OPTIMIZATION SUSTAINED RELEASE TABLET FUCOIDAN PURIFIED EXTRACT FROM BROWN SEAWEED (*Sargassum polycystum*) AS IMMUNOMODULATOR

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ABSTRACT

Fucoidan is a sulfate polysaccharide compound that is widespread in the sea, especially in brown algae. Previous fucoidan pharmacokinetics studies have shown· that fucoidan nanoparticles have the effect of increasing the immunomodulatory ability of fucoidan, but fucoidan consumed orally has a half-life in plasma of about 3 hours so that to reduce the amount of fucoidan supplement use in a day, fucoidan can be formulated into sustained release tablet preparations. The purpose of this study was to determine the optimal composition of Na. Alginate and HPMC as polymers in sustained-release tablet preparations containing purified fucoidan from brown seaweed to produce tablets with characteristics that meet the compendial requirements of the Indonesian Pharmacopoeia and can maintain fucoidan release in plasma with only one consumption per day to improve user compliance. Fucoidan extraction from brown seaweed was carried out using hot extraction. Purified fucoidan tablets were prepared by the Simplex Lattice Design method using Design Expert version 10.1. The concentration of Na. Alginate and HPMC used as much as 2–5% with 8 formulas, which were then printed on a tablet printing tool. The optimum formula was tested as an immunomodulator in mice with parameters for lymph weight, phagocytosis index, and stimulant index. Optimum formula for Na. alginate: HPMC (3.252:3.748%) with flow speed 8.552 g/sec, compressibility 15,648%, hardness 3.726 kg, friability 0.044%, MC 3.296, Crushing time 21.34 minutes, Q15 34.608%, Q45 34.824%. The optimum formula for the immunomodulatory effect had a significant effect on the lymph weight of mice.

Keywords: Fucoidan Purified Extract, Brown Seaweed, Sustained Release, Tablets, Optimization

INTRODUCTION

The occurrence of a global pandemic due to SARS-CoV-2 and other viral diseases, as well as the search for better and more effective treatments, has encouraged research regarding therapies derived from natural sources (Barboux and Bousta, 2021) that are based on the regulation of the immune response (Sani, 2021). The immune system is an organism's defense mechanism that prevents and eliminates potentially dangerous pathogens (Hertanto *et al*., 2021). In addition, the immune response is enhanced by certain compounds called immunomodulators that influence the cellular and humoral immune systems, either by increasing or inhibiting their functions. Immune stimulants have been widely used to treat

cancer, bacterial and viral infections, and immunodeficiency. Simultaneously, immunosuppressants are used to treat autoimmune diseases and organ transplantation (Catanzaro *et al*., 2018).

The potential of marine sulfated polysaccharides, such as brown algae-derived fucoidan (Fernando *et al*., 2020), as immunomodulators in the prevention and treatment of viruses (Yu *et al*., 2021), such as Covid-19 has received considerable interest (Kwon *et al*., 2020; Yang *et al*., 2021; Yim *et al*., 2021). The main problem that limits its application in therapy is its high molecular weight, and the absorption rate of fucoidan in the intestine is only 0.6% (Lee *et al*., 2012). A study is needed to formulate fucoidan in a delivery system such as sustained release; therefore, fucoidan is not damaged by stomach acid and can be absorbed in the intestine with a higher absorption capacity (Chime *et al*., 2020; Nagamine *et al*., 2015).

The matrix, in the form of tablets consisting of polymers (Baek *et al*., 2017), can be derived from either natural polymers or synthetic polymers (Vasvári *et al*., 2018). It can reduce the dose of drugs, minimize side effects, and improve patient compliance and therapeutic effects (Sari *et al*., 2016). Several studies have succeeded in the formation of encapsulated drugs using the ionic gelation method (Albert *et al*., 2016), including the combination of chitosan with maltodextrin and chitosan with sodium tripolyphosphate (S-TPP) (Kusmayadi *et al*., 2019). The objective of this study was to produce sustained-release tablets with purified fucoidan extract from brown algae using an optimum combination of polimer Na. alginate and HPMC.

The active substance of a drug enters the matrix, and the release of the drug from the matrix tablet can be eroded and diffused. The rate of drug release from the matrix system depends on the type and amount of the polymer used (Vasvári *et al*., 2018). Sodium alginate is a group of polysaccharides that it is one of the good polymers for mucoadhesive preparations. In this study, sodium alginate was combined with HPMC. HPMC use in slow-release setups protects tablets when they come into contact with mucous tissue so that the tablet is not damaged by mucous tissue (Vrbanac *et al*., 2020).

