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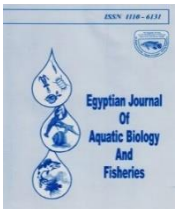

Chlorophyll-a Content Characterization of *Kappaphycus alvarezii* Propagule Ekstract Resulted from Tissue Culture

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ABSTRACT

Seaweed is a macroalgae that is rich in compounds beneficial to health, such as the pigment chlorophyll a. Species *Kappaphycus alvarezii* is a group of *Rhodophyceae* which contains a lot of chlorophyll a. Research on seaweed tissue culture on *K. alvarezii* species *in vitro* over the last few decades has provided many new discoveries in seaweed cultivation activities. One aspect that will be studied through this research is how to characterize the chlorophyll a content of propagule *K alvarezii* extract that resulted by tissue culture. This study aimed to characterize and identify the chlorophyll-a content in *K. alvarezii* propagules resulting from tissue culture to optimize the utilization of seaweed as a raw material for functional food. This research was conducted at the Balai Perikanan Budidaya Laut Lombok (BPBLL) Sekotong, NTB, Indonesia and Laboratorium Kimia Organik Universitas Islam Negeri (UIN) Maulana Malik Ibrahim Malang, Indonesia. This research used experimental methods to characterize the content of chlorophyll a propagule extract of *K. alvarezii* from tissue culture including the value of water content, extract yield value, isolation results (chromatography, spectrophotometry and FTIR) and identification with LCMS. The results of the identification of chlorophyll a by TLC on isolates of *K. alvarezzi* obtained blue green spots which have a Rf value of 0.55. The spectral pattern has a maximum absorption of blue waves at a wavelength of 430nm and a maximum absorption of red waves at a wavelength of 662nm with methanol as solvent. In the LCMS method, it was shown that the molecular weight of chlorophyll a was 893.98m/ z.

INTRODUCTION

Seaweed *Kappaphycus alvarezii* is a red alga widely cultivated for its economic value. The species has considerable phycocolloid benefits as a source of carrageenan and agar. It also requires relatively easy and inexpensive cultivation techniques. The development of research on seaweed tissue culture on *K. alvarezii* or *Eucheuma cottonii* species *in vitro* over the last few decades has provided many discoveries in seaweed cultivation, such as finding superior varieties as seeds and better carrageenan production **(Hurtado *et al*., 2014; Yong *et al*., 2014; Yong *et al*., 2015)**.

The success has supported the availability of seaweed as a raw material for functional food products with health benefits for humans. Seaweed has the potential to be developed as a functional food product because it contains nutrients and bioactive components beneficial to health. Some research results show that *K. alvarezii* has high economic potential; it also contains vitamins, minerals, fiber, sodium, potassium, and

bioactive compounds in the form of secondary metabolites, with pigments as its most essential nutrients **(de Fretes *et al*., 2011; Holdt & Kraan, 2011; Lumbessy *et al*., 2019)**.

In addition to its rich nutritional content, *K. alvarezii* is also rich in photosynthetic pigments and other accessory pigments, namely chlorophyll-a, α-carotene, β-carotene, phycobilin, neoxanthin, and zeaxanthin **(Bixler & Porse, 2011; Madhavarani & Ramanibai, 2014)**. Chlorophyll can be used as a natural pigment product as a health supplement. Several studies on chlorophyll pigment in red seaweed have been conducted **(Reeta & Kulandaivelu, 2000; Aguilera *et al*., 2002; Gudrun & Wincke, 2005; Lee, 2008; Naguit & Tisera, 2009; Sarojini & Narayanan, 2009; Schmidt *et al*., 2010; Chandra, 2012; Lumbessy *et al*., 2021)**. The results of these studies indicate that the varying colors of the thallus are due to the pigment composition consisting of chlorophyll-a, chlorophyll-d, and phycobiliprotein. The varied composition of biopigments in red seaweed can be an opportunity to increase the selling price of seaweed.

Information about photosynthetic pigments in seaweed tissue culture (*in vitro*) has also been known in research by **Lumbessy *et al.* (2018)**, who found that the difference of concentration of liquid PES media give obvious effect to the chlorophyll-a content of *K. alvarezii* propagule. The 10 mlliquid PES treatment in 500ml of sterile seawater gave chlorophyll-a of 61.308µg/ ml. Based on the description above, this study aimed to characterize and identify the chlorophyll-a content in *K. alvarezii* propagules resulting from tissue culture to optimize the utilization of seaweed as a raw material for functional food.

MATERIALS AND METHODS

K. alvarezii propagule from the tissue culture process was conducted at Balai Budidaya Laut Lombok (BBLL) Sekotong, West Nusa Tenggara, Indonesia. Extraction, isolation, and identification of chlorophyll-a of *K. alvarezii* propagule were done at several laboratories, including Laboratorium Kimia Organik Universitas Islam Negeri (UIN) Maulana Malik Ibrahim Malang, Laboratorium Kimia POLINEMA Malang, and Laboratorium Kimia Organik, Universitas Mataram.

