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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF COCOA POD THAT ASSOCIATED IN MALTODEXTRIN IN VARIOUS CONCENTRATION

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ABSTRAK

Cocoa pod extract ((Theobroma cacao L.) has antioxidant and antimicrobial activity that has the potential as a natural food preservative. However, in its use the cocoa fruit skin extract has a disadvantage because the short shelf time and its application to food are limited, efforts are needed to prevent damage and extend shelf life, one of the efforts that can be done is by encapsulating the extract.This study aims to determine the antibacterial activity and antioxidant encapsulation of cocoa peel extract, this study begins with the extraction of cocoa pods with ethanol solvent by comparing cocoa pods : solvent 1: 4 The skin of cacao cocoa fruit used is yellow harvested cocoa fruit, then chopped and dried to form flour.The sample is extracted by maceration with ethanol solvent Antioxidant test is done by DPPH method, while antibacterial test is carried out by the well diffusion method. This study used a completely randomized design method (CRD) with 5 treatments using a maltodextrin concentration of 20% (M1); 30% (M2); 40% (M3); 50% (M4) and 60% (M5). The results showed that the treatment gave the highest yield in the treatment of 60% maltodextrin concentration (M5), while the highest antioxidant activity was obtained in the treatment of 20% maltodextrin (M1) with IC50 75.98 µg / mL and the treatment with the lowest antioxidant activity was obtained at treatment of 60% maltodextrin concentration (M5) with IC50 value 114.89 µg / mL. While for the antimicrobial activity also obtained with the same results, namely treatment of 20% (M1) obtained a higher inhibition diameter compared to treatment at 30%; 40%; 50% and 60% for all types of bacteria. The inhibition diameter in the treatment of the concentration of maltodextrin 20% (M1) for E. coli bacteria is between 4.12 mm - 10.95 mm, Salmonella sp is 2.85 mm - 8 , 25 mm and for Staphylococcusaureus of 5.15 mm - 13.90 mm and the lowest inhibition diameter was obtained in the treatment of 60% maltodextrin concentration (M5) for E. coli bacteria of between 2.0 mm - 4.79 mm, Salmonella sp of 1.15 mm - 4.35 mm and for Staphylococcusaureusat 2.76 mm - 5.17 mm.This study concluded that the encapsulation of cocoa peel extract using 20% maltodextrin had the highest antioxidant and antimicrobial activity when compared with other treatments namely 30% concentration; 40%; 50% and 60% but for the treatment of 20% and 30% there is no difference. Ethanol extract of cocoa pods can be made in the form of encapsulates which are very likely to be used as natural preservatives.

Keywords: Antioxidants, Antimicrobials, Cocoa pods, Encapsulation, Maltodextrin.

INTRODUCTION

One of the efforts made by cocoa farmers in increasing their cocoa production is by pruning the leaves, which aims to maintain a well-formed plant framework, regulate the spread of productive leaves, and will encourage stimulating the formation of new leaves, flowers and fruit. However, the results of leaf trimming for cocoa plants will also have the potential to be a source of environmental pollution if not handled properly, therefore a method is needed to utilize this leaf waste, for example by using it as a source of antioxidants and antimicrobials. This is very possible because cocoa leaves have been reported by several researchers including

Osman et al., (2004) which contain bioactive compounds in the form of phenolic compounds which act as antioxidants. Furthermore, Yang et al. (2011) stated that cocoa pods contain polyphenols, glycoside flavonoids, theobromine and catechins. Cocoa fruit skin can be used as a natural remedy for coconut milk and fresh fish, because the fruit contains secondary metabolites that have antioxidant and antibacterial activity. Chemical preservation techniques utilize antimicrobial abilities, enzyme inhibitors, and antioxidant activity in extracts (Gortzi et al., 2007). However, preparations in the form of extracts have the disadvantages of including a short shelf life and susceptibility to damage. One method of efforts to prevent it is by encapsulation, the encapsulation method aims to increase antimicrobial bioactivity and antioxidant activity (Gortzi et al. 2007 and Sanchez et al., 2010). Encapsulation aims to protect active compounds from degradation which can form toxic compounds and extend shelf life from environmental influences (Anal and Singh, 2007). Therefore, in this study encapsulation of ethanol extract of cocoa fruit peel to determine antibacterial and antioxidant activity.