The aim of this research was to evaluate the physical characteristics and dissolution test of the optimum formulation. The optimum formula was tested as an immunomodulator in mice with parameters for lymph weight, phagocytosis index, and stimulant index.

RESEARCH METHOD

The materials used fucoidan were brown algae (*Sargassum polycystum*) from Pailus Beach, Jepara-Indonesia, fucoidan standard (Sigma Aldrich, catalog no F1890), Na. alginate, HPMC, PVP, Avicel PH 101, magnesium stearate (MKR Chemicals Ltd., Indonesia), ethanol (Mallinckrodt Chemicals), and Sancoidan® (Sanbe Farma, Indonesia).

The tools used in this research were digital scales (O'Hauss), analytical scales, measuring cups, filter paper, magnetic stirrer, stopwatch, centrifuge (PLC series), sonicator (Branson 1800), UV-Vis 1280 spectrophotometer (Shimadzu), pH meter, dissolution tester, hardness tester, and friability tester.

Preparation of Sustained Release Tablets

Fucoidan Purified Extract from Brown Seaweed

The failed drying process, as well as the stirring that will later cause the rupture of nanoparticles if fucoidan will be made nanoparticles first (Kusmayadi *et al*., 2019), is directed at formulating fucoidan purified extract first (Zhang *et al*., 2018) into tablet preparations sustained release, by optimizing components that can act as a matrix in tablets that can release active substances slowly, namely HPMC and Sodium Alginate.

The sustained release formula of the tablets is as follows:

Fucoidan purified extract	= 1.32% x 450 mg*	= 5.94 mg
Sodium Alginat	= range 2 – 5 %	
HPMC	= range 2 – 5 %	
PVP	= 6 mg	

Mg-stearat = 0.5%
Avicel PH101 = ad 200 mg

*450 mg is the dose of the extract already on the market (Sancoidan) multiplied by 1.32% because the estimated purified fucoidan from the extract is the yield obtained.

Table I. Comparative Composition of Sodium Alginate and HPMC in Fucoidan Purified Extract Sustained Release Tablets with Design Expert versi 10.0

Run	Sodium Alginat (%)	HPMC (%)
1	3.5	3.5
2	4.25	2.75
3	3.5	3.5
4	2	5
5	2.75	4.25
6	2	5
7	5	2
8	5	2

The eight formulas were then made into tablets and subjected to physical characteristics tests including flow properties (flow speed), compressibility (%), hardness, brittleness, granule content, tablet crushing time, and dissolution levels of 15 and 45 minutes.

Immunomodulatory Effect

Carbon Ink Suspension

This test was carried out with the aim of observing the effect of the preparation of substained-release tablets of purified fucoidan extract on the immune system of mice. The first step is to make a suspension of carbon ink, carbon ink is taken as much as 1.6 mL and put into a mortar, then added 0.5% sodium carboxymethyl cellulose (S-CMC) suspension dissolved in a 10 mL measuring flask (Sujono *et al*., 2019).

Phagocytosis of Macrophages Using Carbon Cleaning Methods

Tests for non-specific immune responses were based on phagocytic activity, and white male mice were used in this study (Sebayang and Hasibuan, 2021). Mice were divided into five groups. Group I served as a normal control and received distilled water; group II acted as a negative control and received a 0.5% S-CMC suspension; group III received a standard fucoidan drug as an aqueous suspension at a certain dose; group IV received nanoencapsulation containing fucoidan purification extract at a dose of 580 mg/kg; and group V received sancoidan at a dose of 580 mg/kg. Each group was orally administered each medication once a day for seven days. On the eighth day, the entire group was given a suspension of carbon ink at a dose of 10 mL/kg through the tail vein. Blood was collected from the tail at 0, 5, 10, 15, and 20 minutes immediately after injection of the carbon suspension. The blood that has been obtained (25µL) is lysed with heparin 0.25 mL heparin solution and 4 mL 1% acetic acid and absorption is measured using spectrophotometry at 640.5 nm for optical density determination (Anochie *et al*., 2022). Phagocytosis activity was determined based on the ratio of the slope of the linear regression line between transmittance and time in the test and control groups.

