard, and the results were expressed in mg g^{-1}.

Amino acid content was determined using the method of Moore and Stein (1948). To achieve this, 20 µL of the extract was mixed with 170 µL of buffer solution (0.2 M sodium citrate, pH 5, containing 5% ninhydrin and 2% KCN in methylcellulose). Following this, the mixture was incubated for 20 minutes at 100°C. Subsequently, 1.3 mL of 60% ethanol was added to the mixture. The absorbance was then read at 570 nm, and the standard curve was constructed using glycine as the standard, and the results were expressed in mg g^{-1}.

The α-amylase (EC 3.2.1.1) enzymatic activity was measured according to the method of Bernfeld (1955) with modification. For this, 200 µL of the extract sample was mixed with 120 µL of CaCl2 (3 mM) and incubated at 70°C for 5 minutes. After that, 200 µL citrate buffer solution (100mM, pH 5.0) and 100 µL of starch solution (1%) were added to the mixture. After 5 min, 400 µL of 3,5-dinitrosalicylic acid reagent (DNS – 1%) was added to the mixture. The absorbance was then read at 540 nm, and the standard curve was constructed using α-amylase from *Bacillus* sp. (Sigma-Aldrich) as the standard, and the results were expressed in UA mg g^{-1}.

Statistical analyses

The experiments were conducted following a completely randomized factorial design, with one factor being the seaweed liquid extract (LLE, ULE, and water). For seed and seedling assessments, the replications were based on the mean of 100 seeds and five central seedlings, respectively. Each experiment comprised four replications and was independently repeated twice.

After confirming the normality of the data sets using the Shapiro-Wilk test ($p \geq 0.05$) and verifying variance homogeneity using the Bartlett test ($p \geq 0.05$), analysis of variance (ANOVA; $p \geq 0.05$) was performed. Multiple comparisons were conducted using the Tukey and Kruskal-Wallis tests, with significance set at $p \leq 0.05$. When necessary, data transformation was used before analysis to meet ANOVA assumptions. The statistical analyses were carried out using the R software (R Core Team 2020).

Results

Chemical composition of seaweed liquid products

The chromatograms in the positive and negative modes (Fig S1) of LLE (HidroExal® - *L. japonica*) provided the identification of 10 compounds (Table 1). Peak **1** generated a precursor ion in positive more with *m/z* 383.0942 at t_R 0.46 min corresponding to $C_{12}H_{24}O_{11}$, no fragmentation was observed and by literature assessment, the compound was assigned as maltitol, a sugar derivative identified from *L. japonica* (Kuwada

et al. 2005). Peak **2** provided a precursor ion in negative mode with *m/z* 133.0119 at t_R 0.49 min corresponding to $C_4H_6O_5$. The compound was identified as malic acid, the present organic acid as well as others are described as a part of algal chemical components (Li et al. 2022). Peak **3** provided a precursor ion in positive mode with *m/z* 293.0977 at t_R 0.57 min corresponding to $C_{10}H_{16}N_2O_8$. Online database assessment identified the compound as edetic acid (DRUG BANKa 2023) with *m/z* 132.0653 $[C_5H_9NO_3 + H]^+$ as a fragment match used for identification and a database score of 69.40/100.

Table 1
Chemical profile of *Laminaria japonica* liquid extracts (LLE).

	t_R (min)			ESI(+) $[M + H]^+$	ESI(-) $[M-H]^-$	Fragment	
Peak No		**Identification**	**Molecular formula**	**(m/z)**	**(m/z)**	**ESI(+)**	**ESI(-)**
1	0.46	Maltitol	$C_{12}H_{24}O_{11}$	383.0942 (-3.7)			
2	0.49	Malic acid	$C_4H_6O_5$		133.0119		
3	0.57	Edetic Acid	$C_{10}H_{16}N_2O_8$	293.0977 (-0.88)			
4	0.64	Germicidin N	$C_{11}H_{16}O_4$	235.0938 (-3.4)			
5	5.08	NI		573.2509			
6	6.44	NI			447.1336		
7	6.66	Sphingosine	$C_{18}H_{39}N_1O_3$	318.3005 (-0.9)			
8	6.66	2-Amino-1,3,4,5-eicosanetetrol	$C_{20}H_{43}N_1O_4$	362.3280 (2.8)			
9	7.43	NI	$C_{24}H_{31}O_6$	415.2137 (3.9)		437.1924 (-3.7), 453.1677 (-0.4)	
10	14.00	Long chain ceramide (Cer(d18:0/15:0))	$C_{33}H_{67}N_1O_3$	526.5196 (-0.43)*			
* Database: LipidMAPS							

Peak **4** provided a precursor ion in positive mode with *m/z* 235.0938 $[M + Na]^+$ at t_R 0.64 min corresponding to $C_{11}H_{16}O_4$, the peak was signed as germicidin N a metabolite identified in a plant as a product of endophytic fungi *Streptomyces* sp metabolism (Li et al. 2013). Peaks **7** and **8** provided precursors with *m/z* 318.3005 and 362.3280 as a coelution at t_R 6.66 min corresponding to molecular formulas $C_{18}H_{39}NO_3$ and $C_{20}H_{43}NO_4$, no fragmentation was observed and based on literature information, these molecular formulas correspond to the structures of sphingosine. Phytosphingosine is related to marine microalga cell

composition (Shiels et al. 2021). Moreover, 2-Amino-1,3,4,5-eicosanetetrol is reported to be found in certain endemic plants (Rummun et al. 2021) and fungi metabolism (Chen et al. 2018).