Equipment for extraction included cool box, plastic basket, clean cloth, fan scissors, blender, spoon, analytical balance, baking pan, oven, desiccator, measuring cup, beaker glass, spatula, Erlenmeyer, glass funnel, separating flask (separating funnel), rotary vacuum evaporator, freezer, stative, column chromatography, magnetic stirrer, hot plate, nitrogen gas tube, dropper pipette, volume pipette, suction ball, capillary tube, ruler, cutter, pencil, tweezers, marker, cuvette, sample bottle, test tube, test tube rack, micro pipette, centrifuge tube, centrifuge, spectrophotometer.

The chemicals used consist of $CaCO_3$, acetone, methanol, diethyl ether, n-hexane, ethyl acetate, distilled water pH 7, salt saturation, silica Gel F-254 (60 mesh), nitrogen gas, sea sand, plates TLC (Thin Layer Chromatography). All chemicals used are in the PA category (Pro-analysis).

1. Sample preparation

The sample of *K. alvarezii* propagule from the tissue culture process was prepared by first cleaning the sample, expanding the surface, and reducing water content. Cleaning was done by washing the sample using running clean water to remove any residual of the liquid PES fertilizer attached to the propagule walls, which could interfere with the extraction process. The surface expansion of the sample was done by cutting the sample into 0.5 – 1.0cm to make the water content in the material easily evaporate. Then, the samples were aerated at room temperature in a light-tight room to reduce the moisture content

2. Water content analysis

Water content analysis in the sample was done using the method described by **AOAC (2000)**. The method analyzes the moisture content of the material from the evaporation of the water content through heating using an oven at 105^0C until it reaches a constant weight for ± 3 hours. Water content analysis was carried out based on the reduction in mass or initial sample weight due to water evaporation. The calculation of the water content analysis of the material was carried out using the following formula:

$$\%\ Water\ content\ = \frac{B - C}{B - A} \text{ x } 100\%$$

Note: A = weight of the weighting bottle (g)
B = weight of the weighting bottle + initial sample (g)

3. Pigment extraction

The sample extraction process was done using the method by **Lumbessy *et al*. (2021)** with some modifications to maximize the extraction process. Extraction started with weighing 100g of the sample added with $CaCO_3$ as a neutralizing agent during extraction. Next, the sample was mashed with mortars to expand the surface of the material and maximize the extraction process. The sample maceration process was carried out by adding 400mL of methanol: acetone (7:3 v/v) before being incubated for 24 hours at room temperature in a room impermeable to light. After being left for 24 hours, the sample was filtered using Whatman filter paper number 42 to separate the filtrate and residue. The filtrate was evaporated using a rotary vacuum at 32^0C with a speed of 10 rpm to evaporate most of the solvent to obtain a crude pigment extract in the form of a dark green paste. The weighing was then carried out to calculate the yield value **(Ummat *et al*., 2020)** as follows:

$$\%\ \text{Yield} = \frac{\text{weight of dry extract}}{\text{weight of dry sample}} \text{ x } 100\%$$

4. Isolation of the chlorophyll-a compound (Lumbessy *et al*. 2021)

Isolation of the chlorophyll-a compound from the sample of *K. alvarezii* propagule from the tissue culture was done using the stratified column chromatography method with silica gel as the stationary phase and hexane as the mobile phase: ethyl acetate ±

200mL. The isolation process started by dissolving ± 0.3 - 0.4g dry crude extract into ± 3mL of hexane: ethyl acetate mobile phase (8: 2 v/v). The polarity of the mobile phase was continuously increased with a volume of ± 200mL from the ratio of hexane: ethyl acetate (8: 2 v/v), hexane: ethyl acetate (7: 3 v/v), hexane: ethyl acetate (6: 4 v/ v), to a ratio of hexane: ethyl acetate (5: 5 v/v). All fractions with bands of different colors formed were accommodated in a test tube according to their respective colors while continuing to add the mobile phase little by little so that the silica gel did not dry out.

