Bulgarian Journal of Agricultural Science, 28 (No 6) 2022, 1088–1098

Isolation, purification, and characterization of auxin from red algae (*Eucheuma spinosum*) and its application in cayenne pepper (*Capsicum frutescens* L.) plants

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Abstract

Sari, R. P., Maksum, I. P., Hindersah, R., Ishmayana, S. & Soedjanaatmadja, U. M. S. (2022). Isolation, purification, and characterization of auxin from red algae (*Eucheuma spinosum*) and its application in cayenne pepper (*Capsicum frutescens* L.) plants. *Bulg. J. Agridc. Sci., 28 (6)*, 1088–1098

Eucheuma spinosum is a kind of seaweed that belongs to the Rhodophyceae (red algae) class. Red algae itself is a low-level plant that does not have different skeletal arrangements such as roots, stems, and leaves. In red algae, it is believed that besides polysaccharides there is also a plant hormone namely auxin. Auxin is a hormone in plants which have functioned as a regulator of cell enlargement and has the potential to be used as a biostimulant (hormone fertilizer). There are several types of auxins including indole acetic acid (IAA) and indole butyric acid (IBA). This study aims to isolate, purify, and characterize auxin in red algae (E. spinosum) and determine its effect on the growth of chili plants (*C. frutescens* L.). Isolation and characterization were carried out by thin-layer chromatography, adsorption column chromatography, High Performance Liquid Chromatography (HPLC). Infrared spectrophotometry (IR) and Mass Spectrometry (MS) were used for characterization. Biological test with chili plant as bioindicators with various treatment namely control, ETAC-21/ETAC-12, 1% NPK, IAA isolate, and crude extract from red algae and proximate tests were carried out including protein content, fat content, ash content, water content, and carbohydrate content on the fruit. The total protein pattern was synthesized tested using the Sodium Deodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) method to determine the effect of adding IAA isolate to the expressed protein. The results of reversed-phase HPLC analysis stated that the auxin content in red algae was 2.43 mg/g dry weight. The results of IR and MS state that the IAA samples with IAA standards are relatively the same. The results of the biological test on cayenne pepper showed that IAA isolates could stimulate the growth of root length and root dry weight, and increase the number of chilies and chili seeds compared to the control plants. The addition of IAA isolates gave the average protein content, fat content, ash content, moisture content, and carbohydrate content were 6.18, 4.66, 2.96%, 81.46, and 4.73%, respectively. IAA isolate from red algae (*E. spinosum*) gave a distinctly different pattern of protein synthesis in cayenne pepper (*C. frutescens* L.) compared to the control plants, as shown on the SDS- PAGE result.

Keywords: red algae; auxin; *Eucheuma spinosum;* phytohormone; *Capsicum frutescens* L.

Introduction

Fitohormon is a part of the growth and development system in plants which can affect the physiological response of plants at low concentrations. Naturally, the plant has contained growth hormones such as auxin, gibberellins, and cytokinin which are termed endogenous hormones. It is known that there are eight phytohormones, including auxin, gibberellin, cytokinin, abscisic acid, ethylene, jasmonic acid, salicylic acid, and brassinosteroids (Soedjanaatmadja et al., 2020; Romamenko et al., 2015). There are several auxins namely Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), and Naphthalene Acetic Acid (NAA). Auxin (i.e., IAA) is synthesized and found on the apical meristem of the buds, young leaves, in the embryo of seed and developing fruit. Main physiological activities of the hormone were induction of elongation growth, apica dominance, initiation of root formation, differentiation and branching roots, phototropism and geotropism (Soedjanaatmadja et al., 2020). The addition of exogenous hormones is needed to overcome the lack of endogenous hormones in plants characterized by growth below normal (slow), flower loss, and also small fruit size. By adding exogenous hormones, then the problem of lack of endogenous hormones in plants that are a problem in the agricultural sector can be overcome (Rachman et al., 2017).

Indonesia is a maritime country, there are approximately 45% species of seaweed consisting of 452 species of red algae, 196 species of green algae, and 134 species of brown algae, but only a few types of seaweed are known to have high economic value (Amaranggana & Wathoni, 2017). Many species of microalgae are the most promising and popular objects of biotechnology, one of it is used as a bio-fertilizer (exogenous hormonal fertilizer). The cells are rich in vitamins, proteins, carbohydrates, fatty acids, enzymes, pigments, macro and microelements, biologically active compounds with valuable medicinal properties (Romamenko et al., 2015). The benefits of seaweed or microalgae as a source of organic material and fertilizer nutrients have led to its use as a substance to increase soil fertility for centuries, most of that is used for nutritional supplements and as biostimulants or biological fertilizers to increase plant growth and yield. Several commercial seaweed extract products are available for use in agriculture and horticulture (Khan et al., 2009). In various algal taxa, essentially all known phytohormones were detected in concentrations comparable with their content in higher plants (Tarakhovskaya et al., 2007). Therefore, red algae are used in this study to optimize the potential of algae in Indonesia. There's a type of red algae that the outer skin surface is slightly rough because it has rough serrations and spots, the talus is cylindrical, the branching of the talus has a pointed tip and is overgrown with protrusions in the form of soft spines arranged in regular rotation around the branches. Auxin and auxin-like compounds are also reportedly found in marine algae (Khan et al., 2009).

In this research, auxin was isolated from *E. spinosum* because the process of growth and development of algae and higher plants is controlled by the hormonal system.

