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Antibacterial And Antioxidant Activities Of Ethanol Extracts Of Cocoa Husk (*Theobroma cacao L.*) With Maltodextrine In Various Concentration

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Abstract. Cocoa husk (*Theobroma cacao L.*) has antioxidant and antimicrobial activity which has the potential as a natural preservative of food, but in its use the cocoa rind extract has a disadvantage because of its short shelf life and limited application to foodstuffs, therefore prevent damage and extend the shelf life, one of the efforts that can be done is to encapsulate the extract. This study aims to determine the antibacterial and antioxidant activity of encapsulated cocoa peel extract, this study begins with the treatment of extraction of cacao pods with ethanol solvent by comparison of cacao pods powder : 1: 4 solvent. Cocoa husk used are yellow harvested fruits, then chopped and dried to form flour. The sample was extracted by maceration with ethanol solvent. Antioxidant testing was carried out by DPPH method, while the antibacterial test was carried out by 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) and repeated 3 times. This study concluded that encapsulant extract of cocoa husk using maltodextrin 20% had the highest antioxidant and antimicrobial activity compared to other treatments, namely 30% concentration; 40%; 50% and 60% but for treatment 20% and 30% there is no difference. Ethanol extracts of fruit peels can be made in the form of encapsulants which are very likely to be used as natural preservatives.

Keyword: Cocoa husk, antioxidant, antimicrobial, encapsulation, maltodextrin

1. INTRODUCTION

One of the efforts made by cocoa farmers in increasing their cocoa crop production is by doing leaf pruning, this is to maintain the well-formed plant skeleton, regulate the spread of productive leaves, and will encourage stimulating the formation of new leaves, flowers and fruit. However, the results of pruning leaves for cocoa plants will also have the potential as a source of environmental pollution if not handled properly, therefore it is necessary to do a way to utilize this leaf waste for example by using it as a source of antioxidants and antimicrobials. This is very possible because cocoa husk have been reported by several researchers (1). Cocoa husk have polyphenols, flavonoids glycoside, theobromine and catechins (2). Cocoa pods can be used as natural care ingredients 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 (3). However, preparations in the form of extracts have disadvantages including short shelf life and susceptibility to damage. One method of efforts to prevent it is 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 that can form toxic compounds and extend the shelf life of environmental influences (Anal and Singh, 2007). Therefore, in this study encapsulation of ethanol extract of cocoa pods was conducted to determine antibacterial and antioxidant activity.

2. MATERIALS AND METHODS

Materials and tools

A. Tools

The tools used are: Blender, Whatman no. 41 filter paper, filter device, rotary evaporator, analytic scales, measuring flask, glass jar, measuring pipette, micro pipette, nitrogen gas tube, sway shaker, aluminum foil, sample bottle and Erlenmeyer, oven, desiccator, Erlenmeyer, test tube, magnetic stirrer, and a set of SHIMADZU Model UV 160 Spectrophotometer.

B. Material

Materials used include: cacao fruit peel from the type taken from the district cocoa farmer Sigi Biromaru, 2,2-diphenyl-1-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 amoxicillin, chloramphenicol, microbes test *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus* (Microbiology laboratory collection Medical Faculty Universitas Brawijaya Malang).

C. Work procedures

Sample Processing

Cocoa husk used were taken from Desa Makmur, Palolo Sub-district, Sigi Regency, before processing cocoa pods that had been taken were cleaned and chopped first and then dried at 60 ° C until dry which was marked when squeezed was easily broken. Furthermore, it is carried out by using a grinding device and sieved with a size of 100 mesh and then packed in plastic.

Maceration Extraction of Cocoa pods

Each sample of 100g cocoa pods flour was macerated 3 times using 96% ethanol Solvent at a ratio of 1: 4

Encapsulation

The encapsulation process uses spray drying method which begins with making a suspension solution (50 g) between the ethanol extract of cocoa peel and maltodextrin with a variety of maltodextrin concentrations of 20%, 30%, 40%, 50% and 60% (w / v) to the solvent and ethanol extract of cocoa pods 80%, 70%, 60%, 50% and 40% (w / w) to the coating, then homogeneous suspension using homogenizer for 5 minutes at a speed of 13 000 rpm. The resulting suspension was then encapsulated using a spray dryer with an inlet temperature of 150°C and an outlet temperature of 70°C, with a flow of feed of 10 ml / minute.

