Comparison of Physicochemical Characteristics and Antioxidant Activity of Gels from Foam Mat Dying and Ethanolic Extract of Red Spinach (Amaranthus curentus)

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Comparison of Physicochemical Characteristics and Antioxidant Activity of Gels from Foam Mat Dying and Extract of Red Spinach (*Amaranthus curentus*)

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Abstract

Red spinach (RS) contains anthocyanins as antioxidants but is photolabile. Foam mat drying (FMD) is a technique used to protect color pigments. This study aimed to compare the physicochemical characteristics and antioxidant activity of gels containing FMD and ethanolic extract of RS (EERS). FMD and EERS were made and tested for total anthocyanins (TA) and IC50. EERS (F1-F3) and FMD (F4-F5) were each incorporated into gels as an active substance with variations of 1% (F1 and F4), 3% (F2 and F5), and 5% (F3 and F6). The gel was tested for organoleptic, pH, viscosity, spreadability, adhesion and % inhibition. TA and IC50 data were analyzed using the independent t-test. Quantitative data of EERS and FMD gels were analyzed using multivariate tests. FMD gel had a dark red color, while EERS gel was brown. TA of FMD was significantly higher (8.33 ± 0.25/100gr) ($p < 0.05$) than EERS (10.45 ± 0.15/100gr) in line with lower IC_{50} of FMD (35.67 ± 1.87) ($p<0.05$) compared to EERS (47.88 ± 2.45). The pH of FMD and EERS gels were at 5.32-5.77. Increasing the concentration of both FMD and EEBM affects the viscosity, spreadability and adhesion of the gel. The % inhibition results of FMD gel were significantly higher (58.75± 2.12 to 64.72±2.01%) ($p<0.05$) than EERS gel (31.75± 2.13 to 50.12± 3.01%) in all formulas. RS FMD gel has better physicochemical characteristics and antioxidant activity than the extract.

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INTRODUCTION

Red spinach contains anthocyanin pigments, which effective as antioxidants [1,2]. The anthocyanin pigment in red spinach has unstable characteristics, and it is easily oxidized and degraded due to the influence of temperature, light and pH [2,3]. High temperature and intense light exposure will degrade the color of anthocyanin[3]. At alkaline pH, anthocyannin will form 2 colorless compounds [4]. According to Moilati et al. (2020), the antioxidant activity of red spinach ethanol extract was potent at IC_{50} of 2.82 ppm [5]. Research by Ni'am et al. (2022) shows that under the antioxidant activity test, red spinach extract sheet mask can act as a potent antioxidant at IC50 of 68.55 ppm [6].

One method of extracting active substances from natural ingredients is the extraction. Several studies show that extraction results from natural ingredients show rapid color changes so that the extract becomes dark[7]. One process to increase the color intensity of natural materials is Foam Mat Drying (FMD). The FMD process uses maltodextrin as a foaming agent, and then dried at low temperature [8]. The advantages of the FMD drying

method include very low temperatures and vacuum air conditions[9]. This method helps keep bioactive components sensitive to high temperatures and oxidation from experiencing rapid degradation. In addition, the FMD method can produce products with good characteristics, such as color, aroma, and taste, similar to fresh vegetables[10]. The FMD process also provides quick drying in less than 24 hours. The resulting product has a good crystalline powder, low water content, and intense color.

Increasing the efficacy of natural ingredients can be done by making gel. Gel with carbopol 940 has a pH tendency of < 5, so it is suitable for active substances that are easily degraded in alkaline conditions[11]. Carbopol 940 also shows good gel stability during storage[12].

Based on the above background, it is necessary to conduct studies comparing the physical characteristics and antioxidant activity of FMD and red spinach extract gels.

MATERIALS AND METHODS

Materials

The material used in this research was fresh red spinach obtained from the Bandungan area, Semarang Regency. Red spinach was determined as a plant at the Semarang College of Pharmaceutical Sciences (STIFAR), declared *Amaranthus cruentus*. The gel base was made from carbopol 940, Tween 80, Span 80, sorbitol, liquid paraffin, triethanolamine (TEA), methylparaben, propylparaben, and distilled water with pharmaceutical grade purchased from Multi Kimia Raya Chemical. Dimethyl sulfoxide (DMSO) used in this research was an analytical grade purchased from Sigma Aldrich by PT Kairos.