A long-chain ceramide was assigned to peak **10** showing a precursor ion of m/z 526.5196 at t_R 14 min with a molecular formula of $C_{33}H_{67}N_1O_3$. Identification was performed by computational metabolomic software. This compound is part of cell membrane components.

The chromatograms in the positive and negative modes (Fig. S2) of ULE (*U. prolifera* liquid seaweed product) provided the identification of 17 compounds (Table 2). Peak **1** generated a precursor ion in positive more with *m/z* 181.0712 at t_R 0.42 min corresponding to $C_6H_{14}O_6$, no fragmentation was obtained and by literature search, the compound was assigned as mannitol (Torres-Benítez et al. 2022). Peaks **2** and **4** showed their precursor ions at *m/z* 383.0942 $[M + H]^+$ and 381.0801 $[M + K]^+$ at t_R 0.46 and 0.53 min corresponding to $C_{12}H_{24}O_{11}$ and $C_{12}H_{22}O_{11,}$ respectively. The first provided a match with the mass spectra database MS FINDER® which indicated the structure of maltitol with a score of 8.45/10. The second was identified as sucrose. The identified saccharides are well-known in marine alga (Kuwada et al. 2005).

Table 2
Chemical profile of *Ulva prolifera* liquid extract (ULE).

Peak No	t_R (min)	Identification	Molecular formula	ESI(+) [M + H]$^+$ (m/z)	ESI(-) [M-H]$^-$ (m/z)	Fragment ESI(+)	Fragment ESI(-)
1	0.42	Mannitol	$C_6H_{14}O_6$		181.0712 (-0.6)		
2	0.46	Maltitol	$C_{12}H_{24}O_{11}$	383.0942 (-3.7)		367.1228, 205.0693	
3	0.49	Malic acid	$C_4H_6O_5$		133.0119 (4.5)		
4	0.53	Sucrose	$C_{12}H_{22}O_{11}$	381.0801 (0.5) [M + K]$^+$			
5	0.56	Mesaconic acid	$C_5H_6O_4$		129.0188 (0.0)		
6	0.64	NI		283.0440			
7	0.71	2-Isopropylmalic acid	$C_7H_{12}O_5$		175.0601 (-2.9)		
8	1.15	Guanine	$C_5H_5N_5O_1$	152.0171			
9	1.23	NI			276.0903		
10	5.08	NI		573.2509			
11	6.58	Hexadecasphinganine	$C_{16}H_{35}N_1O_2$	274.2754 (2.9)			
12	6.66	Phytosphingosine	$C_{18}H_{39}N_1O_3$	318.3005 (-0.9)			
13	6.66	2-Amino-1,3,4,5-eicosanetetrol	$C_{20}H_{43}N_1O_4$	362.3280 (2.8)			
14	10.40	2,3 didehydro-Gibberellin A_9	$C_{19}H_{22}O_4$	315.1564 (-10.2)			
15	11.76	NI	$C_{11}H_{24}N_4O_1$	267.1641 [M + K]$^+$ (20.2)		251.1874 [M + Na]$^+$ (-10.4)	
16	13.00	MG(16:0/0:0/0:0)	$C_{19}H_{38}O_4$	353.2667 [M + Na]$^+$ (-1.4)[a]			

Databases: a) KNApSAcK,ChEBI,SMPDB,YMDB,FooDB, LipidMAPS,UNPD NPA,COCONUT,MINE; b) LipidMAPS

Peak No	t_R (min)	Identification	Molecular formula	ESI(+) [M + H]$^+$ (m/z)	ESI(-) [M-H]$^-$ (m/z)	Fragment ESI(+)	Fragment ESI(-)
17	14.00	Long chain ceramide (Cer(d18:0/15:0))	$C_{33}H_{67}N_1O_3$	526.5196 (-0.43)[b]			
Databases: a) KNApSAcK,ChEBI,SMPDB,YMDB,FooDB, LipidMAPS,UNPD NPA,COCONUT,MINE; b) LipidMAPS							

Peaks **3** and **5** provided precursor ions in negative mode at t_R 0.49 and 0.56 min with *m/z* 133.0119 and 129.0188 corresponding to molecular formulas $C_4H_6O_5$ and $C_5H_6O_4$, identified as mallic and mesaconic acids. The compounds are reported by literature for playing important roles in germ cell formation and the release of *U. prolifera* (He et al. 2019) and *Laminaria japonica* (Li et al. 2022). Peak **7** provided a precursor ion at t_R 0.71 min with *m/z* 175.0601 corresponding to the molecular formula $C_7H_{12}O_5$, its structure was identified as 2-isopropylmalic acid. This assigned structure is consistent with those identified in algae species such as *Spirulina platensis*, *Ulva fasciata*, *Sargassum hornschuchii* and *Pterocladia capillacea* (Ghallab et al. 2022).

Peaks **11**, **12** and **13** provided precursor ions with *m/z* 274.2754, 318.3005, and 362.3280 at t_R 6.58 and a coelution at 6.66 min. Corresponding molecular formulas $C_{16}H_{35}NO_2$, $C_{18}H_{39}NO_3$, and $C_{20}H_{43}NO_4$ led by a literature search to the structures of sphingosines namely hexadecasphinganine (Abeytunga et al. 2008), phytosphingosine (Shiels et al. 2021) and 2-amino-1,3,4,5-eicosanetetrol (Chen et al. 2018). The compounds have been identified as part of the chemical components of cell membranes in some species of fungi (Rupčić and Marić 1998; Chen et al. 2018).