RESEARCH METHODOLOGY

The tools used are: Blender, Whatman No. 41 filter paper, filter tool, rotary evaporator, analytical scales, measuring flask, glas alarm, measuring pipette, micro pipette, nitrogen gas tube, swaying shaker, aluminum foil, sample bottle and ephendorf, oven, desiccator, Erlenmeyer, test tube, magnetic stirrer, and a set of tools SHIMADZU UV 160 Spectrophotometer.

While the materials used included : cocoa pod from the types of action taken from the cocoa farmers in Sigi Biromaru district, 2,2 diphenyl-2-picrylhydrazyl /DPPH (Sigma), hexane pa (Merck), ethyl acetate pa (Merck) and ethanol pa (Merck), Nutrient Broth, Nutrient agar, NaCl, Aquadest, Hilton Agar, paper disc (Oxoid), Dimethyl sulfoxide (e-Merck), antibiotics amocyxilin, chloramphenicol, microbes test *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus* (collection of Microbiology laboratories Medical Faculty Universitas BrawijayaMalang).

Sample processing of the cocoa pods used were taken from the Village of Makmur, Palolo Subdistrict, Sigi Regency, before processing cocoa pods that had been taken cleaned and chopped first then dried at 60oC until dry which was marked when squeezed easily broken. Afterwards, it was carried out by using a grinder and sieved with a size of 100 mesh and then packed in plastic.

Extraction of Cocoa pod by maceration: Each cocoa pod flour 100g sample was macerated 3 times using ethanol 96% solvent at a ratio of 1: 4.

Encapsulation. The encapsulation process uses the spray drying method which begins with making a suspension solution (50 g) between the ethanol extract of cacao fruit peel and maltodextrin with variations in the concentration of maltodextrin 20%, 30%, 40%, 50% and 60% (w / v) against solvents and the ethanol extract of 80% cocoa peel, 70%, 60%, 50% and 40% (w / w) on the coating, then homogenizing the suspension using a homogenizer for 5 minutes at a speed of 13 000 rpm. The resulting suspension was then encapsulated using a spray dryer with 150oC inlet temperature and 70oC outlet temperature, with a feed flow of 10 ml / minute.

Measurement of antioxidant activity was carried out using the DPPH assay method. The ability to capture free radicals from the material that has been extracted by measuring the decrease in absorbance of the methanol DPPH solution at a wavelength of 517 nm, in the presence of the extract tested (Krings and Berger, 2001). The initial concentration of DPPH solution was 0.1 mM and absorbance readings were carried out after 30 minutes. If the absorbance drops very dramatically (the solution turns yellow) before 30 minutes, it is necessary to dilute the sample solution carefully. Antioxidant activity is expressed as% = {(Akontrol-Asampel)/ (Akontrol)} X 100%.

Antibacterial activity test, agar diffusion method (Ayad et al., 2000). This test was conducted to determine the antibacterial potential of cocoa leaves with perforation method by culture of test bacteria planted 1 ose in 10 ml of liquid media then incubated at incubator at 37 ° C for 24 hours. Then from the culture 100 µL was taken and mixed into 20 mL of media so that the temperature was 45oC, then left at room temperature until the media was condensed, then on the hole made 8 mm diameter using a micropipette. Next, 100 µL filtrate of cocoa peel extract from various ingredients /solvents ratios were adjusted according to the determined concentration (103.104, 105 and 106µg / ml) and incubated at 37oC for 24 hours. The bright zone formed around the wellbore is measured using a caliper. The test bacteria used were Escherichia coli, Salmonella sp and Staphylococcus aureus.

Research Design. The study design used a Completely Randomized Design (CRD) of five (5) treatments with three (3) replications. The treatment included five (5) concentrations of the use of maltodextrin ie 20% (M1); 30% (M2); 40% (M3); 50% (M4) and 60% (M5) and repeated 3 times to obtain 15 experimental units.

Variables observed. The variables observed in this study were preceded by capping, extracting cocoa pods with ethanol solvents and encapsulation processes followed by testing of antioxidant and antimicrobial activity from 5 types of treatment.

RESULTS AND DISCUSSION

Cocoa pod extraction. Extraction of cocoa pods was done by maceration method using ethanol solvent with a ratio of 1: 4 solvent with 48 hours extraction time. Ethanol solvents used can damage the cell walls of the cocoa peel so that polar or non-polar compounds can be dissolved in ethanol and during the maceration process diffusion occurs (Basset et al., 1994). The yield of crude extract obtained was 11.56%. The crude extract obtained is a thick reddish brown liquid.

