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Antioxidant Activity of Fraction Mikroencapsulation of Kesumba Keling Seeds (*Bixa orellana* L.) *Bixa orellana* L.

Annisa Nanda Fatina, Yuvianti Dwi Franyoto, Lia Kusmita, Ika Puspitaningrum, Mutmainah*
Departmen of Pharmaceutical Science, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang Semarang, Indonesia
email: mutmainah.stifar@gmail.com
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ABSTRACT:
Fraction of kesumba keling seeds (*Bixa orellana* L.) contains the compound bixin which is known to have antioxidant properties. To improve stability and acceptability, this fraction was microencapsulated using gum arabic and maltodextrin. This study aims to determine the antioxidant activity of the microencapsulated fraction of kesumba keling seeds (*Bixa orellana* L.). The microencapsulated formulation used gum arabic and maltodextrin encapsulants. Evaluations carried out on the results were yield, solubility of microcapsules, flow rate, efficiency of microcapsules and antioxidant activity. As a parameter, the antioxidant activity was tested using the DPPH method (1,1-diphenil-2-picrylhydarzyl) which was measured using UV-Vis Spectrophotometry with several variations in concentration. The results obtained showed a solubility of 85.558%, flow rate of 4.596 g/s, efficiency of microcapsules 99.628%,. The conclusion obtained is proven to be microencapsulated fraction of kesumba keeling seed (*Bixa orellana* L.) has antioxidant activity with an IC50 value of 3.264 ppm very strong category
KEY WORDS: *Bixa orellana* L., microencapsulation, DPPH

I. INTRODUCTION

Antioxidants are compounds that are able to inhibit damage caused by free radical oxidat [1]. Many studies of antioxidants are of plant origin because common synthetic compounds such as BHA and BHT are thought to be carcinogenic [2]. Kesumba keling (*Bixa orellana* L.) is one of the many plants found in Indonesia and has the potential as an antioxidant. In traditional medicine, this plant is used as antidiabetic, antioxidant, anti-inflammatory, and anticarcinogenic [3]. Seed of kesumba keling (*Bixa orellana* L.) contains the compound bixin which is known to act as an antioxidant by scavenging free radicals.

The use of bixin compounds has several disadvantages, namely it is easily degraded by oxygen, light, and temperature [4]. To overcome these shortcomings, microencapsulation can be used. The microencapsulation method is a technique used to protect bioactive compounds from various factors such as evaporation, oxidation, degradation, temperature, humidity, and light so as to extend the shelf life of the product and avoid damage [5]. The principle of microencapsulation is mixing between the water phase, the core substance and the coating material phase until a stable emulsion is formed, then the process of attaching the coating material to the surface of the core material and the process of reducing the particle size. The choice of microencapsulation method depends on the application and specific parameters, such as desired particle size, physicochemical properties of the core and coating, release mechanism, process cost, etc.[2].

Freeze drying is one of the drying methods for encapsulation which has advantages in preservation, quality of drying products, especially for heat sensitive materials [2]. Encapsulation can be achieved as a homogeneous core in a matrix solution which is then co-lyophilized, resulting in an irregular shape [6]. Freeze drying is a suitable method for the encapsulation of antioxidants to maintain their properties because antioxidants are easily damaged by heat and light [2]. Selection of coating material for microencapsulation of the petroleum ether fraction of kesumba keling seed (*Bixa orellana* L.) which is based on the use of a combination of gum arabic and maltodextrin for microencapsulation in the concentration ranges of 0-10% and

10-20% [7,8]. This study aims to examine the antioxidant activity of the microencapsulated fraction of kesumba keling seeds (*Bixa orellana* L.).

II. RESEARCH METHODS

Object : The object of this research is the physical characteristics of the antioxidant activity of the microencapsulated fraction of kesumba keling seeds (*Bixa orellana* L.). Physical characteristics include organoleptic test, microencapsulated yield, microencapsulated efficiency, moisture content, solubility, flow rate, followed by SEM. *(Scanning Electron Microscopy)* and PSA (*Particel Size Analyzer*).

Tool : separating funnel, rotary evaporator (Heidolph), silica plate GF254, chamber, cuvette, volume pipette, filler, volumetric flask, spectrophotometer (UV-1280 Shimadzu), Freeze Dryer (Thermo Scientific), funnel, Moisture Meter (Shimadzu), Scanning Electron Microscope (Jeol JSM 6510 LA) and Particel Size Analyzer (HORIBA scientific SZ-100).

Material : sample of kesumba keling seeds (Bixa orellana L.), acetone, hexane, H2SO4, KOH, FeCl3 (Merck), NaCl, 70% ethanol, Mayer's reagent, Dragendorff's reagent, and filter paper, gum arabic, maltodextrin, gelatin, CMC, tween 80, aquadest.