Peak **14** was identified using a mass selection tool, with a precursor ion at t_R 10.40 with *m/z* 315.1564 corresponding to the molecular formula $C_{19}H_{22}O_4$, identified by literature as 2,3 didehydro-gibberellin A_9 (Hedden and Sponsel 2015) (Fig. S3), the compound is obtained by dehydrogenation across the 2,3-position of gibberellin A_9. Chromatogram and mass spectra for the identified phytohormone are provided in supplementary material. The last metabolite was signed as peak **15** with a precursor ion at t_R 11.76 min. The compound showed precursor ion $[M + K]^+$ and adduct ion $[M + Na]^+$ corresponding to the molecular formula $C_{11}H_{24}N_4O_1$. By literature assessment no compound could be matched and the metabolite could not be identified.

A software-based knowledge provided by MS-DIAL and MS-FINDER indicated the presence of metabolites **16** and **17**, database scored 77.78 and 53.78 on a scale of 100. Database information is presented in Table 1. The metabolite structures are related to monoacylglyceride and ceramide, both related as part of the membrane cell.

Effect of seaweed liquid products on seeds germination.

GP from seeds soaked with water remained around 66% at 9 das (Fig. 1A). In seeds soaked with LLE at 2 and 4 mL/L, as well as with ULE at 1, 2, and 4 mL/L, the GP was 1.2 and 1.3 times higher, respectively, compared to the control treatment (Fig. 1A). Over 9 das, GP in seeds presoaked with water was 12% at 1 das to 66% at 8 das (Fig. 1B). On the other hand, GP in seeds presoaked with LLE or ULE was 1.2 times higher at 5 das, and this increase was consistent up to 8 das (Fig. 1B).

GI and SVI of seeds presoaked with water remained around 71.4 and 12.7, respectively, at 9 das (Table 3). In contrast, GI in seeds presoaked with LLE or ULE was 1.2 times higher, while SVI was 1.9 times higher than seeds soaked with water (Table 3). No differences were detected in MGT between seeds presoaked with water, LLE or ULE at 9 das (Table 3).

Table 3
Germination index (GI), mean germination time (MGT) and seedling vigour index (SVI) of common bean at 9 days after soaking seeds with *Laminaria japonica* (LLE) or *Ulva prolifera* (ULE) liquid extracts or water.

	GI	MGT (days)	SVI
Water	71.4 ± 6.5b	5.5 ± 0.06ns	12.7 ± 1.8b
LLE	88.4 ± 7.2a	5.5 ± 0.09	23.7 ± 2.5a
ULE	92.3 ± 9.2a	5.4 ± 0.11	24.3 ± 1.7a
Letters indicate significant difference between the means of the same column (Tukey's test, $p \leq 0.05$).			
ns: indicate non-significant difference between means of the same column by analysis of variance (ANOVA; $p \leq 0.05$).			

Effect of seaweed liquid products on seedling development

Seeds soaked with water did not exhibit seedlings with secondary roots formed at 3 das (Fig. 2A and B), whereas treatment using LLE or ULE exhibited 34 seedlings with this root type (Fig. 2A and B). At 4 das (Fig. 2A and C), seeds with control treatment showed 28 seedlings with secondary roots formed, while in seeds soaked with LLE or ULE the number of these seedlings was 35% higher (Fig. 2A).

Primary root length was 3 and 3.5 cm at 3 and 4 das, respectively, in seedlings from seeds soaked with water (Fig. 3A). At 3 das, primary root length was 46% higher in seeds soaked with LLE than in control treatment, while this root length in ULE treatment was 22% higher than LLE treatment (Fig. 3A). Difference in secondary root length was observed only 4 das, when seedlings from seeds soaked with water exhibited 0.4 cm of length (Fig. 3A). At this moment, secondary root length was 2 times higher in seeds soaked with LLE than the control treatment, being ULE treatment 1.5 times higher than LLE treatment (Fig. 3A).

The thickness of primary root seedlings from seeds presoaked with water was 2 mm at 3 and 4 das (Fig. 3B). Conversely, this thickness was 25% higher in seedlings from seeds soaked with ULE. The number of secondary roots in seedlings from seeds soaked with water was 1 at 4 das, in contrast, this number was 4 and 5 times higher in LLE and ULE treatment (Fig. 3C).

Fresh-weight roots, shoots and seedling from seeds soaked with water were 0.95, 4.28 and 5.23 g at 9 das (Table 4). On the other hand, fresh-weight roots were 70 and 58% higher, shoots were 22 and 18%, and seedlings were 32 and 25% in LLE and ULE, respectively. No differences were detected in dry-weight roots, shoots and seedlings between seeds presoaked with water, LLE or ULE at 9 das (Table 4). Length of roots, shoots and seedlings from seeds soaked with water were 7.6, 7.7 and 15.3 cm at 9 das (Fig. 4A). In contrast, fresh weight roots were 76 and 84% higher, shoots were 40 and 45% and seedling were 58 and 64% in LLE and ULE, respectively (Fig. 4A and B).