Seaweeds, especially red algae, i.e., *G. coronopifolia* and *E. cotonii*, are a potential source of phytohormones and can be used as biostimulant agents to improve the quality and quantity of crop production. The phytohormones in red algae, especially auxin, cytokinins, and gibberellins, can be applied to improve agriculture products, especially to increase productivity of horticultural crops (Soedjanaatmadja et al., 2020). As far as we were aware, there were no other studies that have isolated phytohormones from *E. spinosum.* In previous studies, isolation of auxin from *Sarconema filiforme* was carried out, where IAA was found 13.21% in the extract (Shoubaky & Salem, 2015). Other studies also found IAA and Indole Acetamide (IAM) as free auxin in 11 red algae collected from the Brazilian coast (Yokoya et al., 2010), while the extract of *Caulerpa racemose* contained 979.71 mg/L of giberellin (GA3), and 15.57 mg/L of auxin (NAA) at 10 ppm concentration , respectively (Dumale et al., 2018). Therefore, it can be assumed that *E. spinosum* also contains phytohormone auxin.

Chili is one of the horticultural commodities that are widely cultivated by farmers in Indonesia because it has several health benefits and has a high selling price as a result of the large market demand for chili (Pitaloka, 2017). Considering chili is a seasonal plant, its availability is quite rare and not evenly distributed. It can be said, it occurs as a result of the ineffective distribution of agricultural products from surplus areas to be distributed to the deficit areas. Therefore, to optimize the potential of each region in Indonesia, it is necessary to find an innovation that can increase the productivity of a plant, not only in quantity but also in quality. Even though chili is not the main food in Indonesia, price fluctuations provide inflation for the national economy (Yanuarti & Afsari, 2016). To overcome this problem, agriculture in Indonesia should find some innovation to increase chili productivity. The existence of innovations in the field of food biochemistry is expected to help agricultural products in Indonesia. One of the applications of agricultural technology is using growth regulators where phytohormones are applied for this purpose. The plants naturally have contained endogenous hormones in very low concentrations, which makes the plant growth rate slow. Therefore, the addition of exogenous hormones must be performed to improve plant growth

rate and also improve the quantity and quality of the fruits.

Based on the described background, the present study was directed to determine auxin content in red algae (*E. spinosum*) and determine its application on cayenne pepper (*Capsicum frutescens* L.) plants.

Materials and Methods

Materials: Red algae (*E. spinosum*) was purchased from marine algae supplier in Surabaya, Jawa Timur, Indonesia. Cayenne pepper seeds (*C. frutescens* L. Var Prentul Super) as a bioindicator for the biological assay were obtained from the Departement of Agriculture Cultivation, Faculty of Agriculture, Universitas Padjadjaran. Planting media was prepared from soil: husk: manure (3:2:1). All the chemicals used are of pro-analytical grade. The instruments used in this study were glass tools, Kjeldahl distillation apparatus, Soxhletasi apparatus, seeding box, desiccator, thin layer chromatography, vacuum evaporator (Buchi), HPLC (Alltech 8011/2), glass column, C-18 nucleosil ODS column, bio light fluorescence lamp, analytical balance, pH meter, polybag, FTIR (Shimadzu 8400), mass spectrometer (Waters, Xevo Q-Tof MS) and furnace (Thermolyne).

Preparation of extract: First, remove all the dirt on the algae then wash thoroughly and dry it. Furthermore, the dried algae were milled to pieces, and 200 grams of the algae were macerated with 1200 mL methanol for 4 × 24 hours at room temperature. The methanol extract was collected and concentrated with a vacuum evaporator at a temperature below 40°C until concentrate was obtained. Then the concentrate was dissolved with distilled water to 100 mL to obtain a crude extract.

Extraction and partial purification: As much as 25 mL of the crude extract was alkalized with 0,2 N potassium hydroxide solution to pH 8 and extracted with ethyl acetate (40 mL × 2). The water layer was then acidified with 3 N hydrochloric acid to pH 2.5 and then extracted again with ethyl acetate (20 mL × 2). The ethyl acetate fraction was evaporated to dryness in a vacuum evaporator. The residue is dissolved in 5 mL of methanol to obtain methanol extract.

Analytical Thin Layer Chromatography (ATLC): Analytical thin-layer chromatography was performed using plates coated with silica gel GF-254. This plate was cut to the size of 6 cm × 2 cm lower and the upper border was marked 0.5 cm of each end. As many as 3-4 drops of methanol extract were placed with a capillary tube on the lower mark and standard was placed next to it. The plate was eluted with a mixture of ethyl acetate: methylene chloride (6:4) until the eluent move to the upper limit. The result was observed under UV light at a wavelength of 254 nm.

Adsorption column chromatography: The methanol extract was put into a glass column containing G-60 silica gel, then eluted with a mixture of ethyl acetate: methylene chloride (6:4), then fractions of 2 ml were collected. The fractions were analyzed by analytical thin-layer chromatography and the Rf value of each fraction was compared to the Rf value of the auxin (IAA) standard. The fractions that have the same Rf as the standard were combined.

Preparative Thin Layer Chromatography (PTLP): As much as 1 mL of the collected fractions were spotted on a glass plate (20 × 20 cm) that has been coated with silica gel GF-254 and 1000 ppm auxin was used as standard. The glass plate is eluted with a mixture of ethyl acetate: methylene chloride (6:4) until the eluent rises to the top line. Spots with the same Rf with the standard are scraped off and suspended in 2 mL methanol, decanted then the supernatant was concentrated by vacuum evaporator. The residue obtained was dissolved in 1 mL methanol to obtain IAA isolate.

Identification of auxin isolates by Reversed-Phase HPLC, FTIR, and MS: A total of 10 µL fraction containing auxin which had been purified by preparative TLC, was analyzed by Reversed-Phase (RP) HPLC; using Alltec 8011/2 (C-18 nucleosil ODS column) with UV detector at a wavelength of 254 nm. Analiyzed by FTIR (Shimadzu 8400), and analyzed by Mass Spec. MS (Waters type Xevo Q-Tof MS). The buffer elution for RP HPLC was performed with an isocratic method using 35% methanol in acetate buffer (pH 3.5) with a flow rate of 0.7 mL/minute. The RP HPLC, FTIR, and MS results were compared to the IAA standard.