5. Identification of the chlorophyll-a compound

a. Analysis of chlorophyll-a by thin layer chromatography (TLC)

As much as 5 – 10µl of chlorophyll-a isolate was taken using a capillary tube and spotted on the prepared TLC plate with a size of 5 x 10cm. The TLC plate was inserted upright into a chamber containing the mobile phase, namely a mixture of 10mL of hexane : diethyl ether : acetone (5 : 3 : 2, v/v), and it was then closed. We waited until the eluent reached the upper limit, then the TLC plate was taken with a tweezer. The color spots formed were then calculated for their Rf values.

b. Analysis of chlorophyll-a by a spectrophotometer

The sample and blank solution were prepared in a special cuvette or test tube. The spectrophotometer was turned on, and the wavelength was set in the 400 – 700nm range. Then the sample solution and blank solution cuvette were inserted into the spectrophotometer slot. The maximum absorbance of the sample solution at a specific wavelength was read and recorded. In the same way as the sample, the absorbance of the standard chlorophyll-a solution was measured at the same wavelength in the sample. It was then used to calculate the chlorophyll-a content of the sample with the following formula **(Lichtenthaler, 1987)**:

$$\text{Chlorophyll-a} = 10{,}05\ A_{660{,}6} - 0{,}97\ A_{642{,}2}$$

c. Analysis of chlorophyll-a by FTIR (Liu *et al.*, 2006)

Two milligrams of the sample were mixed with 200mg of KBr to make pellets. Pellets were made using a Shimadzu hand press with a working pressure of 8 tons for 10 minutes. FTIR spectrum measurements were carried out using a Tensor 37 FTIR Spectrometer equipped with a DTGS detector. A personal computer equipped with OPUS Software version 4.2. was used to control the work of the spectrometer in producing spectra in the range of 400 – 4000cm^{-1}; the spectrum was generated at a speed of 30 seconds with a resolution of 4cm^{-1}. To increase the signal or noise ratio, 32 spectra were combined and averaged. Analysis of the functional groups of a sample was carried out by comparing the absorption bands formed in the infrared spectrum using a correlation table and using the spectrum of the reference compound (which is already known).

d. Analysis of chlorophyll-a by LCMS (Sangeetha *et al*., 2009)

The sample was analyzed using LCMS with a 3200 Qtrap mass spectrometry detector, Phenomenex C18 column (50 mm x 2.0 mm) at 30^0C. Two types of mobile phase were used, namely eluent A: Aqua Bidest and ammonium formate and eluent B: methanol and ammonium formate. The flow used was a gradient elution of 30 - 90% eluent B. The sample injection volume was 10µL, with a flow rate of 0.5mL/ min and an analysis time of 15 minutes. The separation of compounds was monitored with DAD at 254 and 190nm and a mass spectrometry detector. Mass spectrometry (ESI) analysis was performed on an LCMS-8030 triple quadrupole mass spectrometer. LC-MS was characterized by negative and positive ionization modes with spectra obtained over the mass range of 50-1000 m/z.

6. Data analysis

Data were analyzed descriptively and displayed in the form of pictures, narration, and tables.

RESULTS

1. Water content analysis

The water content analysis of the sample of *K. alvarezii* propagule from the tissue culture process was done using the method described by **AOAC (2000)**. Water content was identified by reducing the initial mass or weight of the sample due to the evaporation of water along with heating in the oven. Then, the mass reduction data were substituted into the water content calculation formula. The calculation results showed that the average water content of fresh *K. alvarezii* propagules used in this study was 87.05%. **Ahmad *et al*. (2012)** stated that the water content in fresh seaweed varies between 75.95 – 96.03%. Meanwhile the water content of the red seaweeds ranged between 76.56 and 81.13% **(Nurshahidah *et al*., 2020)**.

2. Yield value of the extract

The extraction was done using the method by **Lumbessy *et al*. (2021)** with some modifications to maximize the extraction process. The result was a crude extract of dry pigment with a dark green color. According to **Ncube *et al*. (2008)**, extraction is a method of separating bioactive compounds from plant tissue using solvents, especially organic solvents. During extraction, the solvent undergoes diffusion into the plant tissue and dissolves components that have the same polarity as the solvent.

The solvents used at this stage were methanol and acetone. **Torres *et al*. (2014)** showed that methanol is the most efficient solvent for extracting carotenoids and chlorophyll-a in *Gracilaria tenuistipitata* Var. *Liui*. It is further said that all marine organisms have a high-water content and this water content forms a barrier against the penetration of these solvents. This helps explain why the more polar solvents give the best extraction results because they can break down the cell walls of organisms with high water content.

After being extracted, the yield value was then analyzed **(Ummat *et al*., 2020)**. The calculation results showed that the dry extract yield of fresh *K. alvarezii* propagules used in this study was 2.1056%. According to **Usov (2011)**, the cell walls of red algae consist of cellulose, agar, carrageenan, porphyran, and selaran. Compared to the seaweed *Eucheuma spinosum*, the cell walls of *Eucheuma cottonii* or *K. alvarezii* are more easily penetrated by solvents.

3. Chlorophyll-a isolation results

Isolation of the chlorophyll-a pigment from fresh *K. alvarezii* propagules was carried out with reference to **Lumbessy *et al*. (2021)**. Chlorophyll-a was separated from other pigments present in *K. alvarezii* propagules by a column chromatography method with hexane: ethyl acetate (7: 3 v/v) as the mobile phase and silica gel as the stationary phase. The hexane: ethyl acetate solvent mixture of 7: 3 v/v showed the separation of chlorophyll-a began. Table (1) shows that the hexane: ethyl acetate eluent with a ratio of 7: 3 was able to separate fucoxanthin properly, and a green color resulted.