Encapsulation of cocoa pods extract. Amendment of encapsulation of ethanol extract of cocoa pods . The results of the diversity analysis showed that the treatment of the concentration of the coating material (maltodextrin) on the ethanol extract of different cocoa pods was very significant (P <0.01) to yield the encapsulation of the ethanol extract of cocoa pods. The average value of encapsulate extracts can be seen in Table 1.

Table 1 shows that the concentration of cacao fruit peel extract in different encapsulates is very real (P <0.01) with respect to yield produced. The yield value of various concentrations of coating material (maltodextrin) increases with increasing concentration of the coating material used. The highest rendement value was obtained at 60% maltodexy concentration and 20% lowest. The yield of yields in this study is in line with the research reported by Yusrista (2010) on encapsulation of mangosteen peel extract which found that the higher the concentration of maltodextrin solution was added, the higher the yield of the resulting yield. While Rahmalia (2008) reported that encapsulation in vanilla extract, showed the greater the number of coatings (maltodextrin) the greater the yield of encapsulated products produced. This is presumably because the number of coatings plays a significant role in the encapsulate yield, because the water in the material will evaporate during the drying process of encapsulated products. According to Frascareli et al., (2011) that maltodextrin is a good binder because it produces low viscosity at high total solids, it will facilitate the drying process and will produce a high yield. The more maltodextrin used, the greater the yield produced. On the contrary, the higher the extract used, the higher the viscosity formed due to the resulting encapsulation yield will decrease (Young et al., 1993). Murti (2012) states that high viscosity will also cause the material to become more sticky. This makes the amount of material left behind is printed during the granulation process so that the resulting yield is low.

Antioksidan Activity Test. Based on the results of variance analysis (table 2), it was shown that the treatment of maltodextrin concentration as coating material gave a very significant difference (P <0.01) on antioxidant activity. The difference in antioxidant activity of the extract was thought to be caused by differences in the number of cocoa fruit skin extracts for each concentration of maltodextrin, where the higher the concentration of maltodextrin the lower the ethanol extract of the coated cocoa fruit skin. While the one acting as an antioxidant is a bioactive component found in cocoa peel extracts such as alkaloids, flavonoids, polyphenols, tannins and quercetin. Andayani *et al* (2008) stated that compounds which have antioxidant activity generally have hydroxyl groups substituted in ortho position and para -OH and -OR groups. Furthermore Widyawati *et al*., (2010), reported that antioxidant activity is influenced by various factors including differences in the ability to transfer hydrogen atoms to free radicals, the chemical structure of antioxidant compounds, and the position of hydroxyl and methyl groups in rings where more molecules have hydroxyl groups will be stronger in capturing free radicals because of its ability to donate larger hydrogen atoms.

Table 1. Average Value of Yield of Ethanol Extract of Cocoa Pods Encapsulated Products (%).

Concentration of maltodextrin in microcapsules (%)	Rendemen/Recovery (%)			
	Deuteronomy 1	Deuteronomy 2	Deuteronomy 3	Average
20 (M1)	33,14	32,11	31,17	32,14
30 (M2)	46,89	45,78	48,51	47,06
40 (M3)	49,89	52,23	52,2	51,44
50 (M4)	62,34	59,6	63,82	61,92
60 (M5)	69,8	71,2	69,0	70

Note: Different letters behind the average value show very significant differences (P <0.01)

Table 2. Antioxidant Activity (IC 50) Encapsulates the Ethanol Extract of The Cocoa Pods at Various Concentrations of Maltodextrin.

Concentration of maltodex - trin in microcapsules (%)	IC 50 (µg/mL)			Total	Average
	1	2	3		
M1 (20%)	76,45	77,23	73,99	227,67	75,89
M2 (30%)	77,21	75,89	76,73	229,83	76,61
M3 (40%)	79,81	77,29	79,84	236,94	78,98
M4 (50%)	105	98,9	97,57	301,47	100,49
M5 (60%)	115,23	116,21	113,23	344,67	114,89
Cacao Pods Ekstrak	75,91	76,38	75,65	227,94	75,98

Table 3. Diameter of inhibition (mm) of ethanol extract of cocoa pods and encapsulation of cocoa pod extract at various concentrations (%) of several test bacteria.