Planting and biological assay: Cayenne pepper seeds were soaked in water for 1 hour, the submerged seeds were collected, then stored on cotton that had been moistened by ETAC-21 (commercial Biostimulant for vegetative phase) and left in the dark place for 1-2 days until they germinate. Sprouts were transferred to seeding boxes and stored for one week. Cayenne pepper seeds that have grown to the same height were transferred into polybags containing a mixture of planting media (soil: husk: manure = 3:2:1) and stored in a place exposed to sunlight and protected from rain. Plants were watered every morning and afternoon and treated by spraying with auxin isolate (IAA), crude extract, 1% NPK, ETAC-21 at the beginning of sprouting, and then when flowers appeared the spraying was replaced with ETAC-12 (com-

mercial Biostimulant for generative phase) every two days until harvest time. The control group was only sprayed with water.

Proximate analysis of cayenne pepper (C. frutescens* L. *Var Prentul Super): Proximate analysis of the harvested cayenne pepper was done to determine the nutritional content of the fruits based on the modified method of AOAC (1990). This analysis includes determination of total proteins content by Kjeldahl method, total fats content by Soxhlet method, ash content by gravimetric method, water content by weight-loss method, and carbohydrates content by difference method.

All experiments were repeated three times. Statistical analysis performed using Analysis of Variance (Anova) and Fisher test (Kim, 2017), using Minitab Stastical Sofware ver.20.3.0.0.

SDS-PAGE protein analysis of cayenne pepper (C. frutescens* L. *Var Prentul Super):

Protein content in cayenne pepper characterized by SDS-PAGE, according to Laemmli method (1970), and Malik et al. (2009).

Results and Discussion

Analytical Thin Layer Chromatography: Analytical thin-layer chromatography was performed to determine the standard Rf. This Rf value is used to determine the right solvent mixture to purify auxin compounds. Thin-layer chromatography belongs to solid-liquid chromatography, which in this study silica gel GF-254 was used as the stationary phase and the solvent used was a mixture of ethyl acetate: methylene chloride (6:4) as the mobile phase. Silica gel GF-254 is used because auxin has a natural chromophore group so that the use of silica gel GF-254 can provide fluorescence at wavelength 254 (Lu et al., 2010). After the calculation, it was determined that the standard Rf value was 0.52, which this value stated that the solvent used was good enough. Compared with an IAA standard that was indicates that there is an IAA auxin compound in the methanol fraction. So it is necessary to further purify it by adsorption column chromatography.

Adsorption column chromatography:

Fractions of 2 mL were collected and analyzed for their content using analytical TLC and the result is presented in Figure 1. The fractions that have a spot with Rf value similar to Rf of IAA standard were the fractions of 5 to 10. This indicates that the fraction contains auxin compounds (IAA). The research was continued with a preparative thin-layer chromatography (TLC) procedure to obtain purer auxin compounds.

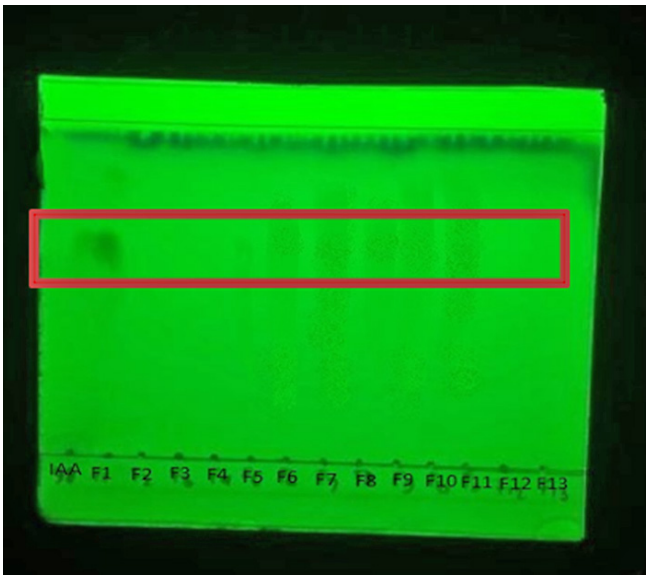

Fig. 1. The result of analytical thin layer chromatography using an ethyl acetate: methylene chloride solvent (6:4 v/v) as mobile phase. The plate used was silica gel GF-254. The spot was observed under UV light at wavelength of 254 nm. Lane 1 = IAA standard, Lane 2 = IBA standard, Lane 3 – 15 = fraction 1 to fraction 13 (in order from left to right) (A)

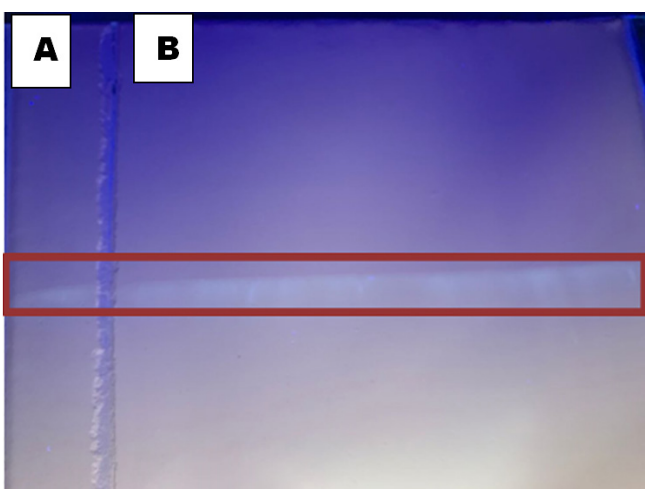

Fig. 2. The result of preparative thin layer chromatography of isolate fractions containing auxin (from the result of adsorption column chromatography), using an ethyl acetate: methylene chloride solvent (6:4 v/v) as mobile phase. The plate used was silica gel GF-254. The spot was observed under UV light at wavelength of 254 nm. IAA standard (A) and fractions of adsorption column chromatography (B)

Preparative Thin Layer Chromatography: Figure 2 shows preparative TLC plate observed under UV light at wavelength 254 nm. Then the parallel stains were scraped off and suspended in methanol. After that, the mixture was centrifuged and decanted to separate the isolates obtained from the KLTP results. The results obtained are concentrated with a vacuum evaporator. The residue was dissolved in 1 mL of methanol for further analysis by reversed-phase high performance liquid chromatography (RP-HPLC) to obtain the concentration of the auxin compound (IAA).