Table 4
Fresh and dry weight of roots, shoots and seedling of common bean at 9 days after soaking seeds with *Laminaria japonica* (LLE) or *Ulva prolifera* (ULE) liquid extracts or water.

	Fresh weight (g)			**Dry weight (g)**		
	Root	Shoot	Seedling	Root	Shoot	Seedling
Water	0.95 ± 0.16b	4.28 ± 0.33b	5.23 ± 0.39b	0.06 ± 0.02b	0.83 ± 0.08ns	0.89 ± 0.09ns
LLE	1.61 ± 0.11a	5.19 ± 0.38a	6.80 ± 0.38a	0.11 ± 0.02a	0.76 ± 0.14	0.87 ± 0.15
ULE	1.50 ± 0.09a	5.02 ± 0.30a	6.51 ± 0.42a	0.09 ± 0.03ab	0.78 ± 0.12	0.87 ± 0.14
Letters indicate significant difference between the means of the same column (Tukey's test, $p \leq 0.05$).						
ns: indicate non-significant difference between means of the same column by analysis of variance (ANOVA; $p \leq 0.05$).						

Effect of seaweed liquid products on the biochemical of seedlings

The total protein content in roots and shoots was 0.28 and 0.42 mg mg^{-1}, respectively, for seeds soaked with water, LLE, or ULE at 6 das (Table 5). At 9 das, the total protein content in these parts of the plant from seeds soaked with water was 0.17 and 0.27 mg mg^{-1}. Conversely, at 9 das, the total protein content in roots and shoots from seeds soaked with LLE and ULE was, on average, 47% higher than that in the control treatment (Table 5).

Table 5
Protein, carbohydrate and amino acid content and α-amylase activity of common bean at 6 and 9 days after soaking (das) seeds with *Laminaria japonica* (LLE) or *Ulva prolifera* (ULE) liquid extracts or water.

	6 das							
	Protein (mg g^{-1})		Carbohydrate (mg g^{-1})		Amino acid (g mg^{-1})		α-amylase (UA g mg^{-1})	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Water	0.28 ± 0.09ns	0.42 ± 0.15ns	1.26 ± 0.24ns	1.31 ± 0.17ns	52.86 ± 8.10b	88.60 ± 17.93b	0.34 ± 0.15b	0.89 ± 0.31ns
LLE	0.29 ± 0.08	0.39 ± 0.16	0.84 ± 0.38	1.35 ± 0.17	79.32 ± 10.25a	132.94 ± 16.33a	0.76 ± 0.23a	0.80 ± 0.19
ULE	0.36 ± 0.09	0.49 ± 0.13	1.10 ± 0.38	1.31 ± 0.12	78.68 ± 15.38a	117.18 ± 32.47ab	0.66 ± 0.10a	0.89 ± 0.32
	9 das							
Water	0.17 ± 0.04B	0.27 ± 0.04b	1.31 ± 0.21a	0.78 ± 0.21b	53.46 ± 9.37ns	69.32 ± 15.45b	0.32 ± 0.13b	0.84 ± 0.22ns
LLE	0.32 ± 0.07A	0.44 ± 0.07a	0.91 ± 0.15b	1.47 ± 0.35a	59.66 ± 7.82	141.78 ± 32.78a	0.36 ± 0.16b	0.68 ± 0.23
ULE	0.38 ± 0.14A	0.41 ± 0.06a	0.78 ± 0.19b	1.47 ± 0.21a	62.80 ± 10.75	140.07 ± 34.29a	0.84 ± 0.25a	0.79 ± 0.31

ns: indicate non-significant difference between means of the same column by analysis of variance (ANOVA; $p \leq 0.05$).

Lowercase and capital letters indicate significant difference between the means in the same column of each das by Tukey ($p \leq 0.05$) and Kruskal-wallis test ($p \leq 0.05$), respectively.

For statistical analysis, α-amylase root data at 9 das data were transformed to √X.

At 6 das, roots and shoots exhibited carbohydrate contents of 1.26 and 1.31 mg mg^{-1}, respectively, in seeds soaked with water, LLE, and ULE (Table 5). The carbohydrate content in these parts of the plant from seeds soaked with water was 1.31 and 0.78 mg mg^{-1}, respectively, at 9 das. In contrast, the carbohydrate in roots from seeds soaked with LLE and ULE was, on average, 31% lower compared to the control treatment, while in shoots, it was 47% higher at this time (Table 5).

The amino acid content in roots and shoots from seeds soaked with water was 52.86 and 88.60 mg mg^{-1}, respectively, at 6 das (Table 5). Conversely, at the same time, the amino acid content was 33% higher in roots from seeds soaked with LLE and ULE, while in shoots, this biochemical content was 25% higher only in seeds soaked with LLE. At 9 das, the amino acid content in shoots from seeds soaked with water was 53.46 and

69.32 mg mg^{-1} (Table 5). On the other hand, only the roots of the LLE and ULE treatments were 51% higher than the control treatment.

The α-amylase enzymatic activity in roots from seeds soaked with water was 0.34 and 0.32 UA mg mg^{-1} at 6 and 9 das, respectively (Table 5). In contrast, the activity of this enzyme was, on average, 49% higher in roots from seeds soaked with LLE and ULE at 6 das, and 62% higher in the ULE treatment at 9 das. In shoots from seeds soaked with water, LLE and ULE, the α-amylase enzymatic activity was 0.89 and 0.84 UA mg mg^{-1} at 6 and 9 das, respectively (Table 5).

