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THE BIOMASS CHARACTERISTICS OF *Hydropuntia eucheumatoides* (HARVEY) IN THE TIDAL BEACH OF HON RUA ISLAND, NHA TRANG BAY: POTENTIAL SOURCE FOR EXPLOITATION, CULTURE, AND FOOD PROCESSING

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ABSTRACT

The study of biomass characteristics of *Hydropuntia eucheumatoides* (Harvey) (local name in Vietnam, Rong Cau chan vit) in Hon Rua - Nha Trang bay is a comprehensive study of its resources. The results of the study have described in detail the morphological characteristics, reproductive organs, weight and coverage of its in quadrat and habitats. As a result, other has successfully stored seaweed algae under laboratory condition for 30 days. The results can be shown that determined the appropriate harvesting time and direction to exploit and conserve resources and propagate this seaweed. In addition, the main chemical and carbohydrate components of this seaweed have also been identified which has shown great potential for their application in the processing of high value food and medicine products in Vietnam in the future.

Keywords: Cultivation, carbohydrate, conserve, exploit, *Hydropuntia eucheumatoides.*

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INTRODUCTION

Hydropuntia euchuematoides (Harvey) (local name in Vietnam, Rong Cau chan vit) is a species of red seaweed; it has a narrow distribution that appears only in some tropical waters such as the East Sea, Indian Ocean. Therefore the research on this species is still limited, and there have been some published *H. euchuematoides* such as Carlos Frederico (2004) studying the genetic classification of this species, Gurgel (2018) the construction of the taxonomic level of *Hydropuntia* and *Gracilaria* genera. Another report by Vo et al. (2015) about the effect of irradiance and temperature on the photosynthesis of *H. eucheumatoides* was carried out. Similarly, Cherbadgy & Propp (2010) carried out the storage of *H. euchuematoides* for 24 hours and light conditions of 36.2 μmol/m^{2}s, supplemented with PO_4, 0,08 μM, NH_4, 0,29 μM, at the temperature of 29 ^oC to assess photosynthesis ability and C:N:P ratio. All articles have yet to show the great benefits of this seaweed biomass. Therefore, this study will point out that *H. euchuematoides* is a potential biomass source for food exploitation, cultivation and processing.

According to the publication by Huynh Quang Nang (1998), the seaweed *Hydropuntia eucheumatoides* is named *Gracilaria eucheumatoides*. This publication has shown that Vietnam's seaweed resources have a total of 15 species of genus *Gracilaria*. But recently, according to the update of the world's seaweed taxonomy, 5 of these 15 species have been renamed *Hydropuntia*, including (*H. eucheumatoides*, *H. edulis, H. fisferi*, *H. divergens, H. changii*). Of which, *H. eucheumatoides* is the size largest and most widely used. In Vietnam, *H. eucheumatoides* grows mainly on the South Central Coast. This species grows naturally on some beaches of Quang Ngai, Khanh Hoa, Ninh Thuan province etc. *H. eucheumatoides* is used as food for making sugar soup, mannequin and as raw materials for processing beverage products (Vo Thanh Trung et al., 2019). Besides this, *H. eucheumatoides* is known to be the raw material for agar extraction, the agar content of this seaweed species has been proved to be large so it is easy to purify agar (Le Nhu Hau & Nguyen Huu Dai, 2010). Due to the importance of this species, we evaluated the biomass characteristics of *H. eucheumatoides* on the tidal beach of Hon Rua Island, Nha Trang Bay. Investigation and assessment of *H. eucheumatoides* resources in Hon Rua Island using field measurement methods such as determination of weight, coverage in quadrat and assessment of habitat characteristics of this species were carried out. Besides, we determine the environmental parameters such as pH, temperature, salinity, total nitrogen, total phosphorus and parameters of chemical composition, carbohydrate content of raw materials.

The purpose of the investigation and evaluation of this seaweed resource was to find suitable living conditions in the natural environment, from which there is a scientific basis for the management, exploitation, conservation, farming and food processing of products has high value.

