Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 May 08; 10(A):1184-1187.
https://doi.org/10.3889/oamjms.2022.9803
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Biochemistry*

Stability Evaluation of *Caulerpa racemosa* as Novel Halal Capsule-Shell

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Edited by: Slavica Hristomanova-Mitkovska
Citation: Permatasari HK, Nurkolis F, Barazani H, Satria P, Prima EN, Alfaien PA, Choirunnisa NL, Kumalawati DA, Solihah J, Pramesti DI, Bolang A. Stability Evaluation of *Caulerpa racemosa* as Novel Halal Capsule-Shell. Open Access Maced J Med Sci. 2022 May 08; 10(A):1184-1187. https://doi.org/10.3889/oamjms.2022.9803
Keywords: Nanoencapsulation; *Caulerpa racemosa*; Capsule shell; Chitosan; Drugs; Functional food
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Received: 14-Apr-2022
Revised: 25-Apr-2022
Accepted: 28-Apr-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The capsule shell is generally made from mammalian gelatin; according to Europe gelatine manufacturers of Europe, about 80% of the base material of the capsule shell comes from pork skin and 15% from cow skin. This raises a great deal of concern in the community, especially on the religious aspect. Muslims are prohibited from consuming anything made from pork, while Hindus are forbidden from consuming anything made from beef. To reduce public concern, an alternative medicine shell made from natural ingredients is necessary.

AIM: This study aims to discover the potential of a new medicinal shell, which is made from the stem of sea grapes (Caulerpa racemosa).

METHODS: This laboratory experimental study used a completely randomized design with three treatment variations, namely, the ratio between the volume of chitosan and tripolyphosphate in Sample 1 (V1) was 2:1, Sample 2 (V2) was 3.5:1, and Sample 3 (V3) is 5:1. Each sample was replicated 3 times (triples) and used the analytical method of the association of official analytical chemists. The dough is then printed on capsules of size O (300–500 mg; 0.5 g).

RESULTS: The results obtained are the particle size, polydispersity index, and zeta potential of V1, V2, and V3 that are significantly different ($p < 0.0001$, $p = 0.0004$, and $p < 0.0001$) based on the one-way Analysis of variance test.

CONCLUSION: From these results, the V3 nanocapsule variant is the best variant and has the potential to be an alternative to drug capsule shells.

Introduction

Indonesia is an archipelagic country with 17,508 islands and a long coastline on each island spanning 81,000 km. Indonesia is also a country with abundant flora, approximately 60% of the total flora in the world is found in Indonesia [1]. However, unfortunately, although there are many medicinal plants in Indonesia, not all have been fully utilized. In fact, Indonesia has enormous potential in the pharmaceutical sector. The use of local Indonesian plants can also not only be used as raw materials for medicine, but also as alternative raw materials for capsule shells [2].

Capsules are solid preparations in the form of small tubes containing drugs in the form of powders, granules, or liquids. Capsule shells are generally based on mammalian gelatin. According to data from one of the gelatin supply companies, namely, Europe gelatine manufacturers of Europe, it is known in 2018 that almost 80% of the gelatin produced comes from pork skin, 15% comes from a thin layer of cowhide (split), and 5 the other % comes from fish, pork, and beef bones [3]. The use of gelatin as a capsule shell raises a lot of concern in the community, especially in the religious aspect. Muslims are prohibited from consuming anything made from pork, while Hindus do not consume anything made from beef [4]. Furthermore, for Muslims, the halal use of gelatin from beef bones is still questionable, because it is feared that the slaughter process is not in accordance with Islamic law [5].

One of the plants in Indonesia that can be used as an alternative to capsule shells and is guaranteed to be halal is seaweed [2]. Seaweeds are widespread and can be found in all regions of the Earth, from polar to tropical regions. Indonesia is an archipelagic country with a long coastline and is located in the heart of the Coral Triangle, being the center of the highest marine

biodiversity in the world [6]. This is what helps Indonesia to become a country very rich in maritime products, especially seaweed. In 1912, van Bosse documented 782 species of seaweed in Indonesia. It was recently reported that around 1000 species of seaweed can be found in Indonesia [7], [8].

According to data on seaweed production and exports, Indonesia is second after the Philippines, with a potential for seaweed development of up to 1.11 million ha and an estimated production of up to 167,937 MT per year [9]. In fact, Indonesian waters are called "the world's seaweed warehouse" by seaweed experts, because Indonesia produces about 56% of the world's total seaweed production [2]. Among several tropical seaweed species, one of the many types of seaweed that is expected to have bright prospects for development is *Caulerpa racemosa* (known as sea grapes or green caviar) which is an underexploited seaweed resource in Indonesia [10], [11], [12], [13].