Discussion

Seaweeds contain several organic compounds in their structural composition with potential applications in agriculture. Some of these compounds have effects on the physiological and biochemical metabolism of seeds, seedlings, and plants (Battacharyya et al. 2015; Castellanos-Barriga et al. 2017; Ali et al. 2021). The use of algae extracts on plants and their impacts have been widely studied in agriculture; however, there is not complete knowledge of their potential effects on agronomic plants (Rayorath et al. 2008; Hernández-Herrera et al. 2016; Castellanos-Barriga et al. 2017; Gupta et al. 2022). In this way, our studies contribute to knowledge regarding the chemical characterization of two algae-based products and their effects on seed germination and seedling growth, including modifications in their biochemistry.

Laminaria and *Ulva* liquid extracts exhibit several chemical compounds, including sugars, organic acids, and phytohormones, that have the potential to affect plants and induce metabolic changes. Mannitol, maltitol, and sucrose are sugars found in algae (Kuwada et al. 2005; Battacharyya et al. 2015; Torres-Benítez et al. 2022). Mannitol is an osmo-protector that plays a vital role in osmoregulation (Kuwada et al. 2005). External application of mannitol has been shown to increase antioxidant enzyme activity in wheat and protect the roots against lipid peroxidation under stressful conditions (Seckin et al. 2009). Therefore, this substance can affect roots. Furthermore, mannitol residue can be present in the terminal reducing of laminarins (Vera et al. 2011), which confer the property of inducing resistance against plant pathogens (Vera et al. 2011; Ali et al. 2021).

Sucrose appears to be a nutrient that can increase lateral root formation and primary root elongation (Xu et al. 2010; Roycewicz and Malamy 2012). In our research, seedling quality and seed germination from seeds soaked with sucrose (0.1, 0.2 or 0.4 g/L) did not exhibit a difference compared to the control treatments (data not shown). Thus, the sugars present in seaweeds can stimulate the growth of seedlings and roots, while also potentially promoting a resistance to abiotic and biotic stress.

Organic acids are present in several seaweeds and play important roles in their germ cell formation (He et al. 2019). In plants, the addition of malic acid to nutrient solutions was able to protect Al-sensitive wheat seedlings from phytotoxic Al concentrations, particularly influencing root apices (terminal 3–5 mm of the root) (Delhaize et al. 1993). It is suggested that the chemical compounds present in LLE and ULE can have an effect on roots and provide resistance against abiotic stress. In contrast, some organic acids, such as edetic acid, 2-isopropylmalic acid and mesaconic acid, have not been described in the literature regarding their effects on seeds or plants. Therefore, further research is necessary to better understand their potential

effects on plants. Additionally, organic acids can be considered an emerging category of biostimulants (Gupta et al. 2022), making them interesting chemical compounds for new tests and research on plants.

Germicidin (Li et al. 2013) and sphingosine (Veerman et al. 2010), found in two liquid seaweed extracts, such as hexadecasphinganine and phytosphingosine, have demonstrated antimicrobial efficacy, suggesting potential effects on plants or microorganisms. Thus, conducting in vitro tests against common bean pathogens may provide valuable information about the antimicrobial potential against phytopathogens of interest. Besides that, sphingosines can exhibit bioactive properties (Abeytunga et al. 2008; Veerman et al. 2010). Amino acids in seaweed biostimulants, such as 2-Amino-1,3,4,5-eicosanetetrol and guanine present in both liquid extract, can function as bioactive and elicitor components in plants (Ali et al. 2021; Gupta et al. 2022).

Gibberellin is an important phytohormone produced by higher plants, also found in seaweeds (Rayorath et al. 2008; Battacharyya et al. 2015; Ali et al. 2021; Gupta et al. 2022), responsible for promoting the growth and elongation of cells in plants (Rayorath et al. 2008; Hedden and Sponsel 2015). However, high concentrations can have inhibitory and deregulatory effects on growth properties (Hedden and Sponsel 2015). Nevertheless, the levels of Gibberellin A9 in ULE might promote seed germination, as well as the growth of roots and shoots in common beans. Although not detected, low gibberellin contents have been reported in *Laminaria* spp liquid extract (Ertani et al. 2018), suggesting a possible presence and effect on plants of this hormone in LLE. Laminarin is another compound found in *Laminaria* spp. that, upon contact with seeds, causes significant alterations in metabolism (Wu et al. 2016).

Seed germination is the initial step for successful seedling establishment and achieving optimal agronomic yield (Gupta et al. 2022) and liquid extracts derived from *L. japonica* or *U. prolifera* (LLE or ULE) appear to impact these processes. Additionally, this process affects crop production and determines the success or failure of future yields, beginning with imbibition and breaking dormancy seed (Rayorath et al. 2008; Gupta et al. 2022). Furthermore, seed germination involves hydrolysis of stored reserves, activation of enzymes, and emergence of the radicle and plumule (Rayorath et al. 2008; Gupta et al. 2022).