Identification of auxin isolates by Reversed-Phase HPLC:

Figure 3 shows that at the retention time of 16.957 minutes, one peak appears from the IAA standard. The peak indicates that the IAA standard used is 100% pure. When compared with the results of the auxin sample in Figure 4, there is one dominant peak with a retention time of 16.746 minutes which has a peak and time that is relatively the same as the IAA standard. This difference in retention time could be caused by the presence of other peaks that caused a shift

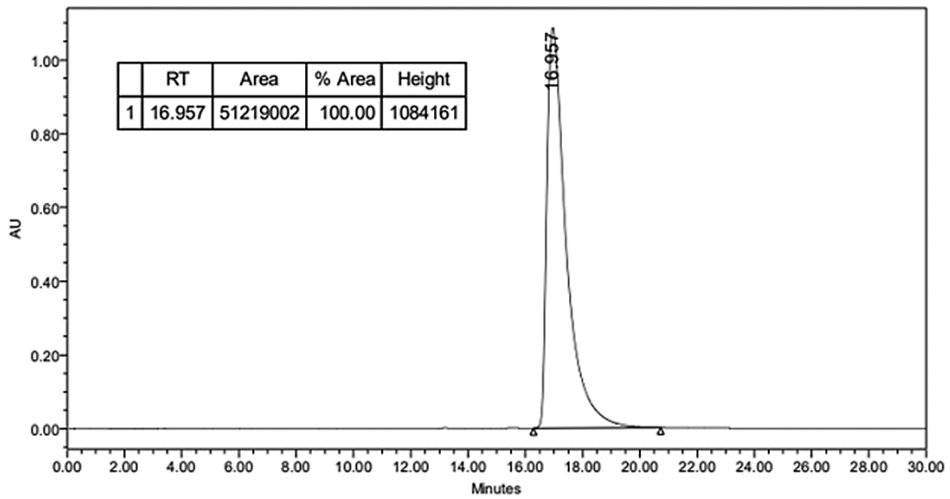

Fig. 3. The standard IAA chromatogram of HPLC results used a reversed phase C-18 nucleosyl ODS column and a UV detector at wavelength of 254 nm. The mobile phase was 35% methanol in acetate buffer pH 3.5 with a flow rate of 0.7 mL/min

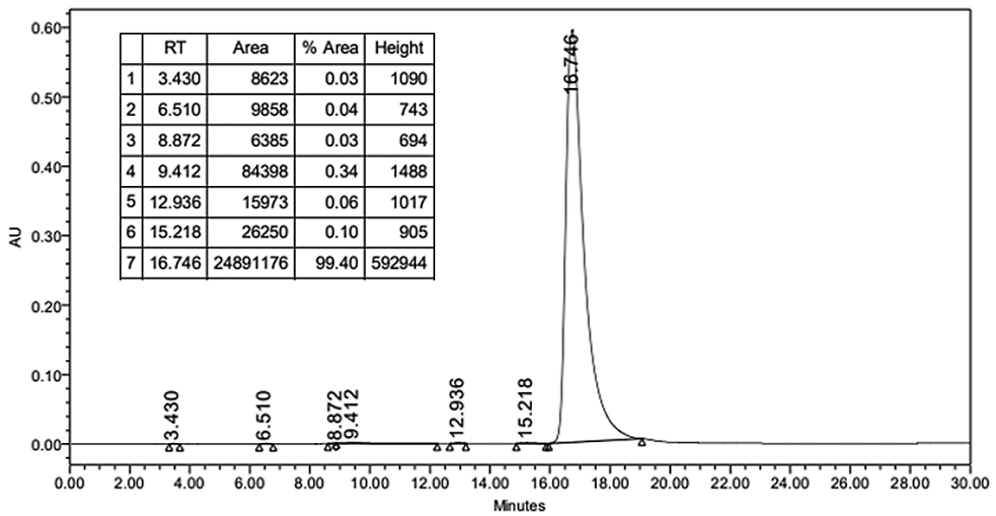

Fig. 4. The IAA isolates chromatogram of HPLC results used a reversed phase C-18 nucleosyl ODS column and a UV detector with = 254 nm. The mobile phase was 35% methanol in acetate buffer pH 3.5 with a flow rate of 0.7 mL/min

in retention time due to the presence of impurities or other components detected in the isolates. After calculation, the concentration of IAA in the red algae (*E. spinosum*) sample was 2.43 mg/g dry weight.

Identification of auxin isolates by FTIR:

The results of the IAA standard using FTIR analysis (Figure 5), show that there are several absorption peaks produced. IAA is known to have 1 carboxyl group and 1 indole ring. The -NH group gives a strong absorption at a wavenumber of 3382.816 cm^{-1} with a sharp band shape. To distinguish it from the -OH group, it can be seen from the shape of the resulting band, the -OH group has a wide band at a moderate intensity, -RCOOH at a wavenumber of 2910.420 cm^{-1}, C=O at a wavenumber of 1688.857 cm^{-1}, and CO at wave number 1204.723 cm^{-1}.