Table 1. Comparison of the eluent used to separate the chlorophyll-a pigment from fresh *K. alvarezii* propagules on column chromatography

Combination of solvent types	Eluent ratio	Note
Hexane : ethyl acetate	8 : 2	Not yet separated
Hexane : ethyl acetate	7 : 3	Separated
Hexane : ethyl acetate	6 : 4	Separated
Hexane : ethyl acetate	5 : 5	Separated

4. Chlorophyll-a identification results

a. Thin layer chromatography (TLC) results

Analysis of the chlorophyll-a pigment from fresh *K. alvarezii* propagules was carried out using the Thin Layer Chromatography (TLC) method to obtain color spots to determine the Retardation factor (Rf) of the compounds forming the chlorophyll-a color spot. The determination of color spots in TLC is based on the Rf value, as was done by **Dimara *et al*. (2012)** using standard chlorophyll-a. The color shown in the separation of pigments in TLC can be used as a basis for pigment identification. Rf analysis needs to be done to strengthen the identification of pigment composition based on color **(Daood, 2003)**. The results of the analysis of chlorophyll-a using the TLC method are presented in **Fig. (1)**.

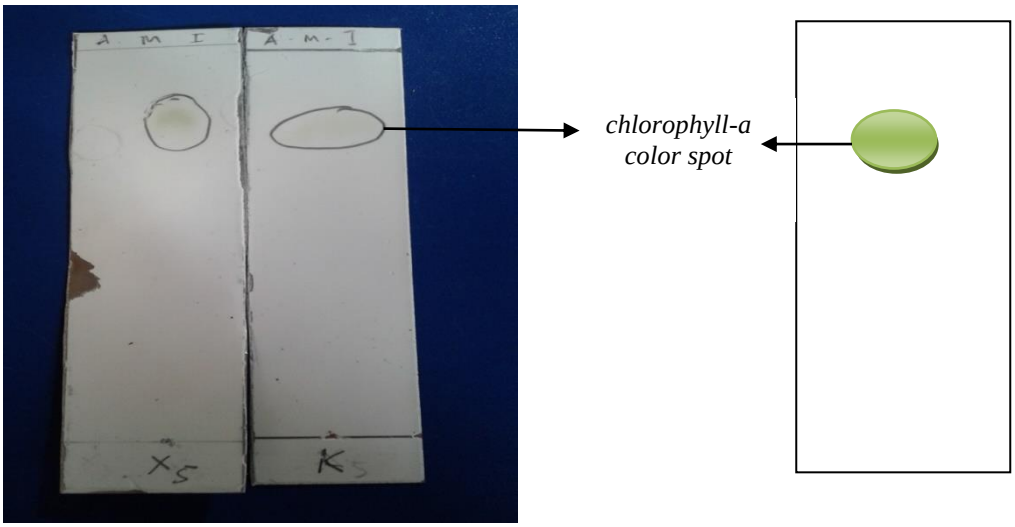

Fig. 1. The results of the analysis of chlorophyll-a from the crude extract using the TLC method *(*X5 = crude extract, K = standard) with a mobile phase of hexane: diethyl ether: acetone (5: 3 : 2, v/v).

b. Results of qualitative analysis of chlorophyll-a with a spectrophotometer

The spectral pattern of chlorophyll-a isolated by column chromatography was compared to the standard spectrum pattern of chlorophyll-a; it has a maximum absorption of the blue wave at a wavelength of 430nm and a maximum absorption of the red wave at a wavelength of 661nm for the standard and 662nm for the results of column chromatography isolation (Fig. 2).

According to **Lichtenthaler and Buschmann (2001)**, the maximum blue wavelength of chlorophyll-a ranges from 428 - 432nm, and the maximum red wavelength of chlorophyll-a ranges from 660 - 665nm. The difference in the maximum red wavelength of chlorophyll-a on the standard and the results of the column chromatography isolation is due to the quality of the solvent or the level of purity of the solvent used for analysis.