Referring to Article 4 of Indonesian Law No. 33 of 2014 concerning halal product guarantee, all products (including drugs) that enter, circulate, and are traded in Indonesia must be halal. Not only in Indonesia, halal products are also being traded in the United Arab Emirates. Because of this, we propose an alternative material to replace mammalian gelatin as a raw material to make capsule shells that is definitely hygienic, halal, guaranteed, and can be accepted by all humans. Therefore, this research was conducted with the aim of innovating seaweed, especially the stem of sea grapes (*C racemosa*), to be used as capsule shells through a series of tests.

Materials and Methods

Sea grapes (*C racemosa*) are obtained from the waters of North Sulawesi at a depth of about 10–20 m above sea level. Fresh sea grapes are dried at room temperature (20–25°C) for 5 h to reduce the moisture content. Botanical identification and verification of samples aim to confirm the species of sea grapes. The drained sea grapes are then mashed with a blender.

Chitosan nano-powder manufacture

Chitosan was synthesized by deacetylation of sea grapes. The sea grape powder was soaked in 600 ml of 50% NaOH (1:20 w/v) then stirred and heated at 120°C for 4 h. The residue and filtrate were separated by filtration, and then the residue was neutralized by washing with distilled water until the pH was neutral. The residue was dried in an oven at 80°C for 24 h.

This laboratory experimental study used a completely randomized design with three treatment variations, namely, the ratio between the volume of chitosan and tripolyphosphate in Sample 1 (V1) was 2:1, Sample 2 (V2) was 3.5:1, and Sample 3 (V3) is 5:1. Each sample was repeated 3 times or tripled and used the analytical method of the association of official analytical chemists [14]. The dough is then printed on capsules of size O (300–500 mg; 0.5 g). Printed using a capsule shell printer machine.

Characterization of capsule shell and nanochitosan

Particle Size, Polydispersity Index (IP), and Zeta Potential

The characterization test which includes P size, IP, and Z potential is carried out at the Pharmacy Laboratory by professional experts who have been certified on the basis of Indonesian national standards.

Management and analysis data

The data obtained in this study came from three formulations of particle size, IP, and zeta potential, which were analyzed using one-way Analysis of variance (ANOVA) at 95% CI (0.05) using Graph pad prism version 9.

Results and Discussion

Nanoencapsulated characteristics

The characteristics of the nanoencapsulated compounds were observed on the basis of the particle size, IP, and zeta potential. In this study, the results of the nanoencapsulated characteristics obtained from the three samples are shown in Table 1.

Table 1: Results of measurement of nanoencapsulated characteristics

	Variants			
	Repetition	V1	V2	V3
Particle size (nm)	1	258.9	190.3	160.5
	2	261.2	199.2	161.2
	3	259.6	193.2	169.2
	Average	259.90	194.23	163.63
	SEM	1.17	4.53	4.83
Polydispers index	1	0.25	0.2	0.18
	2	0.29	0.205	0.178
	3	0.25	0201	0.16
	Average	0.26	0.20	0.17
	SEM	0.02	0.00	0.01
Zeta potential (mV)	1	25.6	28.9	31.5
	2	24.1	27.6	31.9
	3	24.1	28.5	31.5
	Average	24.6	28.33	31.63
	SEM	0.86	0.66	0.23

Each sample was repeated 3 times (triplet) of the chitosan nanoparticle synthesis process for each characteristic. The mean particle size (nm) in Sample 1 (V1), Sample 2 (V2), and Sample 3 (V3) was 259.90 nm, 194.23 nm, and 163.63 nm. The mean IPs V1, V2, and

V3 were 0.26, 0.20, and 0.17. The mean zeta potential (mV) V1, V2, and V3 was 24.6 mV, 28.33 mV, and 31.63 mV. The data obtained from each characteristic were then carried out in statistical tests using the one-way ANOVA test.

P size

In the one-way ANOVA test on particle size data (Figure 1), it was seen that from the three samples, there was a significant difference ($p < 0.0001$). The *post hoc* Tukey HSD test on the particle size data showed a significant difference between all samples with the lowest particle size found at V3 (163.63 nm). The particle size in V3 is smaller compared to other nanoencapsulations made from betel leaf extract, where the nanoencapsulation of betel leaf extract has the lowest size at 165.7 nm [11]. In drug delivery systems for oral preparations, a good particle size is <300 nm [12]. Therefore, V3 is the best condition for the synthesis of chitosan nanoparticles based on their particle size.