The Hon Rua island site was selected for the study because Hon Rua is specific to *H. eucheumatoides* Hon Rua's habitable zone, which is an offshore island that is not heavily influenced by fishermen in the exploitation of this species, so when harvesting biomass and its quality rating is not greatly affected.

MATERIALS AND METHODS

Research location

Hon Rua Island, Vinh Hoa Ward, Nha Trang City, Khanh Hoa province located at the coordinates of 12^{o}17’13.1” to 12^{o}17’027.7” North latitude and from 109^{o}14’25.9” to 109^{o}14’49.3” East longitude with a coastline of about 3 km. Sampling time and ecological study of *H. eucheumatoides* at Hon Rua, Nha Trang was carried out once a month from January to June 2020.

Figure 1. Sampling sites in Hon Rua Island, Nha Trang Bay (area distributed *Hydropuntia eucheumatoides* has red lines) (Map image is quoted from google map and image processed by a map specialist in the Department of Organic Materials from Marine Resources, Nha Trang Institute of Technology Research and Application)

Research subjects

Hydropuntia eucheumatoides (Harvey), 1860 (local name in Vietnam, Rong Cau chan vit). Phylum Rodophyta, class Florideophyceae, order Gracilariales, family Gracilariaceae, genus *Hydropuntia*, species *eucheumatoides* was used in this study.

Research methods

Determination weight seaweed in quadrat following the method of Saito & Abe (1970). Determination coverage following the method of Saito & Abe (1970).

Keeping seaweed in the laboratory: Post-harvest seaweed in the wild will be quickly transported to the laboratory. They are stored in foam containers with ice to stabilize the temperature. After that, seaweed is cleaned to remove algae, sand and gravel and put into tanks of 80 liters of water and kept for 30 days.

Cultivation conditions are as follows: illumination with a light intensity of 100 µmol photon/m^2s with light:dark cycle: 12:12 hours, using natural sea water treated, no nutritional supplement, water change mode every 2 days, temperature 27 ^oC. After 30 days, determine the growth rate of the seaweed.

Methods of analysis

Determining environmental factors such as Temperature: Mercury thermometer, Salinity: determined by Salinometer refractometer (Shibuya, Japan).

Determine the amount of seaweed in the field: the amount of seaweed is measured by electronic scales (KP, Maxel 500 - Taiwan). Determine the total nitrogen by the Nestler-Raynhet method (Samira et al., 2009). Determination of total phosphorus by means of the ammonium molybdate reaction and potassium tartrate (Samira et al., 2009). Determination of ash (Nguyen Van Mui, 2007). Determination of lipid by soxhlet machine (Nguyen Van Mui, 2007). Determination of total carbohydrates by the Dubois et al. (1959). Determination of agar by Suthasinee et al. (2015). Determination of cellulose by Myoung et al. (2010). Determination of starch by the method of Thianming et al. (2008). Determination of monosaccharide content by shimadzu liquid chromatography system, ICE-AS1 column anion, RID-6A detection, glucose and galctose standard by Yanagisawa et al. (2011).

Method of calculation

Determination of daily volume growth by Brinkhuis (1985).

Data processing

The data collected were compared to the statistically significant difference ($P < 0.05$) between the mean of the experimental treatments by one way test - ANOVA and Post-hoc test by Tukey on the Excel 2013 software. The data presented are average ± standard deviation.

RESULTS

Biomass characteristics of *Hydropuntia eucheumatoides*

Shape

Figure 2. Hydropuntia eucheumatoides grows in the wild

H. euchuematoides scattered, fat, hard, flattened, with few branches, awl-shaped branches, short or long, on the margins has convex mounds.