Lymphoid Organ Index Testing

Mice were treated with ether for several minutes until they lost consciousness. The mouse was dissected and its lymphoid organs were removed. The lymphoid organs were weighed and compared with the control group and organ index (%).

RESULTS AND DISCUSSION

Table II. Physical Characteristics Test Results of Fucoidan Purified Extract Sustained Release Tablets

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
Flow speed (g/s)	7.02	9.51	9.15	8.2	8.3	8.07	7.63	6.66
Compressibility (%)	10	19.44	12.9	13.51	19.44	18.42	21.05	18.42
Moisture Content (%)	2.99	3.38	3.93	2.72	2.8	3	3.78	3.6
Hardness of Tablets (kg)	1.95	3.81	4.61	3.23	4.64	3.85	3.56	3.03
Friability (g)	0.0157	0.0214	0.0158	0.0051	0.0386	0.0223	0.0135	0.0198
Disintegrating time (minutes)	6.43	26.29	41.42	7.45	19.07	7.44	29.21	29.49
Dissolution Time 15 minutes (%)	34.4	37.65	30.79	39.51	35.41	34.53	40.86	37.52
Dissolution Time 45 minutes (%)	35.41	36.77	33.02	44.58	35.41	35.16	44.03	44.11

Figure 1. Fucoidan purified extract sustained release tablets run 1–8

The results of each response were then statistically analyzed by analysis of variance (ANOVA) using Design Expert 13.0. Each test response produced a mathematical model equation that described the coefficients of each excipient and its combinations. This was used to assess whether there was an interaction in both combinations of matrix excipients, namely, A for Na-alginate and B for HPMC (Table III).

Table III. Mathematical Model Equations Results of Statistical Analysis of Each Test Response

Response	Model Equation
Flow Speed	Y = 0.48 A + 0.69 B+ 0.36 AB
Compressibility	Y = 5.65 A + 4.53 B – 1.73 AB
Moisture Content	Y = 0.57 A + 0.28 B + 0.02 AB
Hardness	Y = 0.29 A + 0.42 B + 0.09 AB
Friability	Y = -0.001877 A - 0.001447 B + 0.002789AB
Disintegrating Time	Y = 2.60 A – 4.42B + 2.48AB
% Dissolution Rate 15 minute	Y = 8.74 A + 7.92 B – 2.00AB
% Dissolution Rate 45 minute	Y = 11.61 A + 10.26 B – 3.45AB

The flow time is the time required for a certain number of granules to flow through a funnel. A good flow time of less than 10 seconds for 100 grams of granule (Kusmayadi *et al*., 2019) or in other words, a good granule velocity of more than 100 grams per 10 seconds. The results of the flow velocity equation are presented in Table III. The addition of Na alginate at a certain proportion can affect the flow properties of the granules to be longer because Na alginate is hygroscopic; thus, there are granules attached to the funnel wall, and the flow time required by the granules to exit the funnel is long.

Moisture in the granule can affect the flow time of the granule, and high humidity can affect its ability to be pressed because it causes the granule period to nemepel on the *die* or *punch* when pressing. The results of the granule moisture content model are listed in Table III. The results showed that Na-alginate can affect the percentage of moisture because of the nature of alginate, which has a stronger hydrogen bond than HPMC, allowing it to bind more water.

Hardness tests were carried out to determine the resistance of tablets to withstand various mechanical shocks during manufacturing, packing, and distributing; the greater the hardness value, the greater the ability to survive.

Friability is a good parameter for determining the durability of tablets during the packaging process, and the distribution of brittleness is tested to determine which tablets have a brittleness of less than 1%. Small brittleness indicates that tablets are not easily brittle because fragile tablets erode or flake and can even experience erosion during packaging and shock during distribution. The results of the fragility test model equation are listed in Table III. HPMC was more able to increase the mass density so that the brittleness was small because HPMC has a large bulk density of Na alginate.

Table IV. Optimum Formula Validation with One Sample T-Test

Parameters	Test Results	Prediction Results	Significance	Conclusion
Friability	0.044±0.0357	0,022	0,205	Not significantly different
Hardness test	3.726±0.2231	3,735	0,186	Not significantly different
Compressibility	15,648±0,1108	14,287	0,165	Not significantly different
Moisture content	3.296±0.0868	3,234	0,115	Not significantly different
Disintegrating time	21.34±0.7821	22,096	0,175	Not significantly different
Dissolution test (15 minutes)	34.608±0.1214	33,721	0,185	Not significantly different
Dissolution test (45 minutes)	34.824±0.2204	34,064	0,177	Not significantly different

From the test results, *a one-sample t-test* showed that the results did not differ significantly from the point prediction of the Design Expert application. These results indicated that testing sustained-release tablets with *a one-sample t-test* was valid.