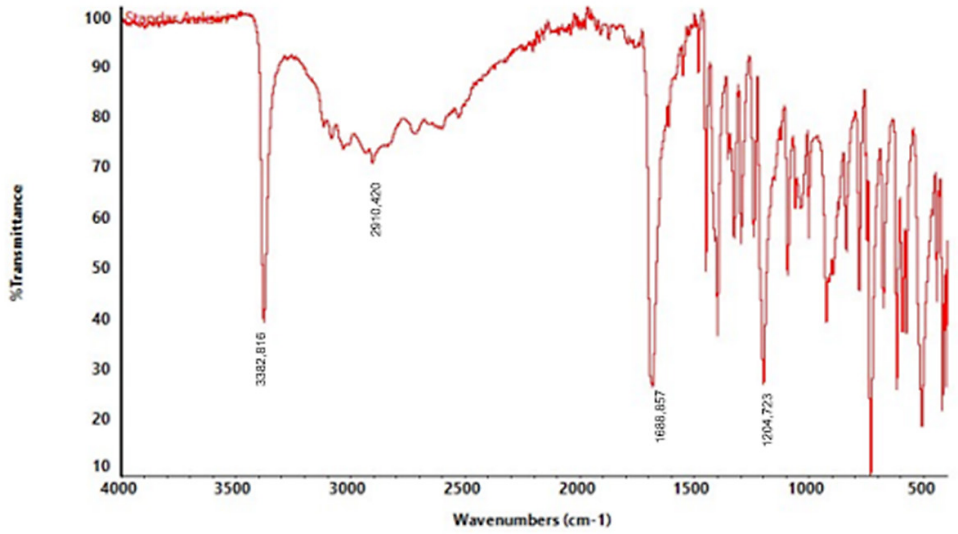

Fig. 5. The IR spectrum shows the standard IAA absorption peaks. The -NH group gives absorption at a wave number (v) of 3382.816 cm^{-1}, -RCOOH at 2910,420 cm^{-1}, C=O at 1688,857 cm^{-1}, C-O at 1204.723 cm^{-1}

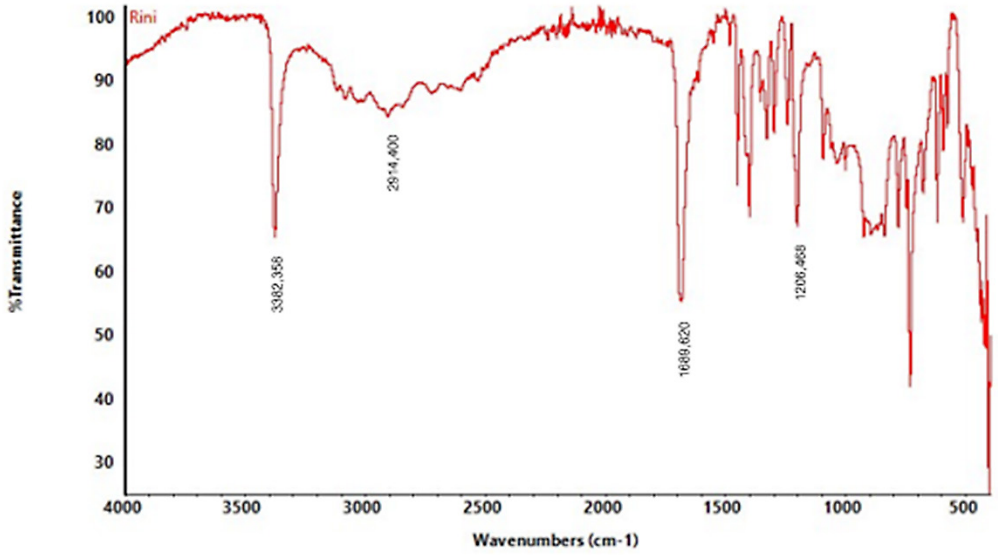

Fig. 6. The IR spectrum shows the absorption peak of IAA isolates from red algae. The -NH group gives absorption at a wave number (v) of 3382.358 cm^{-1}, -RCOOH at 2914.400 cm^{-1}, C=O at 1689.620 cm^{-1}, C-O at 1206.468 cm^{-1}

While the results of the FTIR analysis of the IAA sample in Figure 6 show the absorption of the -NH group at a wavenumber of 3382.358 cm^{-1}, the -RCOOH group at a wavenumber of 2914.400 cm^{-1}, the C=O group at a wavenumber of 1689.620 cm^{-1}, and the CO group at a wavenumber of 1206.468 cm^{-1}. The absorption results from the IAA standard and IAA samples can be said to have the same or similar results. To be able to confirm whether the compound is an IAA compound, the research is continued with mass spectrometry, to determine the molecular weight of the IAA sample.

Identification of auxin isolates using Mass Spectrometry:

The results of mass spectrometry on the auxin sample can be seen in Figure 7. IAA has a molecular weight of 175.2 g/mol. In the mass spectrum, the sample has a molecular weight of 174.0558 g/mol, this indicates that the sample contains pure IAA compounds. IAA compounds are more stable in the negative ion [M-H], so the analysis was carried out in negative mode. The weight of IAA obtained from the sample is reduced by 1 which indicates that the IAA compound in the sample is reduced by 1 H^+ ion.