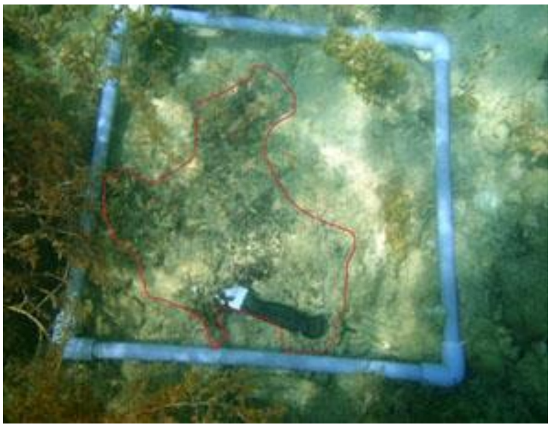

Figure 3. The coverage of *Hydropuntia eucheumatoides* coverage in quadrat (red background line)

Figure 4. Hydropuntia eucheumatoides is harvested

Figure 5. Weight of seaweed in the quadrat

Stems form narrow, branching is alternate and dichotomous; holdfast. Structure of stems consists: the core is the main axon, formed by large cells, surrounded by one or several rows of cylindrical fin cells. The skin consists of 2−4 rows of cells closely linked together, the more out of the cell the smaller (Figs. 4, 5).

Reproductive organs

Figure 6. Adult *Hydropuntia eucheumatoides* contains Spore pouches

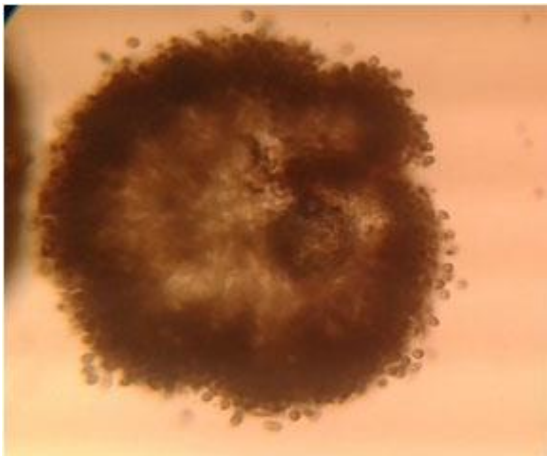

Figure 7. Cross-sectional surgery of Spore pouches

Spore pouches are conical. Spore pouches contain spherical or oval spores, spores in the form of spore, bispore, tetraspore (Figs. 6, 7). From the characteristic of *Hydropuntia euchuematoides* containing spore pouches with a large number of spores, it is potential for research and production of this species by spore collection method. This will have a positive impact on the conservation and removal of this species.

Previously, there was a rapid propagation of seaweed in the form of spore reproduction and the research object was *Gracilaria parvispora* (Edward et al., 1996).

Habitat

Figure 8. Gravel bottom

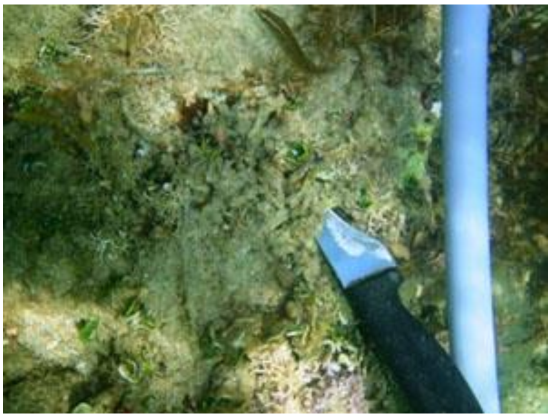

Figure 9. Hard bottom are dead coral reefs that have a flat surface

The habitat is depicted (Figs. 8–11). *H. euchuematoides* living in areas with a hard bottom are dead coral reefs that have a flat surface Figure 9. The bottom such as sandy, finger-type corals or gravel is not generally suitable for growing *H. euchuematoides*. This seaweed grows on the hard bottom because this type of flat substrate is suitable for it to crawl to the plane of the substrate, helping it not to be swept away by tidal waves. In contrast, If it develops on the bottom of sand, gravel and finger stick coral, it will be swept away by the impact of the tidal wave, resulting in no growth.