Table V. Test Results of Normality of Immunomodulatory Effects Using *IBM SPSS*

Parameters	Drug Groups	Significance	Information
Lymph Weight	Normal Group	0.097	Normally distributed
	Negative Group (CMC-Sod)	0.141	Normally distributed
	Positive Group (Sancoidan)	0.200*	Normally distributed
	Fucoidan Tablet Group 70 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 1540 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 2310 mcg/kgBB	0.200*	Normally distributed

	Brown Seaweed Ethanol Extract Tablet Group 58.5 mg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 117 mg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 175.5 mg/kgBB	0.199	Normally distributed
Hepar Weight	Normal Group	0.200*	Normally distributed
	Negative Group (CMC-Sod)	0.200*	Normally distributed
	Positive Group (Sancoidan)	0.106	Normally distributed
	Fucoidan Tablet Group 770 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 1540 mcg/kgBB	0.008	Not normally distributed
	Fucoidan Tablet Group 2310 mcg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 58.5 mg/kgBB	0.085	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 117 mg/kgBB	0.080	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 175.5 mg/kgBB	0.200*	Normally distributed
Phagocytosis Index	Normal Group	0.076	Normally distributed
	Negative Group (CMC-Sod)	0.200*	Normally distributed
	Positive Group (Sancoidan)	0.200*	Normally distributed
	Fucoidan Tablet Group 770 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 1540 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 2310 mcg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 58.5 mg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 117 mg/kgBB	0.037	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 175.5 mg/kgBB	0.200*	Normally distributed
Stimulation Index	Negative Group (CMC-Sod)	0.200*	Normally distributed
	Positive Group (Sancoidan)	0.200*	Normally distributed
	Fucoidan Tablet Group 770 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 1540 mcg/kgBB	0.200*	Normally distributed
	Fucoidan Tablet Group 2310 mcg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 58.5 mg/kgBB	0.200*	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 117 mg/kgBB	0.070	Normally distributed
	Brown Seaweed Ethanol Extract Tablet Group 175.5 mg/kgBB	0.200*	Normally distributed

From the results (Table V) of normality testing (Pozharitskaya *et al*., 2018), the average results of the test group were normally distributed, except for the fucoidan tablet

group with a dose of 1540 mcg/kg body weight. The results obtained were then subjected to homogeneity testing. The results of homogeneity testing can be seen in Table V.

Table VI. Test Results of Homogeneity of Immunomodulatory Effects Using *IBM SPSS*

Parameters	Significance	Information
Lymph Weight	0.180	Homogeneous
Hepar Weight	0.002	Inhomogeneous
Phagocytosis Index	0.123	Homogeneous
Stimulation Index	0.018	Homogeneous

The homogeneity test was used to test data variance and whether data between two or more groups satisfied the same or different variants. From Table VI, it can be concluded that the homogeneity results for lymph weight, phagocytosis index and stimulant index have the same data group variance (homogeneous), while the hepatic weight test parameter has different (inhomogeneous) variant data groups because of the significance value of <0.05 (Table VI).

Table VII. ANOVA Test Results of Immunomodulatory Effects Using *IBM SPSS*

Test Parameters	Significance	Information
Lymph weight	0.005	Significantly different
Hepar Weight	0.063	Not significantly different
Phagocytosis Index	0.054	Not significantly different
Stimulation Index	0.054	Not significantly different

CONCLUSION

Conclusion the optimum formula was obtained Na. alginate: HPMC (3.252 : 3.748%) with flow speed 8.552 g/sec, compressibility 15,648%, hardness 3.726 kg, friability 0.044%, Moisture Content 3.296 %, disintegrating time 21.34 minutes, Q15 34.608%, Q45 34.824%. The results of the physical characteristics evaluation test were validated with *point predictions* from Design *Experts* using *SPSS software*. The results of the validation of the optimum formula equation are shown in Table IV, and the results of the ANOVA testing (Table VII). The lymph weight test parameters were significantly different, whereas the liver weight parameters, phagocytosis index, and stimulation index results were not significantly different because they had a value of >0.05. In this case, it can be concluded that the immunomodulatory effect has a significant effect on the lymph weight of mice.

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