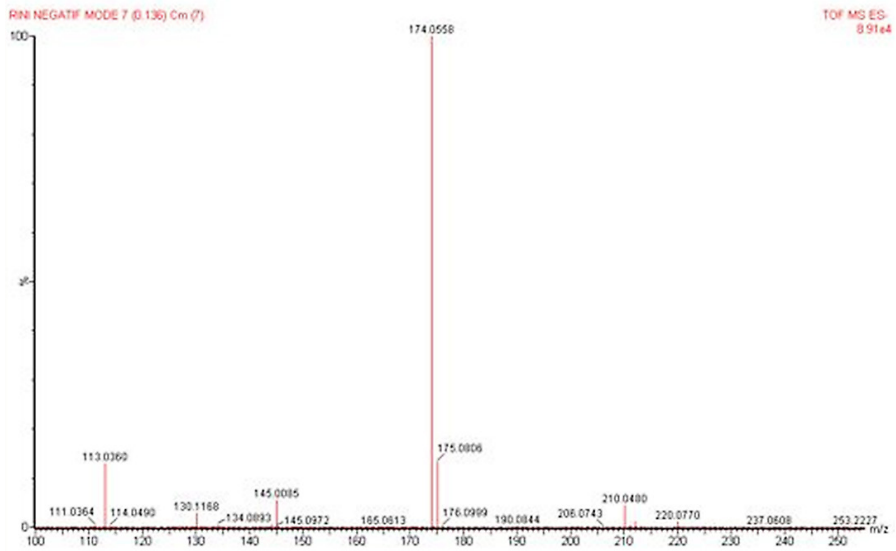

Fig. 7. The mass spectrum shows IAA peaks from the red algae samples. The analysis was carried out in negative mode (M-H)

Table 1. Average of root length (A) and root dry weight (B) of cayenne pepper plants during four weeks of growth. The plants were treated with water (control), commercial biostimulant ETAC-21, 1% NPK, 100 ppm IAA isolate, and 100 ppm crude extract. Error bars indicate standard deviation (SD) of three experiments. Different letters indicate statistically significant differences based on Fisher's post hoc test ($P < 0.05$)

A	Weeks	Root Length (cm)				
		Treatments				
		Control	ETAC-21	1% NPK	IAA isolate	Crude extract
	1	4.96 ± 0.262	5.62 ± 0.131	5.00 ± 0.120	5.59 ± 0.325	5.49 ± 0.276
	2	5.81 ± 0.300	6.82 ± 0.156	6.32 ± 0.156	6.77 ± 0.598	6.66 + 0.468
	3	6.84 ± 0.539	10.01 ± 1.345	8.62 ± 0.464	9.85 ± 0.323	9.74 ± 1.269
	4	7.74 ± 0.827	16.33 ± 4.621	12.54 ± 1.276	15.36 ± 3.646	15.17 ± 2.303
B	Weeks	Root Dry Weight (cm)				
		Treatments				
		Control	ETAC-21	1% NPK	IAA isolate	Crude extract
	1	0.0128 ± 0.003	0.0159 ± 0.00035	0.0142 ± 0.002	0.0153 ± 0.001	0.0154 ± 0.00021
	2	0.0271 ± 0.003	0.0344 ± 0.002	0.0318 ± 0.002	0.0334 ± 0.004	0.0324 ± 0.006
	3	0.0494 ± 0.004	0.1068 ± 0.047	0.0819 ± 0.008	0.1032 ± 0.039	0.1051 ± 0.034
	4	0.2513 ± 0.026	0.6822 ± 0.089	0.4952 ± 0.035	0.6357 ± 0.034	0.6401 ± 0.006

The Effect of auxin isolate on the growth of cayenne pepper plant, on the vegetative phase:

In the vegetative phase, the observation parameters used in cayenne pepper plants were root length and root dry weight. Observations of root length and dry weight were carried out for 4 weeks (28 days) starting from the days after transplanting (DAT) where every week from week 1 to week 4 the root elongation and dry weight of the roots were calculated. Auxin works in influencing cell elongation in plants. In the shoot elongation area, auxin will stimulate hydrogen ion pumping proteins in the cytoplasm so that hydrogen ions will enter the cell wall which causes an increase in cell wall acidity and activates expansion enzymes for cell elongation. This enzyme functions to break down hydrogen bonds between cellulose micro-fibrils and loosen the cell wall structure so that the cell wall weakens and the cell can grow larger (Campbell & Reece, 2010). The results of the bioassay in the first to fourth weeks respectively showed that IAA isolates had a significant effect compared to 1% NPK and control in increasing the root length and root dry weight. Root elongation and the number of secondary roots are used as parameters to determine plant quality from the number of nutrients that can be absorbed from the soil for plant growth. If a plant has more roots, it can be said that the absorption of nutrients from the soil is optimal so that it can improve fruit quality. The average root length in the first week to the fourth week respectively was 5.59, 6.77, 9.85, 15.36 cm, whereas the average root dry weight in the first week to the fourth week respectively was 0.0153, 0.0334, 0.1032, 0.6357 g. In the root length parameter (Table 1A), the P-value at week 1 to week 4 has a P-value < 0.05 (α). In the ANOVA test results, if the P-value is greater than 0.05 (α) (P-value > 0.05) then H_0 is accepted, which means that there is no significant difference in each treatment. So that the root length parameter can be said that the root elongation has a significant difference. While in Table 1B, the first to third weeks do not show a P-value < 0.05 (α). So that at week 1 to week 3 there was no significant difference in root dry weight but at week 4 the P-value < 0.05 (α). So it can be said that there is a significant difference in root dry weight at week 4.

In plants which treated with ETAC-21 and ETAC-12 gave better results compared to plants given other treatments. This is because the ratio of auxin-cytokinin and gibberellins contained in the two biostimulants is formulated properly and optimally. This means that ETAC-21 and ETAC-12 are appropriate multi-compounds for plant growth in the vegetative and generative phases (Soedjanaatmadja, 2008).