Measurement of antioxidant activity was carried out using DPPH assay method. The ability to capture free radicals from the extracted material by measuring the reduction in absorbance of DPPH methanol solution at a wavelength of 517 nm, with the presence of extracts tested (Krings and Berger, 2001). The initial concentration of DPPH solution is 0.1 mM and absorbance readings are 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 $\% = \{(A_{\text{Control}} - A_{\text{sample}}) / (A_{\text{Control}})\} \times 100\%$

Antibacterial activity, agar diffusion method (4), this test was carried out to determine the antibacterial potential of cocoa leaves by perforation method by planting 1 test culture culture in 10 ml of liquid medium then incubating it at an incubator at 37 ° C for 24 hours. 100 µL of the culture was taken from the culture and mixed into 20 mL of medium so that the temperature was 45°C, then allowed to stand at room temperature until the media was compacted, then made a hole with a diameter of 8 mm using a micropipette. Next in the hole, 100 µL of filtrate extracts of cocoa rind

from various material / solvent ratios were determined according to predetermined concentrations (103, 104, 105, and 106 $\mu\text{g} / \text{ml}$) and incubated at 37 ° C for 24 hours. The bright zone formed around the wellbore is measured using a calipers run. The test bacteria used were *Escherichia coli*, *Salmonella sp* and *Staphylococcus aureus*

D. Research Design

The study design used a Completely Randomized Design (CRD) of five (5) treatments with three (3) replications. The treatment consisted of five (5) concentrations of using maltodextrin which was 20% (M1); 30% (M2); 40% (M3); 50% (M4) and 60% (M5) and repeated 3 times so that 15 experimental units were obtained.

E. The variables observed

The variables observed in this study began with the siege, extraction of cacao husk with ethanol solvent and the encapsulation process, which was continued by testing the antioxidant and antimicrobial activity of 5 types of treatment.

3. RESULTS AND DISCUSSION

Extraction of cocoa peel

Cocoa husk extraction was carried out by maceration method using ethanol solvent with a solvent ratio of 1: 4 with extraction time of 48 hours. Ethanol solvents that are used can damage the cell wall in the pods so that polar or non-polar compounds can dissolve in ethanol and during the maceration process diffusion occurs (5). The crude extract yield obtained was 11.56%. The crude extract obtained in the form of a slightly reddish brown thick liquid



Figure 1. Extract of cocoa peel

Encapsulation of cocoa husk extract:

Encapsulate yield of ethanol extract of cocoa husk

The results of the diversity analysis showed that the treatment of coating material concentration (maltodextrin) on the ethanol extract of cocoa husk was significantly different ($P < 0.01$) on encapsulant yield of ethanol extract of cocoa pods. The average value of the yield of encapsulated extract can be seen in Table 1.

Table 1. Average value of extracted encapsulated product extract extract of cocoa husk (%)

Maltodextrin concentration in encapsulates (%)	Rendement (%)			Average
	Replication 1	Replication 2	Replication 3	
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

Description: Different letters behind the average value show a very different difference real ($P < 0.01$)

Table 1 shows that the concentration of cacao fruit peel extract in encapsulants differed significantly ($P < 0.01$) from the yield produced. The yield value of various coating material concentrations (maltodextrin) has increased with increasing concentration of coating material used. The highest yield was obtained at 60% maltodextrin concentration and the lowest 20%. The yield in this study is in line with the research on the encapsulation of mangosteen pericarp extract which found that the higher concentration of maltodextrin solution was added, the resulting yield would increase(7). While reported that encapsulation of vanilla extract showed that the greater the amount of coating (maltodextrin) the greater the yield of encapsulated products produced (8). This is presumably because the number of coatings greatly contributes to encapsulate yield, because the water in the material will evaporate during the process of encapsulating product drying. In the encapsulation formulation of ethanol extract of cocoa peel with a higher amount of cocoa peel extract, it resulted in lower yield. According to Frascareli et al., (2011) that maltodextrin is a good binding material because it produces low viscosity in high total solids, it will facilitate the drying process and will produce high yields. The more maltodextrins used, the greater the yield produced. Conversely, the higher the extract used, the higher the viscosity that is formed, so that the resulting encapsulation yield will decrease (10). high viscosity will also cause the material to become more sticky. This makes the amount of left behind material printed during the granulation process so that the resulting yield becomes low (11).