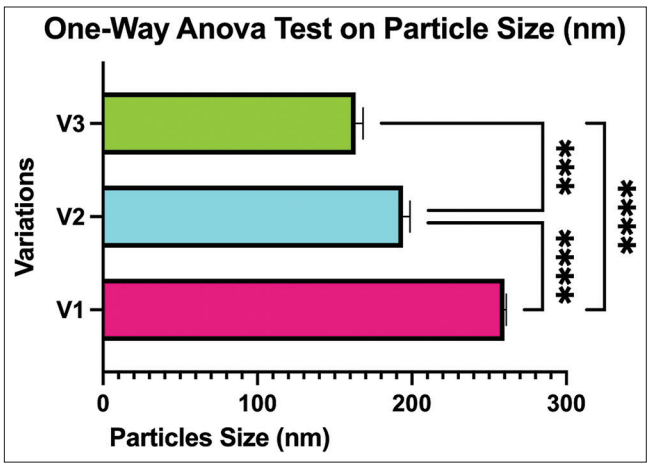

Figure 1: One-way ANOVA test particle size

IP

The IP is a form of expression of the particle size distribution, where IP is a value that expresses the width of the particle size distribution in a preparation. The IP range is between 0 and 1. An IP value of <0.5 is monodispersed, while an IP value of more than 0.5 is polydispersion [13]. In the one-way ANOVA test on IP data (Figure 2), the results were obtained where there was a significant difference between samples ($p = 0.0004$). The three samples have a value of <0.5, which indicates the nature of the three samples is monodispersion.

Z potential

The zeta potential value is generally used to determine the nature of the charge of the particles and the stability of the nanoparticles. A particle is declared

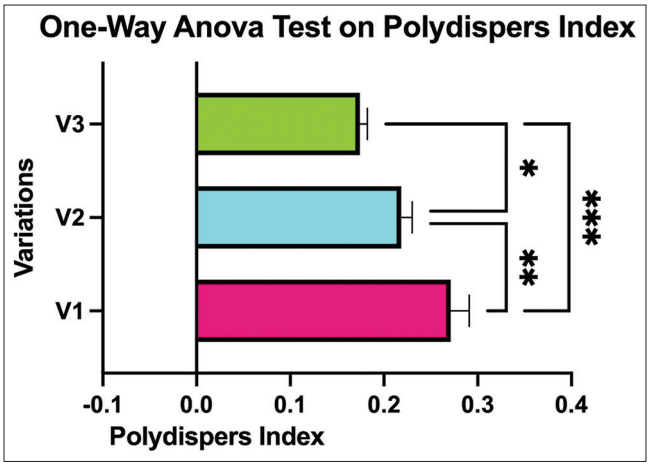

Figure 2: One-way ANOVA test polydispersity index

stable if it has a zeta potential value outside the range of 30 mV [13], [14]. In the one-way ANOVA test on zeta potential data (Figure 3), it was found that the three samples were significantly different ($p < 0.0001$) with the highest value of zeta potential at V3 (31.63), indicating that V3 had stable properties compared to other samples.

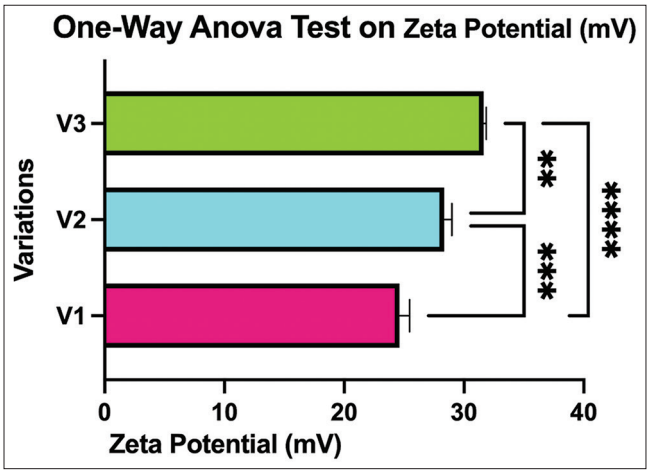

Figure 3: The zeta potential one-way ANOVA test

Conclusion and Practical Implications

Based on the results obtained from this study, it was shown that the optimal conditions for the synthesis of chitosan nanoparticles from the handle of the sea grape (*C racemosa*) were obtained from Sample 3 (V3). The encapsulated shell has potential as a carrier in the delivery of supplements and herbal medicinal extracts on the basis of particle size, IP, and zeta potential, which allows the nanochitosan in the shell nanochitosan to be biocompatible, biodegradable, mucoadhesive, and improve permeation, and can be developed to produce natural-based supplements or drugs. They have no problems in the religious aspect.

Availability of Data and Material

Data will be available by sending an application email to the corresponding author.

Acknowledgments

The authors thank all of the contributors for their outstanding help in research and also in formatting the paper. I would also like to express my gratitude to my two special people, who have provided suggestions-comments in the research and writing of this manuscript, as well as the motivation that has given the authors to keep the passion for research during the pandemic: 1. Professor Hardinsyah, MS, Ph.D. (as President of the Federations of Asian Nutrition Societies; President of the Food and Nutrition Society of Indonesia; and Chair of the South-east Asian Probiotic Scientific and Regulatory Experts Network) and 2. Professor Dr. Nurpudji A Taslim, MD., MPH, Sp. GK (K) (Chair of the Indonesian Clinical Nutrition Physician Association).

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