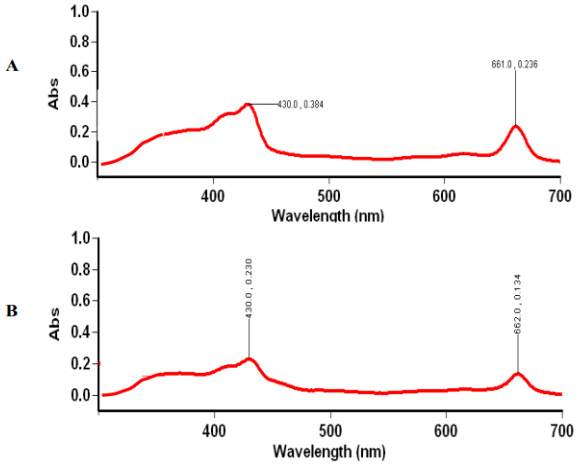

Fig. 2. The spectral pattern of chlorophyll-a at the maximum wavelength **(A)** The standard spectral pattern of chlorophyll-a, **(B)** The spectral pattern of chlorophyll-a as a result of column chromatography isolation

c. Results of qualitative analysis of chlorophyll-a by FTIR

The functional group of chlorophyll-a was analyzed using the spectrophotometric Fourier Transform Infra-Red (FTIR) method. FTIR is useful for studying functional groups in a compound **(Yue *et al.*, 2007)**. Infrared spectra analysis of the fucoxanthin sample of *K. alvarezii* is presented in Table (2) and Fig. (3).

Table 2. The analysis results of the functional group of chlorophyll-a of *K. alvarezii* propagules using Fourier Transform Infra-Red (FTIR) Spectrometer

Absorption peak (cm^{-1})	Vibration of functional groups
3462.44	N-H stretching of secondary amines or imines
2925.63	C-H stretching of C-O-CH_3
2858.13	C-H on N-CH_2-
1731.50	C-O in unsaturated αβ (ester and lactone)
1639.83	C-H on conjugated C-C
1542.31	N-H bending
1462.64	C-H *anti-symmetric* and *symmetric*
1382.88	C-N on tetrapyrrole ring
1273.35	C-O vibration
1125.22	C=N groups
1073	C-C skeletal
742.62	CH_2 rocking

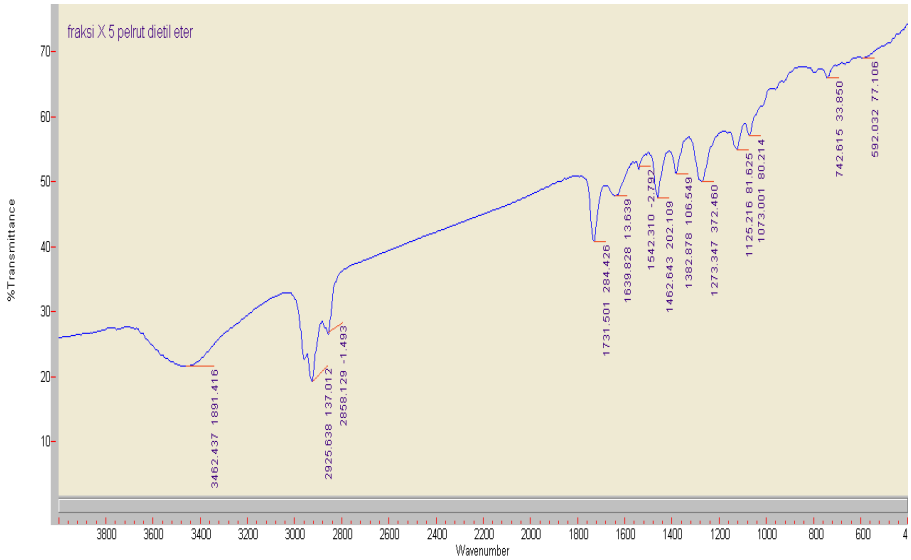

Fig. 3. FTIR spectrometer chromatogram of chlorophyll-a of *K. alvarezii* propagules isolated by column chromatography

The chlorophyll group is characterized by the presence of a ketone oxygen vibration ring in which the hydrogen bonded to water coordinates with the central magnesium atom of the chlorophyll. This condition will give rise to hydrogen

bonded to the carbonyl ester with a peak at 3431.31cm^{-1} **(Konwar & Baruah, 2011)**. The chlorophyll-water aggregate will produce a peak at 1643.30cm^{-1} **(Konwar & Baruah, 2011)**. Additionally, the peak of 1643.30cm^{-1} can also indicate the coordination of ketone oxygen with magnesium **(Dikio & Isabirye, 2008)**. Anti-symmetric and symmetric C-H alkyl group deformation appears at 1417cm^{-1} **(Dikio & Isabirye, 2008)**. Meanwhile, the peak at 1155cm^{-1} indicates the presence of a C=N group **(Konwar & Baruah, 2011)**.

d. Mass spectrum of chlorophyll-a of *K. alvarezii* propagules analyzed by LCMS

Fig. (4) shows that the sample contains the chlorophyll-a compound with the characteristics of 3 molecular ion fragments; the highest abundance (base peak) of these fragments is 893.98m/ z, which is also strongly suspected as the molecular weight of chlorophyll-a. The resulting fragmentation list is presented in Table (3).