Seeds soaked with LLE or ULE exhibit initially enhanced germination parameters and root development. The enhancement of these parameters is likely attributed to the synergistic interactions among a group of molecules present in algae (Hernández-Herrera et al. 2014; Battacharyya et al. 2015; Gupta et al. 2022). Exogenous gibberellin has been shown to modify and enhance the germination and amylase activity of wheat seeds (Wang et al. 2016). This important phytohormone, along with sugars (such as mannitol and sucrose) and organic acids (such as malic acid) present in seaweeds, may strongly influence seed germination and root growth. Additionally, algae contain a rich array of vitamins and minerals that have the potential to nourish the seedling and positively influence these developmental processes (Hernández-Herrera et al. 2014; Battacharyya et al. 2015; Ali et al. 2021).

Soaking seeds with LLA or ULE leads to alterations in the biochemical metabolism of common bean seedlings. Modifications in the number of amino acids and proteins observed in seedlings from seeds treated with LLE or ULE may be involved in nitrogen metabolism (Engel et al. 2023) and protein synthesis (Baud et al. 2002). Engel and coauthors (2023) observed that treatments of soybean seeds with the brown seaweed

Ascophyllum nodosum modified gene expression related to nitrogen metabolism, in addition to increasing seed production. These end-of-cycle results (R5) corroborate our findings, indicating a significant alteration in nitrogen metabolism increasing plant productivity.

The α-amylase is a key enzyme for seed germination and seedling development, playing a critical role in the hydrolysis of starch (Rayorath et al. 2008), exhibiting high activity in roots from seeds soaked with LLE and ULE. During these processes, polysaccharides are broken down into simple sugars such as glucose and fructose through hydrolysis, resulting in energy production and a continuous supply of carbon skeletons for the biosynthesis of primary cellular components (Rayorath et al. 2008). Significant changes in carbohydrate content may be linked to readily available energy resources (Baud et al. 2002) crucial for seedling development, resulting in increased length and fresh weight of seedlings. Furthermore, the synthesis and secretion of α-amylase in the aleurone layer might be substantially induced by gibberellic acid in plants (Rayorath et al. 2008; Hedden and Sponsel 2015), which also is present in ULE. Furthermore, even in the absence of gibberellin, other compounds present in *L. japonica* may increase alpha-amylase activity in plants, as observed in seed imbibition with *Ascophyllum nodosum* (Rayorath et al. 2008). Quantitative and qualitative research provides new insights to better understand the metabolic changes in plants that received treatments with algae.

Conclusion

Our initial research identified 10 and 17 different chemical compounds in two seaweed liquid extracts, based on *Laminaria japonica* and *Ulva prolifera*, respectively. These compounds, when applied to common bean seeds during soaking, can act individually or synergistically to improve seed germination and seedling development, thereby modifying amino acids, proteins and carbohydrates content, as well as α-amylase activity, of seedlings. Sugars such as mannitol and sucrose, organic acids like malic acid, amino acids, and gibberellin may exert effects on common bean seeds and seedlings, particularly on root development. Other chemical compounds found in our extract, such as germicidin, sphingosine, 2-isopropylmalic acid, and 2-Amino-1,3,4,5-eicosanetetrol, have not been fully described in terms of their effects and require further studies regarding their impact on plants.

Our findings suggest that LLE and ULE are potent biostimulants recommended for common bean plants, with a potential priming effect on seeds (Gupta et al. 2022). However, further studies are necessary to determine their possible impact on common bean cultivation in farm settings. In addition, It is possible that the effects of both biostimulants on plants may manifest in plant physiology, particularly in photosynthetic rates (Castellanos-Barriga et al. 2017) and the efficiency of stomatal uptake (Hernández-Herrera et al. 2016).

Declarations

Ethical Approval

Not applicable

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Availability of data and materials

The materials and data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Author Contributions Statement

CFR, FKC, AAS and MBF performed the experiments with seeds and seedlings. GOC analyzed the seaweed extracts using UPLC, wrote the manuscript text portion, and prepared figures 1-2 and supplementary material. CFR performed the statistical analyses. CFR wrote the main manuscript text and prepared figures and table. MBF, LPS and MJS corrected and reviewed manuscript text, figures and table. All authors reviewed the manuscript.