Figure 2. Encapsulation results of various ethanol extracts of cocoa pods from various treatments

Antioxidant Activity

Table 2. Encapsulatory antioxidant activity (IC 50) of skin ethanol extract cacao husk at various concentrations of maltodextrin

Maltodextrin concentration in encapsulates (%)	IC 50 ($\mu\text{g/mL}$)			Total	Rataan
	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
Cocoa pods husk extract	75,91	76,38	75,65	227,94	75,98

Based on the results of variance analysis (table 2) shows that the treatment of maltodextrin concentration as coating material gives a very significant difference ($P < 0.01$) to antioxidant activity. The difference in antioxidant activity of the extract is thought to be due to differences in the amount of cacao pods extract for each concentration of maltodextrin, where the higher the concentration of maltodextrin the lower the ethanol extract of the coated cocoa pods. While those that act as antioxidants are bioactive components found in cacao fruit 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 positions and para to –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 greater ability to donate hydrogen atoms (12).

In table 2 shows the increase in maltodextrin concentration in encapsulants causes a decrease in antioxidant activity, and when compared between the treatment of the use of maltodextrin 20% (M1) with 30% (M2) the results were relatively similar or not significantly different ($P > 0.05$). This is presumably due to the higher use of maltodextrin, the number of extracts in the encapsulate decreases as a result the components that act as antioxidants such as polyphenols, tannins and other active components are also low due to the low antioxidant activity in the encapsulate.

The antioxidant activity of the cacao husk extract is slightly higher than the encapsulation results. Antioxidant activity of cacao husk extract was 75.98 - 94.74 $\mu\text{g} / \text{mL}$ (13), while in the same amount of encapsulant ethanol extract of cacao peel, the range of antioxidant activity ranged from 75, 89 - 114.89 $\mu\text{g} / \text{mL}$. Based on the IC50 values obtained, the ethanol extract of cacao husk and encapsulated has antioxidant activity with this range and is classified as a strong antioxidant.

Antibacterial activity test

Testing of antimicrobial activity at various concentrations of the use of maltodextrin was carried out to see the potential use of peanut butter (maltodextrin) on cacao fruit peel extract against pathogenic bacteria in this case *E. coli*, *Salmonella sp* and *S. aureus* which were measured based on the ability of the extract to inhibit the growth of test bacteria through measurement of the inhibition diameter shown in the clear area around the well area where the wider the clear area in the well area shows the higher inhibitory ability of the extract and encapsulate. Based on the results of the study, ethanol extract of cocoa husk can inhibit all test bacteria, both Gram positive bacteria and Gram negative bacteria with a larger inhibitory diameter than the encapsulated products of ethanol extract of cocoa husk. The results of this study indicate that the antimicrobial activity of ethanol extracts of cacao husk and encapsulated in a broad spectrum because it is able to inhibit Gram positive and Gram negative bacteria. The inhibition of cocoa husk extract and encapsulation results of ethanol extract of cacao husk was 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 the concentration of 0.5% ethanol extract of cocoa husk and the encapsulation results of ethanol extract of cocoa husk showed the smallest inhibitory diameter of all test bacteria and the highest inhibition diameter at 2% concentration. The higher the

concentration of cacao husk extract and encapsulation, the higher the inhibitory diameter, this is in line that the higher the extract concentration, the higher the inhibitory diameter produced (14).

The results of the analysis showed that the concentration of maltodextrin showed very different results ($P < 0.01$). This is because the higher the concentration of maltodextrin used the lower the ethanol extract of the coated cocoa husk due to the smaller diameter of the inhibition produced. Cocoa peel extract contains compounds that act as antibacterials such as flavonoids, polyphenols, tannins and quercetin. The antibacterial inhibition process occurs due to the contact of antibacterial compounds on the cell surface or compounds that diffuse into bacterial cells (Kanazawa, 1995). The diameter data of inhibition of cocoa pods extract at various concentrations of maltodextrin can be seen in Table 3.

Table 3. Inhibition diameter (mm) of ethanol extract of cacao pods and encapsulate extract of cocoa husk at various concentrations (%) of several bacteria.