Figure 10. Finger-type corals bottom

Therefore, research on the substrate will choose the right substrate for this seaweed culture in the sea, thereby making an important contribution to the study of this seaweed biomass model.

Figure 11. Sandy bottom

Weight seaweed* and *coverage in quadrat of H. Eucheumatoides

Weight seaweed in quadrat and coverage of *H. eucheumatoides* at the time of the 1^{st}-6^{th} month, 2020 collection at Hon Rua Island was shown in Table 1.

Table 1. Weight seaweed in quadrat and coverage of *Hydropuntia eucheumatoides* at the time of 1^{st}–6^{th} month collection at Hon Rua Island

Period	Weight seaweed in quadrat (g wet/m^2)	Coverage (%/m^2)
January	1650 ± 25	23 ± 2
February	1780 ± 28	25 ± 2
March	1920 ± 34	31 ± 3
April	2100 ± 41	35 ± 3
May	2100 ± 45	34 ± 3
June	1420 ± 21	18 ± 1

Weight seaweed and coverage in quadrat fluctuations between months tend to increase with the months of the year. The weight of seaweed increased from January to April and by the beginning of May, the weight no longer increased. This shows that the seaweed grows well in the period from February to April every year. And the coverage of seaweed can reach 34–35% on the bottom of coral reefs. Thus, we can see that the weight of algae changes seasonally and reaches a high weight from April to May. This will be an appropriate time for seaweed harvesting, which has important implications for the conservation and exploitation of seaweed in Hon Rua in particular and Nha Trang Bay in general.

Characteristics of environmental factors where *H. eucheumatoides* live

The characteristics of environmental factors of the ecosystem where the *H. eucheumatoides* live are shown in Table 2.

Table 2. Environmental factors where *Hydropuntia eucheumatoides* live in Hon Rua Island from January to June 2020

Period	Temperature (^oC)	Salinity (‰)	Deep (m) (low tide - high tide)	The light (µmol photons/m^2/s)	NH_4-N (mg/L)	PO_4-P (mg/L)
January	19–21	32.5	2–2.5	445	0,31 ± 0,01	0,08 ± 0,002
February	22–24	32.6	1.8–2.4	445	0,32 ± 0,01	0,07 ± 0,001
March	23–26	33.1	1.7–2.4	468	0,33 ± 0,015	0,08 ± 0,002
April	26–28	33.3	1.2–2.4	519	0,32 ± 0,01	0,06 ± 0,001
May	26–29	33.3	1.2–2.4	523	0,32 ± 0,01	0,08 ± 0,002
June	27–29	33.3	1.2–2.4	525	0,34 ± 0,02	0,09 ± 0,003

The ecosystem where *H. eucheumatoides* live fluctuations of environmental factors over time of the year. In which two factors have a large variation in temperature and light, while the other factors have not changed much. The sea temperature from January to March is low at 19−26 ^oC, while from April to June water temperature is high at 27−29 ^oC. Light intensity tends to increase from January to April and is stable from April to June. The intensity of light is changed because of the influence of tidal waves, in January–March of Hon Rua Island, the impact of the northeast monsoon so the intensity of the wave is higher than that from April to June. Large fluctuating tidal waves made the suspension suspense, so when we conducted light measurements, the results from January to March were lower than from April to June. The impact of the northeast monsoon also causes the temperature in January–March to be lower than from April to June.

In addition, the variation in depth between months also affects the growth of seaweed *H. eucheumatoides* on Hon Rua island live at a depth of 1.2−2.5 m. And the tidal fluctuations of January–March are lower than for April - June. The reason for April–June is the period in the summer, the diving waters and the great attraction of the moon, at this time the tidal water level is very low, the sea temperature rises. This greatly affects the growth of seaweed, so by the end of June, the seaweed will wither or a few surviving seaweed.