The generative phase: Parameters in the generative phase are the time of emergence of flowers and cayenne pepper which are calculated after the day of transplanting (HPT) from the seeding tray to the polybag, the number of cayenne peppers and seeds produced, and the weight of cayenne peppers and seeds obtained. In the generative phase, auxin works synergistically with gibberellins and cytokinins. Where cytokinins themselves function to increase the number and size of fruit or seeds, assist flowering, and delay rot in old plants (Rachman et al., 2017). The result of the research showed that the time of emergence of flowers and cayenne pepper was faster in plants treated with 100 ppm IAA isolate compared to 1% NPK and con-

Table 2. Average of flowers emergence and cayenne pepper fruits emergence. The plants were treated with water (control), commercial biostimulant ETAC-21/12, 1% NPK, 100 ppm IAA isolate, and 100 ppm crude extract. Error bars indicate standard deviation (SD) of three experiments. Different letters indicate statistically significant differences based on Fisher's post hoc test (P < 0.05)

Treatments	Average (days)	
	Emergence of flowers	Emergence of fruits
Control	83.67 ± 0.58	106.00 ± 2.65
ETAC-21/ETAC-12	76.67 ± 1.15	94.00 ± 1.73
1%NPK	80.33 ± 0.58	98.33 ± 1.15
IAA isolate	80.67 ± 1.15	96.00 ± 1.73
Crude extract	80.33 ± 1.15	97.33 ± 2.89

Table 3. Average number of cayenne pepper fruits, weight of cayenne pepper fruits, number of seeds, and weight of seeds. The plants were treated with water (control), commercial biostimulant ETAC-21/12, 1% NPK, 100 ppm IAA isolate, and 100 ppm crude extract. Error bars indicate standard deviation (SD) of three experiments. Different letters indicate statistically significant differences based on Fisher's post hoc test (P < 0.05)

Treatments	Average			
	Number of fruits (pcs)	Weight of fruits (g)	Number of seeds (pcs)	Weight of seeds (g)
Control	12.33 ±1.53	12.3446 ± 1.91	277.33 ± 60.09	4.007 ± 0.38
ETAC-21/ETAC-12	20.67 ± 3.06	34.7882 ± 1.83	704.67 ± 43.47	11.0962 ± 1.89
1% NPK	15.00 ± 2.00	21.53 ±3.38	469.33 ± 68.41	6.5190 + 0.54
IAA isolate	18.33 ± 2.52	31.1728 ±4.07	577.00 ± 42.76	10.0848 ± 1.47
Crude extract	19.00 ± 2.00	32.2867 ± 3.09	626.33 ± 22.81	10.7564 ± 1.22

Table 4. Average percentage of protein, fat, ash, water and carbohydrate content of cayenne peppers. The plants were treated with water (control), commercial biostimulant ETAC-12, 1% NPK, 100 ppm IAA isolate, and 100 ppm crude extract. Error bars indicate standard deviation (SD) of three experiments. Different letters indicate statistically significant differences based on Fisher's post hoc test (P < 0.05)

Treatments	Average content (%)				
	Protein	Fat	Ash	Moisture	Carbohydrate
Control	2.93 ± 0.27	2.98 ± 0.436	2.92 ± 0.196	78.40 ± 2.319	12.77 ± 2.76
ETAC-21/ETAC-12	7.11 ± 0.871	5.11 ±0.97	3.12 ± 0.187	80.48 ± 1.965	4.18 ± 1.399
1%NPK	5.49 ± 0.35	4.03 ± 0.206	2.94 ±0.267	79.09 ± 1.494	8.44 ± 1.283
IAA isolate	6.18 ± 0.19	4.66 ±0.714	2.96 ± 0.159	81.46 ± 1.672	4.73 ± 2.299
Crude extract	6.23 ±0.118	4.72 ± 0.585	3.02 ± 0.076	80.50 ± 1.85	5.52 ± 1.34

trol (Table 2). Plants that were given IAA isolate also had a significant effect compared to 1% NPK and control in peppers and seeds produced, and the weight of cayenne peppers and seeds obtained. The time of emergence of flowering and cayenne pepper of plants treated with IAA isolate, was 80 and 96 days, respectively. Furthermore, the mean number of cayenne pepper, the weight of cayenne pepper, number of seeds, and weight of seeds were 18 fruits, 31.18 g, 577 grains, and 10.09 g, respectively.

In the Table 3 shows that the average number of cayenne pepper, the weight of cayenne pepper, number of seeds, and weight of seeds in plants treated with 100 ppm IAA isolate had a significant difference compared to those treated with 1% NPK and control. The addition of biostimulant ETAC-12 and 100 ppm crude extract also gave a significant difference compared to 1% NPK and control. As it has been said that both ETAC-12 and 100 ppm crude extract contains other types of phytohormones besides auxin so that the activity in plant growth in the generative phase is better than 1% NPK and control. The cytokinin composition of ETAC-12 is greater than that of auxin. ETAC-12 also contains gibberellins which function to promote bud development, stem elongation, leaf growth, promote flowering and fruit development, and influence root growth and differentiation (Soedjanaatmadja, 2008).

Proximate Analysis of Cayenne Peppers: The results of the proximate analysis of cayenne peppers are shown in Table 4. According to Ogunlade et al. (2012), protein content, fat content, ash, moisture, and carbohydrate content in cayenne pepper (*C. frutescens* L.) were 3.07%, 2.87%, 1.21%, 85.19%, and 4.62%, respectively. The results of this study showed that cayenne pepper from plants treated with 100 ppm IAA isolate, 100 ppm crude extract, and biostimulant ETAC-21/ETAC-12 had higher protein and fat content and differ significantly from plants treated with 1% NPK and controls. While in this study, plants added with IAA isolates gave an average protein content, fat content, ash, moisture, and carbohydrate content were 6.18, 4.66, 2.96, 81.46, and 4.73%, respectively. In this study, phytohormones those contained in the biostimulants (ETAC-21 and ETAC-12), IAA isolate, and crude extract added to cayenne pepper can increase protein synthesis that can stimulate the activity of enzymes used in metabolic processes. Higher enzyme activity, lead to a higher degradation of carbohydrates for hydrolysis into energy or nutrients needed by plant growth (Yeni & Mulyani, 2014). This is not only indicated by the length and dry weight of the roots, but also by the increased protein content in plants treated with the addition of exogenous hormones. This statement is following the results of this study where the carbohydrate content of plants treated with 100 ppm of the IAA isolate was lower than 1% NPK and control, where the mean was 4.73%.