Maltodextrin concentration in encapsulates (%)	Concentration encapsulates (%)	Clear zone diameter (mm)		
		<i>Ecoli sp</i>	<i>Salmonella sp</i>	<i>Staphylococcus aureus</i>
	0,50	4,12	2,85	5,15
M1 (20)	1,00	5,10	3,78	6,75
	1,50	6,85	5,25	8,15
	2,00	10,95	8,25	13,90
	0,50	3,75	2,75	4,50
M2 (30)	1,00	4,95	3,25	5,80
	1,50	5,85	4,10	7,90
	2,00	9,12	7,79	12,45
	0,50	3,10	2,30	3,45
M3 (40)	1,00	3,60	2,85	4,87
	1,50	4,90	3,90	5,45
	2,00	8,15	5,85	6,66
	0,50	2,15	2,13	3,35
M4 (50)	1,00	3,25	2,45	4,45
	1,50	3,89	3,80	5,30
	2,00	5,50	4,56	6,18
	0,50	2,00	1,15	2,76
M5 (60)	1,00	3,30	2,75	3,80
	1,50	4,20	3,85	4,45
	2,00	4,79	4,35	5,17
	0,50	4,75	3,25	5,70
Ethanol extract of cocoa pods	1,00	5,25	4,85	7,75
	1,500	7,10	6,15	10,15
	2,00	12,85	9,25	14,85

Table 3. shows that before encapsulated cocoa husk extract showed an increase in inhibitory power along with the increasing concentration of ethanol extract of cacao husk on all test bacteria. While the encapsulated cocoa peel extract shows the higher maltodextrin concentration, the lower the inhibitory diameter obtained. The inhibitory power of the treatment M1 (20%) and M2 (30%) are relatively the same at various encapsulate concentrations but for other treatments M3 (40%); M4 (50% and M5 (60%) have decreased inhibition with increasing concentrations of maltodextrin as coating material for all test bacteria.

The results of this study indicate that the encapsulation treatment of cocoa husk ethanol extract can provide protection against bioactive components contained in cocoa husk, because in various treatments the concentration of coating material (maltodextrin) still provides inhibition on bacterial growth as shown by the presence of clear areas around the well, where the biggest inhibitory power of *Staphylococcus aureus* bacteria was followed by *E.coli* and *Salmonella sp.* This difference is thought to be caused by several factors including the ability of diffusion of extract ingredients, concentration of extract, interaction between medium components, and environmental conditions. This suspicion is supported that the concentration of a substance that functions as an antibacterial is one factor that determines the size of the ability of antibacterial substances in inhibiting the growth of bacteria tested (15). It was further 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 test bacteria which affected the work of cacao husk extract in encapsulate as an antimicrobial compound. The presence of antibacterial activity from encapsulants of cocoa husk extract is supported by the results of phytochemical screening which showed the presence of bioactive compounds in extracts of cocoa husk, such as alkaloids, flavonoids, polyphenols, and tannins. Flavonoid compounds reported by some researchers will interfere with the integrity of bacterial cell membranes, tannins work competitively with glycosyltransferase enzymes in reducing saccharide as the basic ingredient of glycosylation. 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. In addition tannins can also react with cell membranes, enzyme inactivation, and destruction or inactivation of the function of genetic material (16;17).

Conclusion

Based on the results of research on the antioxidant and antimicrobial activity of encapsulants of ethanol extract of cocoa husk at various concentrations of maltodextrin, conclusions can be:

1. Encapsulation of ethanol extract of cacao husk at various concentrations of maltodextrin treatment that gave the highest yield was obtained at 60% (M5) concentration and the lowest was obtained at 20% maltodextrin concentration (M1)
2. The treatment that gave the highest antioxidant activity was obtained in maltodextrin 20% (M1) treatment with IC50 value of 75.89 $\mu\text{g} / \text{mL}$ and the treatment with the lowest antioxidant activity was obtained in the treatment of 60% maltodextrin concentration (M5) with IC50 value of 75.89 $\mu\text{g} / \text{mL}$ While for the antimicrobial activity was also obtained with the same result that the treatment at a concentration of 20% (M1) obtained a higher inhibitory diameter than the 30% concentration treatment (M2); 40% (M3); 50% (M4) and 60% concentration treatment (M5) for all types of bacteria.
3. Ethanol extract of cocoa pods encapsulated with maltodextrin at various concentrations still shows its activity both as an antioxidant and as an antimicrobial so that it is very likely to be used as a natural preservative.

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