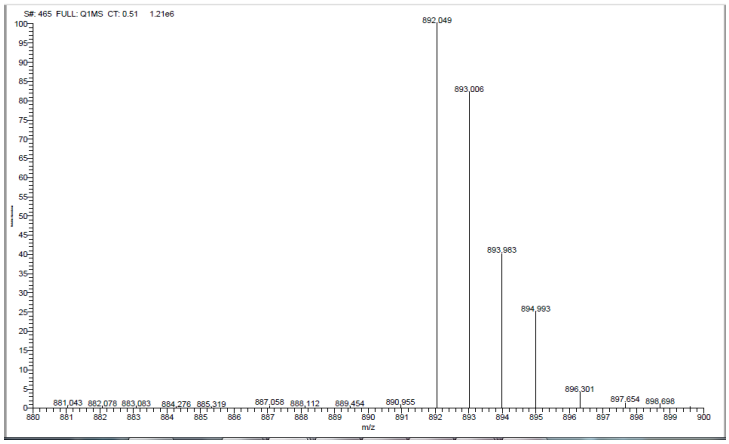

Fig. 4. The chromatogram of chlorophyll-a from LCMS analysis results

Table (3) shows that the first and second peaks are chlorophyll-a with a molecular weight of 893.01 and 893.98m/ z. The third peak has a molecular weight of 894.99m/ z; this peak is suspected as chlorophyll-a with the addition of H^+ ($[M+H]^+$). The last peak, with a molecular weight of 896.30m/ z, is chlorophyll-a with the addition of ion H^+ ($[M+2H]^+$).

Table 3. Molecular fraction of the chlorophyll-a compound in *K. alvarezii* propagules

Ion mass (m/z)	Estimated molecular ion fraction
893.01	$C_{55}H_{72}O_5N_4Mg$
893.98	$C_{55}H_{72}O_5N_4Mg$
894.99	$C_{55}H_{73}O_5N_4Mg$
896.30	$C_{55}H_{75}O_5N_4Mg$

CONCLUSION

The results of the identification of chlorophyll-a with TLC on *K. alvarezzi* isolates obtained green and blue spots with an Rf value of 0.55. The spectral pattern had a maximum absorption of blue waves at a wavelength of 430nm and a maximum absorption of red waves at a wavelength of 662nm with methanol solvent. LCMS shows that the molecular weight of chlorophyll-a was 893.98m/ z.

REFERENCES

Ahmad, F., M. R. Sulaiman, W. Saimon, C. F.Yee & P. Matanjun. (2012) 'Proximate Compositions And Total Phenolic Contents Of Selected Edible Seaweed From Semporna, Sabah, Malaysia', Borneo Science, 31, pp. 85 – 96. https://www.researchgate.net/publication/281545270_Proximate_compositions_and_total_phenolic_contents_of_selected_edible_seaweed_from_Semporna_Sabah_Malaysia

Aguilera, J., Bischof, K., Karsten, U., Hanelt, D. & Wiencke, C. (2002) 'Seasonal Variation in Ecophysiological Patterns in Macroalgae from an Arctic Fjord. II. Pigment Accumulation and Biochemical Defence Systems Against High Light Stress', Marine Biology, 140, pp.1087-1095. https://doi.org/10.1007/s00227-002-0792-y

AOAC International. (2000) 'Official Methods of Analysis of AOAC', Association of Official Analytical Chemists. Washington DC: AOAC International.

Bixler, H.J. & Porse, H. (2011). 'A Decade of Change in the Seaweed Hydrocolloids Industry', Journal of Applied Phycology, 23(3), pp. 321–335. DOI:10.1007/s10811-010-9529-3

Daood, H. (2003) 'Chlorophyll. Encyclopedia of Food Sciences and Nutrition (Second Edition)', Academic Press, pp. 1196-1205

de Fretes, H., Susanto, A.B., Limantara, L., Prasetyo, B., Heriyanto & Brotosudamo, T.H.P. (2011) 'Composition and Content of Pigment, Photostability and Thermostability Studies of Crude Pigment Extracts from Red, Brown and Green Varieties of Red Algae Kappaphycus alvarezii (Doty) Doty', Conference: Proceeding of ICONS At: Malang, Indonesia. https://www.researchgate.net/publication/337729619_Pigment_Composition_Photostability_and_Thermostability_Studies_of_Crude_Pigment_Extracts_from_Red_Brown_and_Green_Varieties_of_Red_Algae_Kappaphycus_alvarezii_Doty_Doty

De Junet, A., G. Abril, F. Guerin, I. Billy, De Wit R. (2009) 'A multi-tracers analysis of sources and transfers of particulate organic matter in a tropical reservoir (Petit Saut, French Guiana)' *River Res*earch and *Appl*ications, 25(3), pp. 253–271. https://doi.org/10.1002/rra.1152