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Figures

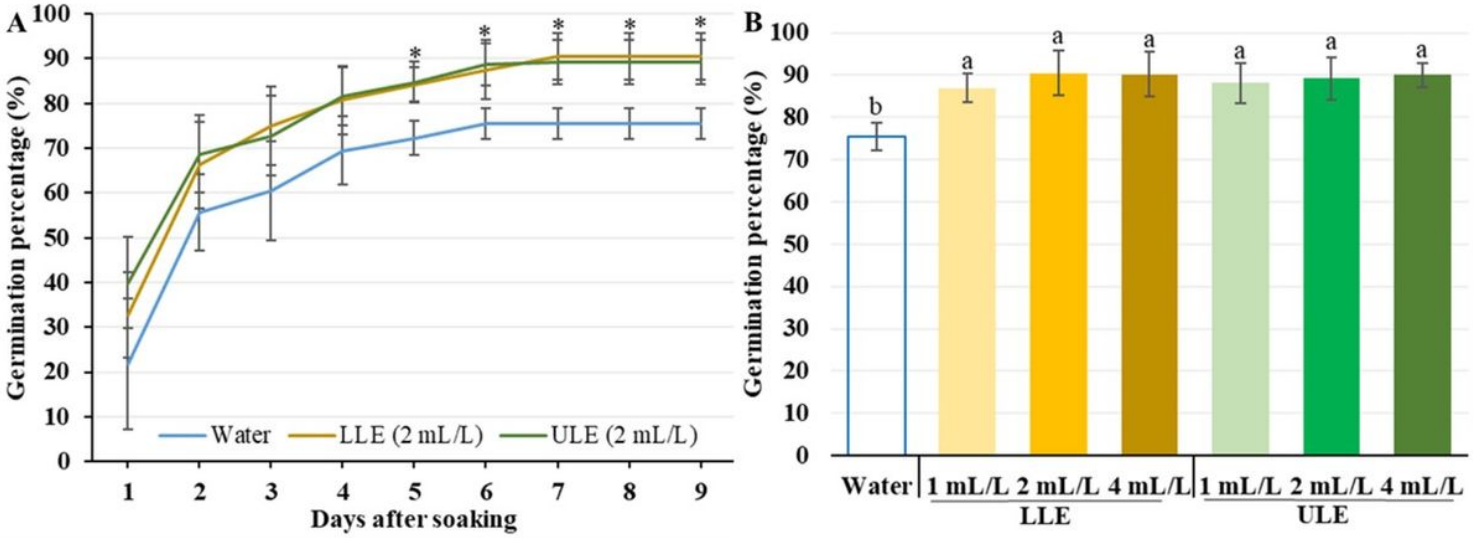


Figure 1

Germination percentage of common bean seeds over time (A) and 9 days after soaking (B) seeds with *Laminaria japonica* (LLE) or *Ulva prolifera* (ULE) liquid extracts or water. Bars represent standard deviation. * and letters indicates significant difference between the means of seaweed liquid products and control in the same day by Tukey test ($p \leq 0.05$).

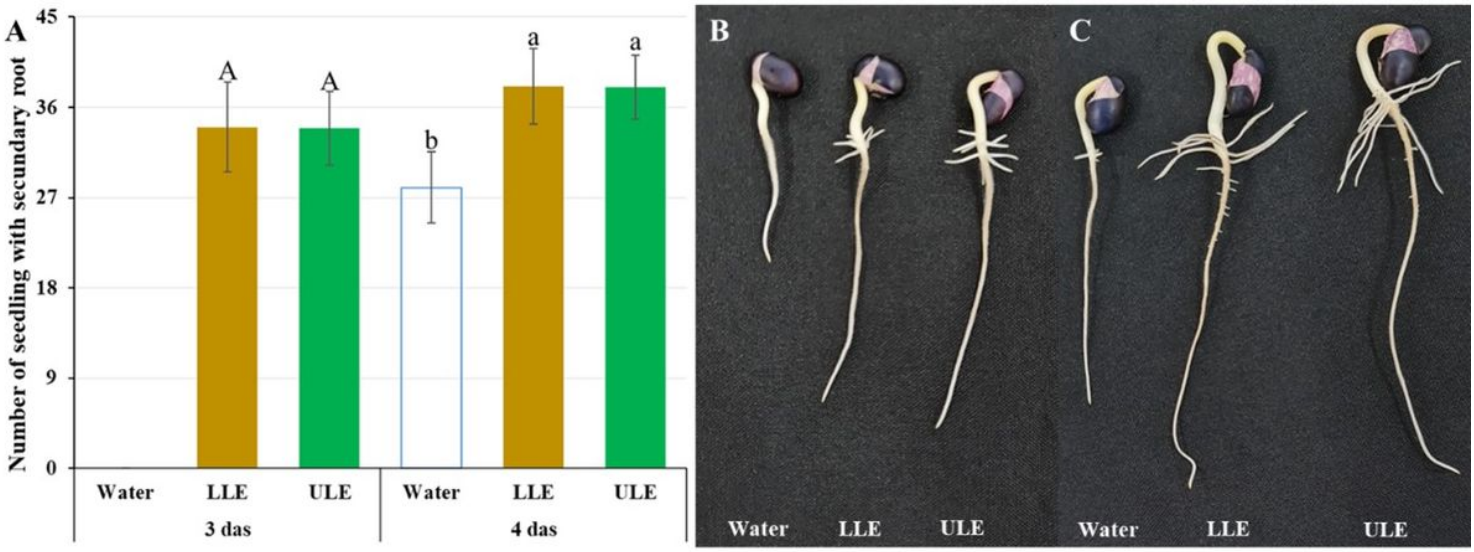


Figure 2

Number of seedlings with secondary root (A) formed of common bean at 3 (B) and 4 (C) days after soaking (das) seeds with *Laminaria japonica* (LLE) or *Ulva prolifera* (ULE) liquid extracts or water. Bars represent standard deviation. Lowercase and capital letters indicate significant difference between the means in the same dat by Tukey ($p \leq 0.05$) and Kruskal-wallis ($p \leq 0.05$) test, respectively.