Measured parameters of salinity and total nitrogen and phosphorus content did not change much from January to June. This shows that the water quality at Hon Rua is quite stable, there is no invasion of fresh water and nutrients flowing down from the island which makes a difference with other islands with small streams of fresh water flowing into the sea. Thus, *H. eucheumatoides* in Hon Rua island grows and develops slowly from January to March and grows well from April to May and growth decreases in June.

Keeping H. eucheumatoides in the laboratory

H. eucheumatoides is a natural seaweed that has not been studied and cultivated, so we initially kept the seaweed variety in the laboratory for 30 days. The results of the study are shown in Table 3.

Table 3. Growth rate of *Hydropuntia eucheumatoides* under kept conditions

Parameter	Value
Original weight (W_o)	100 gr
Weight after 30 days (W_t)	194 gr
Growth rate by weight (% w/day)	2.21 %/day
Number of branches generated	2−3

Thus, it can be seen that in conditions of artificial rearing, *H. eucheumatoides* can still grow and develop normally. From an initial thallus of *H. Eucheumatoides* with a small number of branches after a 30-day retention period, the seaweed has clearly divided sub-branches, the number of branches usually occurs from 2 to 3 branches.

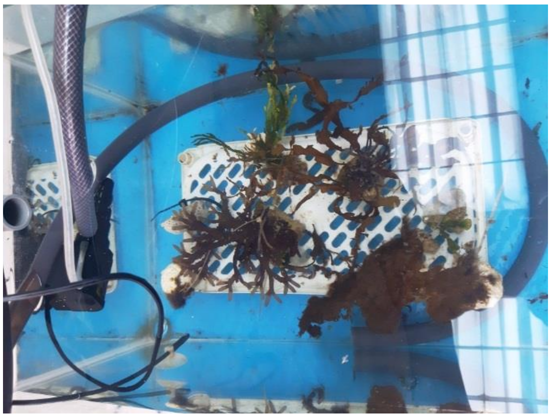

Figure 12. Thallus of *Hydropuntia eucheumatoides* was kept in a laboratory thermostatic tank

Figure 13. Thallus of *Hydropuntia eucheumatoides* grows under keep conditions

The keeping of wild thallus will be active in the production of seaweed in the form of branching, thereby ensuring the supply of seaweed for the commercial seaweed farming model. The growth rate of thallus kept in the laboratory was slower than some other species of seaweed such as *Gracilaria tenuistipitata* with 4.2% growth rate (% day) (Le Nhu Hau & Nguyen Huu Dai, 2010), and *Ulva papenfussii* growth rate 6,5 (% day) (Vo Thanh Trung et al., 2019). Because the two species of seaweeds grow in high nutrient environments such as brackish water or tidal areas, the growth of these two species is higher than *H. eucheumatoides*.

The main chemical composition of *H. eucheumatoides*

H. eucheumatoides is used popular with coastal people and is traded in markets, and supermarkets. But the chemical composition of this species has not been studied and evaluated. Research results in Table 4 provide some chemical information about this seaweed so that it has a scientific basis for the development of production of biological products as well as high value food from this species. Chromatogram of sugar composition of *H. eucheumatoides* was shown in Figure 14.

The results in Table 4 show that *H. eucheumatoides* has the main component of carbohydrate accounting for 80.3%, low lipid and color content of 1.5%, nitrogen-protein content is not high, fluctuating around 6%. This seaweed has a quite high mineral content because the seaweed hulls are calcified by seawater and some sand particles adhere to the surface. The chemical composition of this seaweed has a high carbohydrate content, mainly galactose, accounting for 87.2%, glucose content of 10.3% and the remaining sugars account for 2.5%. The carbohydrates of this seaweed are mainly agar and a little starch and cellulose, so when analyzing the composition of monosaccharid, there will be only two main sugars, galactose and glucose. The high carbohydrate content is a suitable material for the production of biofilm materials used in the treatment of burns and food bags of high economic value. Especially, this algae has the main components of agar and starch which are suitable materials for obtaining agarose and red seaweed starch for high technology applications in the field of health and food. High levels of galactose and glucose are also suitable materials for fermentation technology, in which fermented products such as probiotic beverages, VFAs (volatile organic acids) etc. The applications of red seaweed have been interested in the world and these studies are recorded in the publication of "Seaweeds for Food and Industrial Applications".