Research procedure: Kesumba keling seeds (*Bixa orellana* L.) macerated with acetone solvent and partitioned using acetone : petroleum ether : water (10:5:1). The results of the petroleum ether fraction were tested for phytochemical screening, Thin Layer Chromatography (TLC), color test with H2SO4, spectrum pattern with UV-Vis spectrophotometer, antioxidant activity test and analysis of percent bixin content. Preparation of microencapsulated by mixing maltodextrin, gum arabic, gelatin and water was homogenized for 1 minute, then the mixture of petroleum ether fraction and tween 80 was added and homogenized for 10 minutes [8]. The solution was frozen in the freezer for 24 hours, and dried by freeze drying for 72 hours. The dry samples were ground and sieved using a 24 mesh sieve. Microencapsules are stored in tightly closed containers and protected from light. The microcapsule formula is presented in table 1.

Table 1. Microenkapsul Formula Kesumba Keling Seed Fraction *(Bixa orellana.* L)

Material	Formula (% b/v)
Petroleum eter fraction	0.031
Arabic gum	2.781
Maltodextrin	17.219
Tween 80	0.5
Gelatin	1
CMC	0.4
Aquadest	ad 100

a. Microencapsulation efficiency testing

This test was carried out with 2 grams of sample dissolved in 100 mL of acetone and then the absorbance was measured using a spectrophotometer at λ 502 nm [9].

$$\text{Total uncapsulated bixin} = \frac{A}{2870} \times \frac{100000}{\text{sample weight (mg)}} \times 100 \quad (1)$$

$$\text{Total encapsulated bixin} = 100\% - \text{total uncapsulated bixin} \quad (2)$$

$$\text{Microencapsulation Efficiency} = \frac{\text{total encapsulated bixin} - \text{total unencapsulated bixin} \times 100}{\text{Total encapsulated bixin}} \quad (3)$$

b. Moisture testing

This test is carried out by weighing 0.5 grams of microcapsules and measured in a moisture meter, the start button is pressed and then the resulting number is recorded when the notification sound is heard [2].

c. Solubility test

The test was carried out by dissolving 1 gram of microcapsules in 25 ml of distilled water, the resulting solution was filtered using Whatman No. paper. 42, then the filter paper and residue were dried in an oven at 105°C for three hours and then cooled and weighed [2].

Percent solubility = 100% - percent residue

$$\text{Percent residue} = \frac{\text{filter paper weight and residue} - \text{filter paper weight}}{\text{Sample weight}} \times 100\% \quad (4)$$

d. Flow speed test

The test was carried out with 10 grams of microcapsules inserted into a closed funnel. The cover at the end of the funnel was then opened and the microcapsules were allowed to flow until no microcapsules remained in the funnel, and the flow time was recorded [2].

e. SEM Analysis

The test was carried out with a surface tension of 20 kV, pressure of 88Pa and a magnification of 100 times.

f. PSA Analysis

The test was carried out with 1mL of sample that had been dispersed in water put into a cuvette and measured for 15 minutes to produce globule size and distribution curve (Ariani et al., 2019).

g. Antioxidant activity test

The test was carried out on fractions and preparations made with a series of 0.1 ppm-0.5 ppm concentration fraction and a series of microcapsules with a concentration of 10,000 ppm-30,000 ppm. Take 1 mL of the sample fraction and preparation and then added to the 4 mL 0.1 mM of DPPH solution. The absorbance was measured with a UV-Vis spectrophotometer at λ 517 nm was measured after 30 minutes of incubation [9].

$$\% \text{ Inhibitory} = \frac{[DPPH\ ]_0 - [DPPH\ ]_s}{[DPPH\ ]_0} \text{ X } 100\ \% \quad (5)$$

$[DPPH\ ]_0$ = Initial DPPH concentration

$[DPPH\ ]_s$ = Final DPPH concentration remaining

III. DISCUSSION

The results of the extraction of kesumba rivet seeds obtained yield of 3.72% ± 0.3213. Phytochemical screening test results and TLC for the petroleum ether fraction of kesumba keling seeds (*Bixa orellana* L.) showed that the fraction contained phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, and tannins. The identification of the presence of bixin compounds in the fraction showed positive results in the form of TLC results with an Rf value of fraski and a standard Rf value of 0.38, a purplish blue color in the color test with H_2SO_4 and a spectrum pattern with λ the maximum fraction is at 487 nm, 458 nm.

The results of the microencapsulation characteristic test results of fractions of kesumba keling seeds (*Bixa orellana* L.) showed that high yields could be influenced by the presence of maltodextrin which caused the amount of solids obtained to increase so that the amount of yield produced was also higher [10]. Maltodextrin with low DE (Dextrose Equivalent) has a small number of hydrophilic groups, so that the adsorption of water in the surrounding air is less [11]. Gum arabic contains more hydrophilic groups which are shorter, so it is easy to bind water molecules in the surrounding air and humidity will increase [12]. High humidity reduces the ability of the powder to flow because the powder tends to get wet and sticks to the wall and high humidity causes the powder to be difficult to spread in water because it tends to stick together so that no pores are formed so that it is difficult to wet and the solubility will decrease [10].