The water content in foodstuffs greatly affects the quality and shelf life of these foodstuffs. Therefore, determining the water content of a food ingredient is very important so that in the processing and distribution process it gets the right treatment. The more water content contained, the shorter the shelf life because if a material contains a lot of water content, microbes can grow. Ash is an inorganic residue from the combustion process or the oxidation of organic components of food. Total ash content is part of a proximate analysis that aims to evaluate the nutritional value of a product/food ingredient, especially total minerals. The result of water content and ash content in this study does not have a significant difference which means the addition of IAA isolate did not affect storage time and minerals contained in cayenne peppers are relatively low. However, if the micro mineral content is too high, it can harm the body, so a high total ash content in a food product is an indicator that the product is potentially dangerous for consumption.

SDS-PAGE protein analysis of cayenne pepper (C.frutescens* L. *Var Prentul Super):

In this study, SDS-PAGE was used to analyze the pattern of protein synthesized from the addition of IAA isolates in cayenne pepper (*C. frutescens* L.) compared to control

plants. Plants given IAA isolates or exogenous auxin will increase the absorption of macro and microelements to form chlorophyll which is needed in the photosynthesis process. With the increase in photosynthesis, photosynthate and auxin will move to the roots to help the formation of gibberellins and cytokinins which function in the formation of flowers and fruit (Soedjanaatmadja et al., 2020). When the levels of auxin in plants are high, there will be a transcriptional regulation that produces an enzyme (Mockaitis & Estelle, 2008). Therefore, the SDS-PAGE method was used to compare the protein or enzyme synthesized by cayenne pepper (*C. frutescens* L.) in each treatment variation, namely the addition of ETAC-21/ETAC-12, 1% NPK, IAA isolate, and crude extracts from red algae and control plants qualitatively through the bands formed from the electrophoresis results shown in Figure 8.

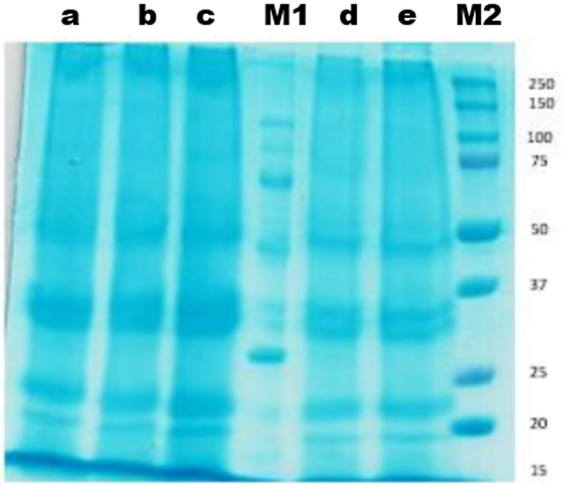

Fig. 8. SDS-PAGE results on cayenne pepper (*C. frutescens* L.) in various treatments, namely Biostimulant ETAC-21/ETAC12 (a), IAA isolate (b), crude extract (c), 1% NPK (d), and control (e) using 90 kDa marker protein (M1) and 250 kDa marker protein (M2)

From the results obtained, there are several variations in the number of bands in cayenne pepper from electrophoresis as described in previous research by Kumar & Tata (2010). Bands in plants treated with IAA isolate, crude extract, and ETAC-21/ETAC-12 gave a thicker band intensity in each band formed compared to plants treated with 1% NPK and control plants. In their research, Kumar & Tata (2010) stated that there are species-specific bands in the band with molecular weights between 29 kDa and 50 kDa for *C. annuum* and *C. frutescens* species.

Conclusions

Auxin (IAA) can be isolated, identified, and characterized, with the results of high performance liquid chromatography showing the concentration of auxin in red algae (*E. spinosum*) of 2.43 mg/g dry weight. The addition of IAA isolates from red algae (*E. spinosum*) can affect the growth of cayenne pepper plants (*C. frutescens* L.) in the vegetative phase compared to the control plants, as seen from the increase in root length and root dry weight. at week 1-4. respectively. IAA isolate from red algae (*E. spinosum*) increased the quality and quantity of fruits and seeds of cayenne pepper, respectively. From proximate analysis, the protein content of cayenne pepper (*C. frutescens* L.) was higher compared to the control plants by an average of 6.18% ± 0.19.and 4.73% ± 2.299. From the SDS result, IAA isolate from red algae (*E. spinosum*) gave a distinctly different pattern of protein synthesis in cayenne pepper (*C. frutescens* L.) compared to control plants.

Acknowledgements

The authors are grateful to the Directorate of Research and Community Service and Innovative Universitas Padjadjaran; Dean of the Faculty of Mathematics and Natural Sciences, Head of the Department of Chemistry, and Head and the entire staff of Biochemistry Laboratory. We are also grateful to the laboratory facilities provided by the Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Padjadjaran and to thank all those who have given advice, motivation, and material support.

Author Contributions

R. P. S. conceived and designed the experiment, performed the experiment, analyzed and interpreted the data, and wrote the manuscript.

I. P. M. conceived and designed the experiments, analyzed and interpreted the data. Wrote and revised the manuscript.

R. H. conceived and experimented, analyzed, and interpreted the data.

S. I. Analyzed and interpreted the data. Wrote and revised the manuscript.

U. M. S. S. conceived and designed the experiments, analyzed and interpreted the data, wrote the manuscript, compiled and revised the manuscript.

Declaration Of Conflicting Interests:

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Funding Support:

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Grant Number: 1347/UN6.3.1/PT.00/2021.

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Received*: April,19, 2021;* **Accepted***: September,03, 2021;* **Published***: December, 2022*