Table 1. Formulation of gel made from red spinach extract and FMD[15]

Bahan	Konsentrasi %					
	F1	F2	F3	F4	F5	F6
Red spinach FMD powder	1	3	5	-	-	-
Red Spinach Extract	-	-	-	1	3	5
Carbopol	0,5	1	1,5	0,5	1	1,5
HPMC	0,5	0,5	0,5	0,5	0,5	0,5
Propilenglikol	10	10	10	10	10	10
Metil Paraben	0,18	0,18	0,18	0,18	0,18	0,18
Trietanolamin	3	3	3	3	3	3
Aquadest	Ad 100					

Extraction of Red Spinach [13]

A total of 1 kg of red spinach was washed, sorted, and dried at 40 °C for 8 hours. The dried red spinach is blended and extracted using the maceration method. Extraction was carried out with 96% ethanol for 3x24 hours. Remaceration is carried out 2x. The liquid extract was concentrated using a rotary evaporator at 50°C for 8 hours. The yield results are then calculated, and the thick extract is formulated into a gel.

Foam Mat Drying (FMD) of Red Spinach[14]

Red spinach leaves are washed to remove dirt on the leaves and attached pests, then air-dried to remove water. Red spinach leaves were added to water in a ratio of 1:1 (100 grams of red spinach: 100 ml) and blended. The blended leaves were mixed with 6% Tween 80 as a foaming agent and mixed using a mixer for 8 minutes. 15% maltodextrin was added to the mixture and stirred again using a mixer for 3 minutes. Red spinach was placed in a tray covered with aluminum foil with a thickness of 1 mm and dried using a tray dryer at a temperature variation of 40°C for 60 minutes. FMD was dried when it reached the constant weight. FMD powder was sieved with no. 60 mesh.

Formulation of gel made from red spinach extract and FMD

The extract gel formula and FMD of red spinach can be seen in Table 1.

Carbopol was dissolved using 10 ml of hot distilled water at 80°C in a glass beaker for 1 hour, then placed in a mortar, and triethanolamine was added to it little by little, stirring until a transparent gel mass was formed (mixture

A). HPMC was developed using 10 ml of hot distilled water at 80°C in a glass beaker for 1 hour, then placed in a mortar. The HPMC that had been developed is placed in a mortar and then stirred until a transparent gel mass forms (mixture B). Methylparaben was added to the mortar and dissolved with propylene glycol (mixture C). Next, mixtures A and B were added to mixture C, sequentially stirring until homogeneous. The extract was dissolved in 5 mL of 96% ethanol and then incorporated into the gel base. Red spinach FMD was dissolved with propylene glycol and added to the gel base. The remaining distilled water was added to the mixture until it is homogeneous [16].

Determination of anthocyanin levels[17]

Red spinach extract and FMD were dissolved in pH 1.0 buffer and pH 4.5 buffer with a ratio of extract or FMD to buffer of 1:5 (v/v). The absorbance of each solution was measured at a maximum wavelength of 512 nm and a wavelength of 700 nm (as a corrector) after being incubated for 15 minutes at room temperature.

Test the physical characteristics of the gel[18]

The physical characteristics tests carried out were organoleptic by direct observation, homogeneity using two glass objects glued together, pH, viscosity, spreadability and adhesion.

Antioxidant activity test[19]

Antioxidant activity tests were carried out on gel samples, FMD poweder and ethanolic extract. Red spinach extract and FMD samples were made with 50, 100, 150, 200 and 250 ppm concentrations. A total of 1 mL of sample was added with 2 mL of DPPH and made up to 5 mL. The sample was read for absorption at a wavelength of 516.12 nm. The absorption results are then calculated to obtain IC_{50}.