Table 4. Chemical composition and carbohydrate content of *Hydropuntia eucheumatoides*

Ingredient	Nito - protein	Ash	Lipid	Carbohydrate	Ingredient carbohydrate		
					Agar	Starch	Cellulose
Content (%)	6.2	12	1.5	80.3	68.3	6.2	3.8

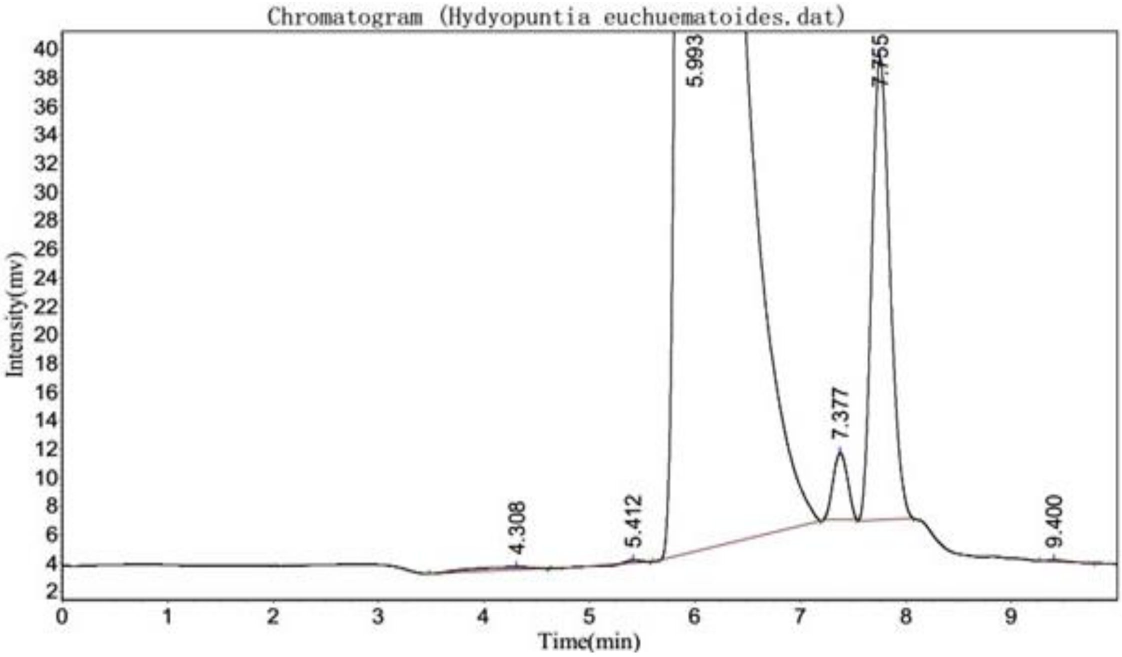

Figure 14. Chromatogram of sugar composition of *Hydropuntia eucheumatoides*

Note: Retention time 7.377 of peak glucose, retention time 7.755 of peak galactose.

CONCLUSION

Described in detail the morphological characteristics, reproductive organs of *Hydropuntia eucheumatoides,* habitats where diatoms live with a hard bottom, corals with flat surfaces; yield 1,400–2,100 g fresh/m^2, coverage 18–30 %/m^2. It has been successfully kept in laboratory conditions for 30 days, with a growth rate of 2.2%/day. The main chemical components have been identified: carbohydrate 80.3%, lipid 1.5%, ash 12%, protein 6.2% and carbohydrate composition including 68% agar, 6.2% starch, 3, 8 cellulose and the monosaccharide composition is glucose 10.3% and galactose 87.2%. This publication contributes to opening the direction of application of *H. eucheumatoides* biomass in the processing of food, medical and pharmaceutical products with high use value.

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