Treatment of maltodextrin concentration (%)	Encapsulate concentration (%)	Clear zone diameter (mm)		
		Ecoli sp	*Salmonella* sp	*Staphylococcus aureus*
M1 (20)	0,50	4,12	2,85	5,15
	1,00	5,10	3,78	6,75
	1,50	6,85	5,25	8,15
	2,00	10,95	8,25	13,90
M2 (30)	0,50	3,75	2,75	4,50
	1,00	4,95	3,25	5,80
	1,50	5,85	4,10	7,90
	2,00	9,12	7,79	12,45
M3 (40)	0,50	3,10	2,30	3,45
	1,00	3,60	2,85	4,87
	1,50	4,90	3,90	5,45
	2,00	8,15	5,85	6,66
M4 (50)	0,50	2,15	2,13	3,35
	1,00	3,25	2,45	4,45
	1,50	3,89	3,80	5,30
	2,00	5,50	4,56	6,18
M5 (60)	0,50	2,00	1,15	2,76
	1,00	3,30	2,75	3,80
	1,50	4,20	3,85	4,45
	2,00	4,79	4,35	5,17
Ethanol extract of cocoa pods	0,50	4,75	3,25	5,70
	1,00	5,25	4,85	7,75
	1,50	7,10	6,15	10,15
	2,00	12,85	9,25	14,85

In table 2 shows an increase in the concentration of maltodextrin in encapsulates causing a decrease in antioxidant activity, and if compared between the treatment of the use of maltodextrin 20% (M1) with 30% (M2) obtained relatively similar results or not significantly different (P> 0.05). This is presumably because the use of maltodextrin is getting higher, the number of extracts in encapsulates decreases as a result of the components acting as antioxidants such as polyphenols, tannins and other active components as a result of which antioxidant activity in encapsulates is low. Antioxidant activity in cocoa peel extract was slightly higher than the encapsulation results. The antioxidant activity in cocoa peel extract was obtained at 75.98 - 94.74 μg / mL (Anwar, K, et al., 2017), whereas in the same amount of ethanol encapsulated cocoa fruit extract, the range of antioxidant activity ranged from 75, 89 - 114.89 μg / mL. Based on the IC50 value obtained, the ethanol extract of cacao fruit peel and its encapsulate have antioxidant activity in that range and are classified as strong antioxidants.

Test for antibacterial activity. Tests for antimicrobial activity at various concentrations of maltodextrin use were carried out to see the potential use of coating (maltodextrin) on cocoa peel extract against pathogenic bacteria in this case *E. coli*, *Slamonella sp* and *S. aureus* as measured by the ability of extracts to inhibit the growth of test bacteria through the inhibition diameter measurements shown by the clear area around the well area where the clearer area in the well area showed higher inhibitory and encapsulate ability. Based on the results of the study, the ethanol extract of cocoa fruit peel can inhibit all test bacteria, both Gram positive and Gram negative bacteria with inhibitory diameters which are greater than the encapsulated ethanol extract of cocoa fruit skin. The results of this study indicate that the antimicrobial activity of cacao fruit skin ethanol extract as well as encapsulate form is classified as a broad spectrum because it can inhibit Gram positive and Gram negative bacteria. The inhibition of cocoa bark extract and the results of encapsulation of ethanol extract of cocoa fruit peel were higher for Staphylococcus aureus (Gram positive) compared to *Salmonella sp* and *E. coli* (Gram negative) bacteria.

Based on the data in table 3 shows that at a concentration of 0.5% the ethanol extract of cocoa pods and the results of encapsulation of ethanol extract of cocoa pods showed the smallest inhibition diameter of all test bacteria and the highest inhibitory diameter was at a concentration of 2%. The higher the concentration of cocoa peel extract and the encapsulation of inhibition diameter are also higher, this is in line with the statement of Pelczar and Chan (2005) that the higher the concentration of extract, the higher the inhibition diameter produced.

The results of the analysis showed that the concentration of maltodextrin showed very different results ($P < 0.01$). This is caused by the higher the concentration of maltodextrin used, the lower the ethanol extract of the coated cocoa pods as a result, the smaller the resulting inhibition diameter. Cocoa pods extract contains compounds that act as antibacterial agents such as flavonoids, polyphenols, tannins and quercetin. Antibacterial inhibition processes occur because of the contact of antibacterial compounds on the cell surface or compounds that diffuse into bacterial cells (Kanazawa, 1995). Data on inhibition diameter of cocoa bark extract at various concentrations of maltodextrin ratios can be seen in table 3.