A gel sample containing 1 gram of red spinach extract and FMD was dissolved in 10 mL of 96% ethanol, then 3 mL was taken and added with 2 mL of 0.1 Mm DPPH solution. Shake until homogeneous and incubate for 30 minutes. The sample was read for absorption at a maximum wavelength of 516.12 nm. The absorption results are used to calculate the percentage of inhibition of the gel.

Data analysis

Data from total anthocyanin content, pH, viscosity, stickiness and spreadability, and percentage of inhibition were analyzed statistically using 2-way ANOVA to compare the physical characteristics of each gel formula and the differences between gels from extract and FMD. Organoleptic data were described descriptively.

RESULTS AND DISCUSSION

The results of red spinach extraction showed that the yield of the thick extract obtained was 38.86%. The extract obtained was dark brown with a distinctive aroma of red spinach and a thick consistency.

The FMD results of red spinach showed that the yield obtained was 92.43%. Red spinach FMD powder was dark red with a distinctive spinach aroma. The physical appearance of the extract and FMD of red spinach can be seen in Figure 1. The % yield was significantly different between extract and FMD. These findings were related to the foaming process, where there has to be a foaming agent added to the red spinach powder [20]. The evaporation method in extraction impacted the extract due to the elimination of the solvent [21].

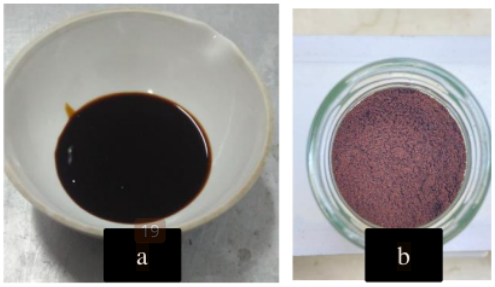

Figure 1. Appearance of red spinach extract (a) and red spinach FMD (b)

The results of total anthocyanin in extract and FMD of red spinach shown in Table II. The statistical analysis results showed a significant difference in anthocyanin levels between red spinach extract and FMD.

Table II. Determination of extract anthocyanin levels and FMD

Sample	% yield	Anthocyanin levels (per 100 grams)*	IC_{50}*
Extract	38,86± 4,20	8,33 ± 0,25	47,88 ± 2,45
FMD	92,43±2,21	10,45 ± 0,15	35,67 ± 1,87

*Samples analyzed three times replication with ± standard deviation

The results showed that the anthocyanin content in FMD was higher than in extract. This finding is because the extraction process takes longer time and higher temperatures. High temperatures cause anthocyanins to be rapidly degraded [22]. Extracting secondary metabolites with a long process can cause the oxidation and degradation of flavonoid compounds. The extraction method or drying at more than 60°C can cause the anthocyanin content to oxidize and change the color into a darker one[23].

The results of the gel formulation made from ethanolic extract and FMD of red spinach can be seen in Table III. The physical appearance of the gel with red spinach extract and FMD can be seen in Figure 2. The red spinach extract gel is dark brown, with a typical red spinach odor and a sticky consistency. Red spinach FMD gel is dark red, has a distinctive smell of red spinach, and quickly forms a thin film when applied to the skin.

The results of the pH characteristics showed that the pH between the extract gel formula and red spinach FMD did not show any significant differences. When compared, the pH of gels made from extracts and FMD did not show any significant differences. Increasing the concentration of red spinach can reduce the pH of the preparation. This is due to the nature of anthocyanins, which tend to be acidic so that they can lower the pH of the gel [24,25].

The results showed an increase in viscosity in line with the increase in extract concentration and FMD of red spinach. Each formula for the extract and FMD of red spinach showed significant differences due to increasing the concentration of the active substance. According to the previous study, it is stated that concentration of natural active substances will affect the viscosity [26]. The results of statistical tests show that gels with the same concentration of active substances do not show any significant differences, so the active ingredients do not affect viscosity.

The spreadability of the extract gel and FMD of red spinach at the same concentration showed no significant differences. Increasing the concentration of active substances can reduce the spreadability of the gel. This is in accordance with previous research, which states that increasing the red spinach concentration can reduce the preparation's spreadability [27]. Spreadability is inversely proportional to viscosity. The higher the viscosity, the lower the spreadability [28].