Dikio E.D. & D.A. Isabirye. (2008) 'Isolation Of Chlorophyll *A* From Spinach Leaves', Bulletin Of The Chemical Society Of Ethiopia, 22(2), pp. 301-304. DOI:10.4314/bcse.v22i2.61302

Gudrun, K. & Wincke, C. (2005) 'Photosynthesis, Photosynthetic Pigment and Mycosporine-Like Aminoacids After Exposure of the Marine Red Alga Chondrus crispus (Gigartinales, Rhodophyta) to Different Light Qualities', Phycologia, 44(1), pp. 95-102. DOI:10.2216/0031-8884(2005)44[95:PPPAMA]2.0.CO;2

Holdt, S. L., & Kraan, S. (2011) 'Bioactive Compounds in Seaweed: Functional Food Applications and Legislation', Journal of Applied Phycology, 23(3), pp. 543-597. DOI:10.1007/s10811-010-9632-5

Hurtado, A.Q., D.A. Yunque, K. Tibubos, & A.T. Critchley. (2009) 'Use of Acadian Marine Plant Extract Powder from *Ascophyllum nodosum* in Tissue Culture of *Kappaphycus alvarezii',* Journal of Applied. Phycology, 21(6), pp. 633–639. DOI:10.1007/s10811-008-9395-4

Konwar, M. & G. D Baruah. (2011) 'On the nature of vibrational bands in the FTIR spectra of medicinal plant leaves', Archives of Applied Science Research, 3(1), pp. 214-221. http://scholarsresearchlibrary.com/aasr-vol3-iss1/AASR-2011-3-1-214-221.pdf

Lee, R. E. (2008) 'Phycology. Fouth Edition', Cambridge University Press, http:/www.cambridge.org/ 97805621864084.

Lichtenthaler, K. H. (1987) 'Methods in Enzymology. Chlorophyll and Carotenoid: Pigments of Photosynthetic Biomembranes', New York: Academic Press.Inc.

Lichtenthaler, H. K. & C. Buschmann. (2001) 'Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy', Current Protocols in Food Analytical Chemistry (CPFA), John Wiley and Sons, New York, F4.2 (1-5). DOI:10.1002/0471142913.faf0403s01

Liu, J. W., Dong, S. L., Liu, X. Y. & Ma, S. (2000) 'Responses of the macroalga *Gracilaria tenuistipitata* var.liui (Rhodophyta) to iron stress', Journal of Applied Phycology, 12(6), pp. 605-612. DOI:10.1023/A:1026523213818

Liu, H. X., S. Q. Sun, G. H. Lv & K. K. C. Chan. (2006) 'Study on angelica and its different extracts by Fourier transform infrared spectroscopy and twodimentional correlation IR spectroscopy', Spectrochimica Acta, Part A Molecular and Biomolecular Spectroscopy, 64(2), pp. 321-326. DOI:10.1016/j.saa.2005.07.026

Lumbessy. S. Y., Sri, A., Happy, N., and Muhammad, F. (2018). 'Concentration of liquid pes media on the growth and photosynthetic pigments of seaweeds Cotonii propagule (*Kappaphycus alvarezii* Doty) through tissue culture'. Russian Journal of Agricultural and Socio-Economic Sciences, *75*(3), pp. 133-144. DOI https://doi.org/10.18551/rjoas.2018-03.15

Lumbessy, S. Y., Andayani, S., Nursyam, H., & Firdaus, M. (2019) 'Biochemical study of amino acid profile of *Kappaphycus alvarezii* and *Gracilaria salicornia* seaweeds from Gerupuk Waters, West Nusa Tenggara (NTB)', EurAsian Journal of BioSciences, 13(1), pp. 303-307. http://www.ejobios.org/download/biochemical-study-of-amino-acid-profile-of-kappaphycus-alvarezii-and-gracilaria-salicornia-seaweeds-6207.pdf

Lumbessy, S. Y., Junaidi, M., Diniarti, N., Setyowati, D. N., Mukhlis, A., & Tambaru, R. (2021, March) 'Identification of chlorophyll pigment on Gracilaria Salicornia seaweed', in IOP Conference Series: Earth and Environmental Science, 681(1), pp. 1-4. IOP Publishing. DOI:10.1088/1755-1315/681/1/012017

Madhavarani, A & Ramanibai, R. (2014) 'In-vitro Antibacterial Activity of Kappaphycus alvarezii Extracts Collected from Mandapam Coast, Rameswaram, Tamil Nadu', International Journal of Innovative Research in science, Engineering and Techhnology, 3(1), pp. 8436–8440

Naguit, M. R. A. & Tisera, W. L. (2009) 'Pigment Analysis on Eucheuma denticulatum (Collins & Hervey) and Kappaphycus alvarezii (doty) Cultivars Cultured at Different Depths', Threshold, 4, pp. 29–37