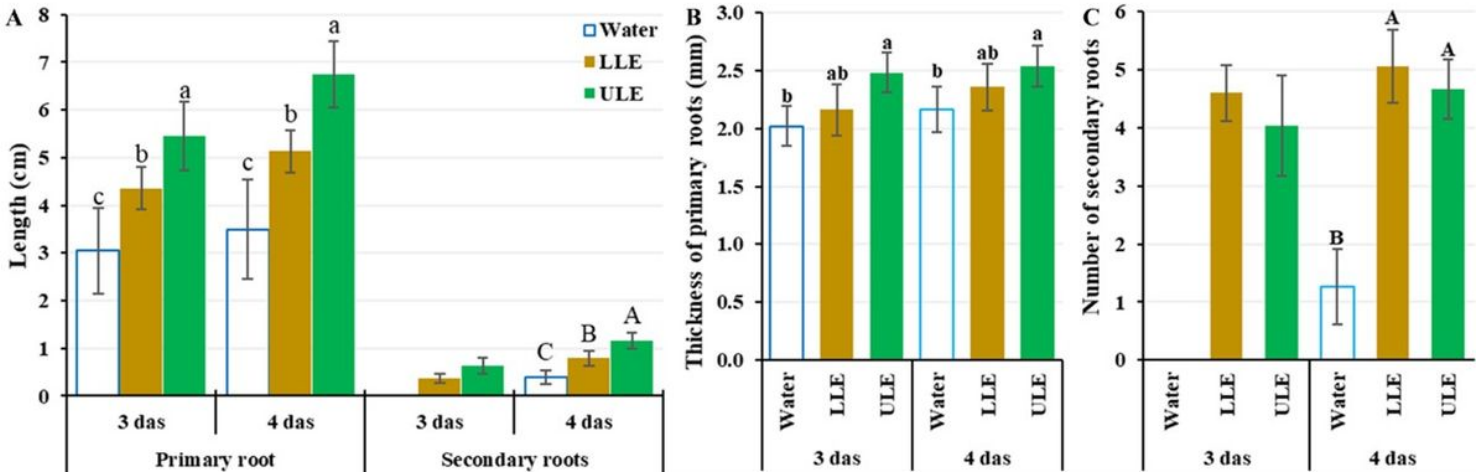


Figure 3

Primary and secondary root length (A), thickness of primary roots (B), and number of secondary roots (C) of common bean at 3 and 4 days after soaking (das) seeds with *Laminaria japonica*(LLE) or *Ulva prolifera* (ULE) liquid extracts or water. Bars represent standard deviation. Lowercase and capital letters indicate

significant difference between the means in the same dat by Tukey ($p \leq 0.05$) and Kruskal-wallis ($p \leq 0.05$) test, respectively.

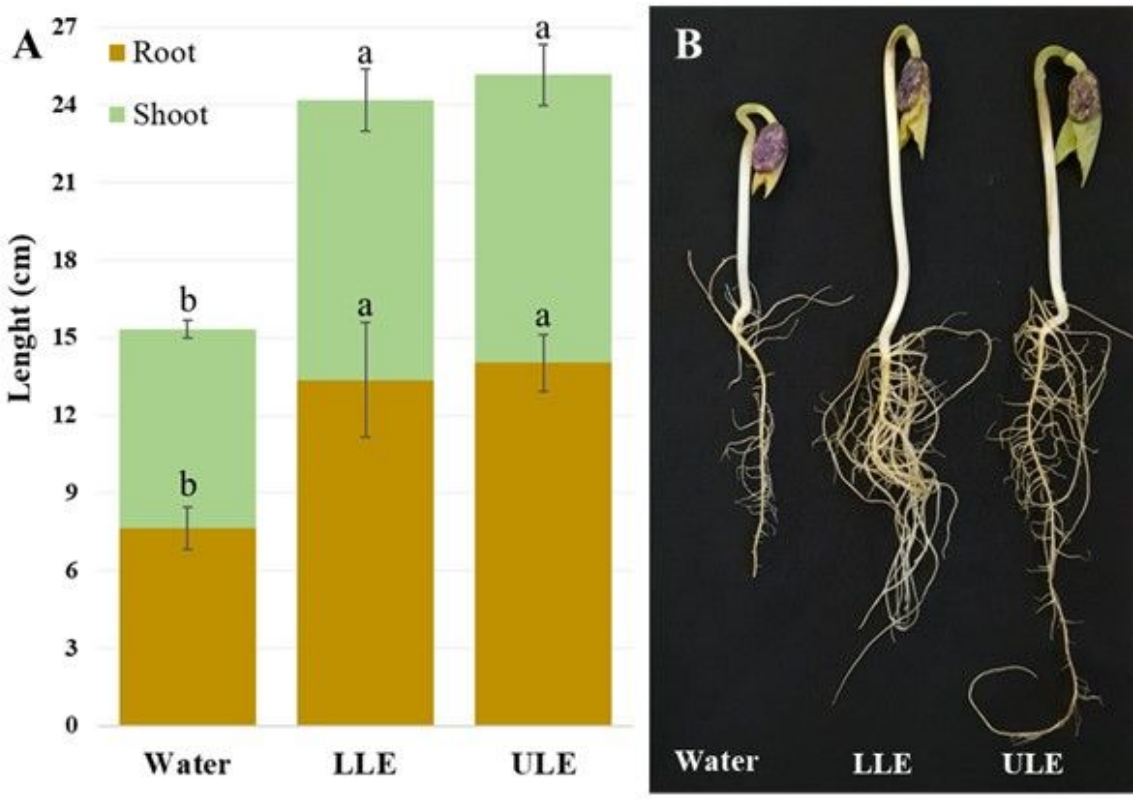


Figure 4

Length of roots, shoots (A) and seedling (B) of common bean at 9 days after soaking seeds with *Laminaria japonica* (LLE) or *Ulva prolifera*(ULE) liquid extracts or water. Bars represent standard deviation. Letters indicate significant difference between the means (Tukey's test, $p \leq 0.05$).

Supplementary Files

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