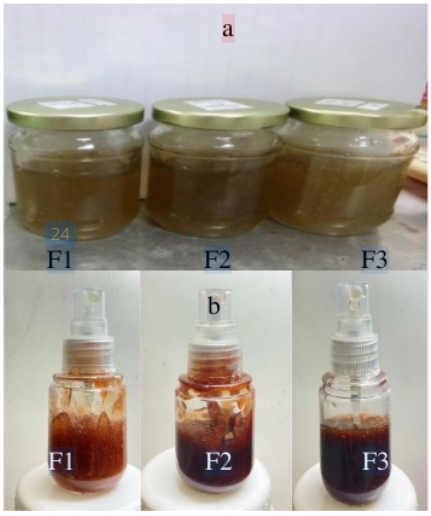

Figure 2. The result of gel made from red spinach extract (a) and FMD powder (b)

The adhesion of red spinach extract gel and FMD at the same concentration and between formulas showed no difference. The results of the adhesion test align with research by Pratama et al. (2020), which shows no effect of increasing the concentration of active substances on adhesion [29]. Research by Puspita et al. (2021) shows that adhesion can be affected if the concentration of the active substance is more than 10%[30].

percentage. The results of the antioxidant activity test of red spinach extract gel and FMD showed that increasing the concentration of active substances could increase the percentage of inhibition [31]. The test results showed that red spinach FMD gel had higher antioxidant activity than red spinach extract gel ($p = 0.0041 < 0.05$). Red spinach FMD gel has higher antioxidant activity because the FMD process is carried out at a lower temperature than the extraction process [24,32]. The FMD process also lasts in 3 hours compared to the extraction that last in 8 hours. This is following previous research that anthocyanins are stable at temperatures < 50°C33.

Table III. Physical characterization and

Parameter*	Formula					
	F1 (2%)	F2 (5%)	F3 (10%)	F4 (2%)	F5 (5%)	F6 (10%)
pH	5,77 ±0,68	5,65 ± 0,72	5,45 ± 0,88	5,68 ± 0,65	5,54 ± 0,71	5,32 ± 0,84
Viscosity (cps)	98,45 ± 2,33[a]	115 ± 4,18[a]	122 ± 8,75[a]	95,21 ± 3,41[b]	120,25 ± 8,14[b]	134,15 ± 5,18[b]
Spreadability (cm)	5,63 ± 0,11[a]	4,77 ± 0,14[a]	3,81 ± 0,12[a]	5,56 ± 0,13[b]	4,68 ± 0,10[b]	3,78 ± 0,11[b]
Adhesion (minutes)	2,28± 0,55[a]	2,33± 0,61[a]	2,41± 0,54[a]	2,31± 0,47[b]	2,45± 0,63[b]	2,32± 0,48[b]
% Inhibition	31,75± 2,13[a,c]	48,66± 3,15[a,c]	50,12± 3,01[a,c]	58,75± 2,12[b,c]	61,19± 2,35[b,c]	64,72±2,01[b,c]

* Data displayed from 3 replication with ±standard deviation
[a] significantly different between each formula of red spinach extract gel
[b] significantly different between each formula of red spinach FMD gel
[c] significantly different between gel from extract compared to FMD at the same concentration

The IC_{50} results of the extract and FMD showed significant differences. IC_{50} FMD of red spinach showed higher results than red spinach extract. The IC_{50} results were in line with the gel inhibition

FMD can increase the stability of color pigments so that the anthocyanin activity of red spinach can be increased34. The FMD process is shorter than extraction and is carried out at low temperatures, making it more efficient and preventing the product from oxidizing[35]. The drying results of FMD showed better characteristics compared to non-FMD. FMD has been widely used as a drying method that is able to preserve the color pigments and contents of plants, especially those containing photolabile active substances[36].

CONCLUSION

Foam mat drying powder of red spinach has a lower IC_{50} than the extract. Gels containing foam mat drying powder of red spinach have good physical characteristics. Gels made from foam mat drying powder of red spinach have higher antioxidant activity than the gels containing red spinach extract.

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