The presence of maltodextin will cause the efficiency of the microencapsulated to be even greater, this is because it has high oxidation resistance [13]. The presence of protein components makes gum arabic have good emulsifying properties and can produce microcapsules that have high retention, thereby increasing the efficiency of microcapsules. [14]. Maltodextrin with DE *(Dextrose Equivalent)* <20 has low stability because it does not have caking strength. Data from the characteristic test results for the fraction of kesumba rivet seeds (*Bixa orellana* L.) more details can be seen in table 2 and figure 1.

Figure 1. Microencapsulesule fraction of kesumba keeling

Table 2. The results of testing the physical characteristics of microcapsules

Testing	Results
Organoleptic Form Smell Color flavor	 powder sweet orange tasteless
Yield (%)	85.49 ± 1.35
Microencapsulation Efficiency (%)	99.61 ± 0.08
Moist content (%)	2.62 ± 0,13
Flow Rate (grams/second)	4.29 ± 0.16
Solubility (%)	85.49 ± 1.35

The morphology of the microcapsules with a magnification of 100x obtained a size of 100 m with a flake-like shape, the results can be seen in Figure 2. The average size of the microcapsules obtained is 1647,2 nm with a polydisperses index value of 1.078, the results can be seen in Figure 3.

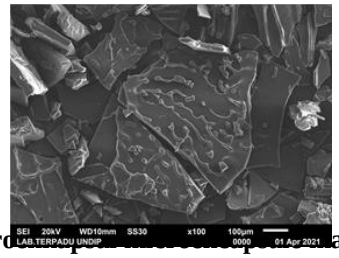

Figure 2. Micr[illegible]agnification 100 x

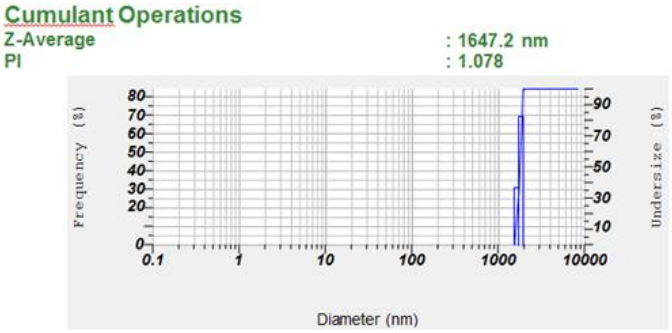

Figure 3. Results of Microencapsul Kesumba Keling Particle Size and Distribution Test (Bixa orellana L.) with Particel Size Analyzer (PSA)

Testing the antioxidant activity of the fraction obtained IC50 of 3.1 ppm and the average percentage of bixin content is 85.48%. The results of testing the antioxidant activity of microcapsules are presented in table 3. Measurement of antioxidant activity in microencapsulated products aims to determine how effective the microencapsules protect the bixin compound in the fraction. Measurement of antioxidants with the DPPH method is simple, fast and does not require a lot of reagents. Antioxidant activity is expressed in IC50. Results for IC50 microencapsulated fraction of kesumba keling seeds (*Bixa orellana* L.) 3,60 ± 0,63 ppm.

Table 3. Test results of antioxidant activity microenkapsul kesumba keling seed fraction (Bixa orellana L.)

Replikasi	Concentration (ppm)	Sample (mL)	Rate correction (ppm)	Abs DPPH	Abs Sample	% Inhibitory
1	10000	0.996	10039.2	0.598	0.484	19.06
	15000	1.494	15058.8		0.481	19.57
	20000	1.992	20078.4		0.479	19.90
	25000	2.490	25098.0		0.476	20.40
	30000	2.988	30117.6		0.473	20.90
2	10000	0.997	10025.2	0.598	0.484	19.06
	15000	1.496	15037.8		0.482	19.40
	20000	1.995	20050.4		0.478	20.07
	25000	2.494	25063.0		0.474	20.74
	30000	2.992	30075.6		0.472	21.07
3	10000	0,998	10022.0	0.668	0.542	18.86
	15000	1.497	15033.0		0.539	19.31
	20000	1.996	20044.0		0.535	19.91
	25000	2.495	25055.0		0.534	20.06
	30000	2.993	30066.0		0.532	20.36

Bixin belongs to the carotenoid group, so the mechanism of action of antioxidants is almost the same as that of carotenoids. Carotenoids can function as singlet oxygen quenchers by extinguishing the harmful potential of singlet oxygen and converting it into triplet oxygen. Excited carotenoids release heat and then return to stable carotenoids [9].

Table 4. Antioxidant Properties based on IC50 values

IC50 values	Antioxidant Properties
50 ppm<	Very strong
50 ppm-100 ppm	strong
100 ppm-150 ppm	medium
150 ppm-200 ppm	weak

Based on table 4, the IC50 value of the microencapsulated test sample for the fraction of kesumba keling seeds (*Bixa orellana* L.) shows an IC50 value of less than 50. According to these parameters, this indicates that the sample is a very strong antioxidant. The smaller the IC50 value, the higher the antioxidant power of the sample [15].

IV. CONCLUSION

Based on the results of research that has been done, it can be concluded that microencapsules of kesumba keling seed fraction (Bixa orellana L.) has antioxidant activity with an IC50 value of 3.60 ppm with a very strong category.

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