The data in Table 3 shows that at a concentration of 0.5% of the ethanol extract of cocoa pods and the results of encapsulation of ethanol extract of cocoa pods showed the smallest inhibition diameter of all test bacteria and the highest inhibitory diameter was at a concentration of 2%. The higher the concentration of cocoa pods extract and the encapsulation of inhibition diameter are also higher, this is in line with the statement of Pelczar and Chan (2005) that the higher the concentration of the extract, the higher the inhibition diameter produced. The analysis showed that the concentration of maltodextrin showed results very significantly different ($P < 0.01$). This is caused by the higher the concentration of maltodextrin used, the lower the ethanol extract of the coated cocoa fruit as a result, the smaller the resulting inhibition diameter. Cocoa pods extract contains compounds that act as antibacterial agents such as flavonoids, polyphenols, tannins and quercetin. Antibacterial inhibition processes occur because of the contact of antibacterial compounds on the cell surface or compounds that diffuse into bacterial cells (Kanazawa, 1995). Furthermore, cocoa bark extract at various concentrations showed an increase in inhibitory power as the concentration of ethanol extract of cocoa pods increased on all test bacteria.

While the ethanol extract of cocoa peel extract showed higher concentration of maltodextrin the lower inhibition diameter obtained. The inhibitory power in the treatment of M1 (20%) and M2 (30%) is relatively the same at various encapsulate concentrations but for other treatments M3 (40%); M4 (50% and M5 (60%) experienced a decrease in inhibition with higher concentrations of maltodextrin as coating agents for all test bacteria. Furthermore, it was stated that the encapsulation treatment of ethanol extract of cocoa peel can provide protection against bioactive components contained in cocoa pods, because in various treatments the concentration of coating material (maltodextrin) still inhibits bacterial growth which is shown by the presence of clear areas around the well, where the greatest inhibition is Staphylococcus aureus then followed by *E. coli* bacteria and *Salmonella sp*. This difference is thought to be caused by several factors including diffusion ability of extract material, extract concentration, interaction between medium components, and environmental conditions. This allegation is supported by the statement of Siswandono and Soekardjo (2000) that the concentration of a substance that functions as an antibacterial is one of the factors that determine the size of the ability of antibacterial substances to inhibit the growth of bacteria tested. Furthermore it was stated that the inhibitory diameter of the extract was also influenced by the type of microorganisms tested due to differences in cell wall structure between the test bacteria that affected the work of cacao pods extract in encapsulates as antimicrobial compounds. The antibacterial activity of the encapsulated cocoa peel extract is supported by the results of phytochemical screening which shows the presence of bioactive compounds in cocoa fruit skin extracts, such as alkaloids, flavonoids, polyphenols, and tannins. Flavonoid compounds reported by several researchers will interfere with the integrity of bacterial cell membranes, tannins work competitively with glycosyltransferase enzymes in reducing saccharide as a glycosylation base material. Glycosyltransferase enzyme is an enzyme that plays a role in the process of adding sugar groups to proteins or lipids. If this enzyme is inhibited, the formation of bacterial polysaccharides is also inhibited. Other effects as antibacterials from tannins include reaction with cell membranes, enzyme inactivation, and destruction or inactivation of the function of genetic material (Yuliantina et al., 2009; Agustin DW. 2010).

CONCLUSION

Based on the results of research on antioxidant and antimicrobial activity from the encapsulated ethanol extract of cocoa pods at various concentrations of maltodextrin, conclusions can be drawn;

1. The encapsulation of the ethanol extract of cocoa pods at various maltodextrin concentrations resulted in the highest yield obtained at a concentration of 60% (M5) and the lowest was obtained at the treatment of 20% maltodextrin concentration (M1)
2. The treatment that gave the highest antioxidant activity was obtained at a concentration of 20% (M1) with a value of IC50 75.89 mg / mL and the lowest antioxidant activity was obtained at a concentration of 60% maltodextrin (M5) with IC50 value 114.89 µg / mL. While for antimicrobial activity also obtained with the same results at a concentration of 20% (M1) with a higher inhibitory meter compared to other concentration treatments for all types of bacteria.
3. Ethanol extract of cocoa pods can be made in the form of encapsulates and is very likelyto be used as a natural preservative.

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