Ncube, N. S., A. J. Afolayan and A. I. Okoh. (2008) 'Assessment Techniques Of Antimicrobial Properties Of Natural Compounds Of Plant Origin: Current Methods And Future Trends', African Journal Of Biotechnology (AJB), 7(12), pp. 1797-1806. DOI:10.4314/ajb.v7i12.58804

Nurshahida, M. S. F., Nazikussabah, Z., Subramaniam, S., Faizal, W and Aini, M. A. N. (2020). 'Physicochemical, physical characteristics and antioxidant activities of three edible red seaweeds (*Kappaphycus alvarezii*, *Eucheuma spinosum* and *Eucheuma striatum*) from Sabah, Malaysia. IOP Conference Series: Materials Science and Engineering. 991. 012001. 10.1088/1757-899X/991/1/012001

Quach, H. T., Steeper, R. L. and Griffin, G. W. (2004) 'An improved method for the extraction and thin-layer chromatography of chlorophyll a and b from spinach', Journal of Chemical Education, 81(3), pp. 385-387. DOI:10.1021/ed081p385

Reeta, J. & Kulandaivelu, G. (2000) 'Effect of Light Intensity on the Saturation of Photosynthesis in Gracilaria Species (Rhodophyta)', Seaweed Research Utilization, 22(1&2), pp. 31-35. http://eprints.cmfri.org.in/7073/

Sangeetha, R. K., N. Bhaskar, S. Divakar & V. Baskaran. (2009) 'Bioavailability and Metabolism of Fucoxanthin in rats : Structural Characterization of Netabilites by LC-MS (APCI)', Molecular and Cellular Biochemistry, 333(1), pp. 299-310. DOI:10.1007/s11010-009-0231-1

Sarojini Y. K., & Narayanan, L. (2009) 'Influence of Environmental Factors on Variationsin Distribution of Photosynthetic Pigments of Macro Algae', Algal Biomass Resources and Utilization, pp.157-163

Sathya, S. (2017) 'Separation of algal pigments by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC)', World Journal of Pharmacheutical Research, 6(16), pp. 1275–1284. DOI:10.20959/wjpr201716-10328

Schmidt, E. C., Nunes, B. G., Maraschin, M. & Bouzon, Z. L. (2010) 'Effect of Ultraviolet-B Radiation on Growth, Photosynthetic Pigment, and Cell Biology of Kappaphycus alvarezii (Rhodophyta, Gigartinales) Macroalgae Brown Strain', Photosynthetica, 48(2), pp. 161-172. DOI:10.1007/s11099-010-0022-7

Torres, I. L., C. Rosa-Ferreira, & S. Munro. (2014) 'The Arf family G protein Arl1 is required for secretory granule biogenesis in Drosophila', Journal of Cell Science, 127(10), pp. 2151-2160. DOI:10.1242/jcs.122028

Ummat, V., Tiwari, B. K., Jaiswal, A. K., Condon, K., Garcia-Vaquero, M., O'Doherty, J., O'Donnell, C., Rajauria, G. (2020) 'Optimisation of Ultrasound Frequency, Extraction Time and Solvent for the Recovery of Polyphenols, Phlorotannins and Associated Antioxidant Activity from Brown Seaweeds', Marine Drugs, 18(5), pp.2-15. DOI:10.3390/md18050250

Usov, A. (2011) 'Polysaccharides of the red algae', Advances in carbohydrate chemistry and biochemistry, 65 pp.115-217. DOI:10.1016/B978-0-12-385520-6.00004-2

Vanitha, A. & Chandra, S. (2012) 'Studies on Photosynthetic Pigments of Some Red Algae of Covelong, Chennai (India)', International Journal of Current Science, pp.149-154

Yong, W. T. L., J. Y. Y. Chin, V. Y. Thien, and S. Yasir. (2014) 'Evaluation of Growth Rate and Semi-Refined Carrageenan Properties of Tissue-Cultured *Kappaphycus alvarezii* (Rhodophyta, Gigartinales)', Phycological Research, 62(4), pp. 316–321. DOI:10.1111/pre.12067

Yong, Y. S., W. T. L. Yong, V. Y. Thien, S. E. Ng, A. Anton, & S. Yasir. (2015) 'Acclimatization of Micropropagated *Kappaphycus alvarezii* (Doty) Doty ex Silva (Rhodophyta, Solieriaceae) in Outdoor Nursery System', **Journal of Applied Phycology,** 27(1), pp. 413–419. DOI:10.1007/s10811-014-0289-3

Yue, J., G. Chen, Q. Yuan, L. Luo, & Y. Gonthie. (2007) 'Hydrodynamics and mass transfer characteristics in gas–liquid flow through a rectangular microchannel', Chemical Engineering Science, 62(7), pp. 2096-2108. DOI : https://doi.org/10.1016/j.ces.2006.12.057