

MICROENCAPSULATION OF FLAVONOIDS FROM GEDI LEAVES (*Abelmoschus manihot* L.) IN β -CYCLODEXTRIN USING COPRECIIPITATION AND KNEADING METHODS

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ABSTRACT

The protection of active compounds such as flavonoids as candidate drug compounds has been the focus of several studies in recent years. This is due to the different physicochemical properties of flavonoids. Furthermore, the function of these flavonoid compounds can be maximized from the initial process of separating them from the plant of origin known as extraction. This study compared the effectiveness and efficiency of using microwave extraction and maceration of Gedi leaves (*Abelmoschus manihot* L.). Then, the extract was encapsulated to protect the unstable flavonoids. There were two methods to encapsulate the extract in this study. They were the coprecipitation and kneading methods. The result shows that microwave extraction assisted has higher product in less solvent and was time-consuming. The coprecipitation also shows better performance in encapsulating the extract. The calculation of the efficiency of encapsulation was done for 3 different volumes of both methods. The addition of 1 mL extract by coprecipitation showed approximately 92.12%. It was also supported by the data of FTIR and UV VIS.

Keywords: Gedi Leaves, Flavonoids, Maceration, Microwave, Microencapsulation, Coprecipitation.

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INTRODUCTION

The flavonoid content in Gedi leaves (*Abelmoschus manihot* L.) is relatively high, around 23-41%, and its antioxidant properties are also quite good.^{1,2} The properties of flavonoids as effective antioxidants in both plants and animals have been studied over the last few decades. Nonetheless, flavonoids still have great potential to counteract the detrimental effects of a large generation of reactive oxygen species (ROS).³ Flavonoids, the most abundant bioactive compounds in plants, have functioned as antioxidants⁴, anticancer⁵, antibacterial⁶, and anti-inflammatory.⁷ The use of flavonoid compounds from natural ingredients in the study of drug development is something that has been done for a long time. However, it can lose significant amounts during the flavonoid extraction process due to its unstable nature at high temperatures.⁸ The choice of procedure and time during the extraction and isolation process plays a major role in maximizing the use or biological activity of natural ingredients such as flavonoids, especially in the development of medicinal compounds. Therefore, there is an urgent need to choose a selective and effective method for the extraction and isolation of the active compounds of these natural products. In recent years Microwave Assisted Extraction (MAE) has been introduced. This modern tool has many benefits and is more efficient than traditional extraction methods. In addition, MAE can minimize several things including cost reduction, extraction time, number of solvents used, energy consumption, and low CO₂ emissions.^{9,10} For flavonoid isolation, MAE has advantages over traditional methods such as reflux and solid-liquid maceration extraction to isolate flavonoids from natural materials such as corn flour samples. There were significant differences in the decomposition of rhamnetin, quercetin, kaempferol, and myricetin after MAE extraction and maceration. This significant difference is due to the high temperature created by microwaves in a closed container and partially decomposed, according to the number of substituents (hydroxylate and methoxylate groups). Based on the relationship between the degradation process and the chemical structure, it is known that the number, type of substituents, and position of the hydroxyl groups affect the stability of flavonoids. The stability of flavonoids could be inclined by a little number of substituents. The presence of sugar in the flavonoid structure can also help stabilize flavonoids during the extraction process.^{11,12} Various microencapsulation designs have been developed to protect the physical properties of flavonoids such as stability, good processing conditions, and absorption through the intestinal tract. It is also called a method

to entrap flavonoids in gastro-resistant polymers and carry them directly to the intestine.¹³ Therefore, microencapsulation is useful to protect new drug candidate compounds.^{14,15} β -cyclodextrin (β -CD) is one of the macromolecules that can be used in the encapsulation of flavonoid compounds. Several studies reported the use of β -CD in the encapsulation of secondary metabolites and essential oils.^{12,14} The encapsulation procedure using β -CD can be carried out in several ways such as co-precipitation and kneading. In addition to determining the appropriate extraction method, this study will also compare the efficiency of the two methods.

EXPERIMENTAL

Material and Methods

Gedi leaves used in the extraction process were fresh Gedi leaves. Other materials were high purity (99%) of β -CD, ethanol absolute ($\text{CH}_3\text{CH}_2\text{OH}$), n-hexane, MgSO_4 anhydrous and ethyl acetate, Shinoda reagent, quercetin, methanol, CH_3COOK , AlCl_3 , and double deionized water. Before the extraction process is carried out, phytochemical tests are carried out first. Extraction is carried out in two stages, namely maceration and Microwave Assisted Extraction (MAE) with microwave specification as 23 L straight microwave oven 3-21D0(S)IN.

Extraction of Flavonoids from Gedi Leaves

Gedi leaf powder which is greenish yellow as much as 200 g is mixed with 600 mL absolute ethanol. This mixture was then stirred with a shaker for 45 minutes by dividing it into two containers. Then each container was macerated for 3 x 12 hours in a dark glass bottle, shaken again, and separated between the powder and the solution if the flavonoid did not show positive results in the dregs. Next, the extract was evaporated with a rotary evaporator around the boiling point of the solvent (ethanol). Therefore, the extract obtained was thick. Samples were stored in dark containers for further processing. The second extraction process is using the Microwave Assisted Extraction method. A total of 100 g of Gedi leaf powder was added to as much as 300 mL of ethanol into a round bottom flask that had been designed to blend with the microwave. Set the microwave power on the mid-low to high for about 30 minutes. Shinoda test on the extract obtained. Then evaporate to get a thicker extract^{16,17}.

Total Flavonoid Content Determination

In both extract samples, a total flavonoid test was performed to compare which method was more effective in flavonoid extraction.¹⁸ 25 mg of Quercetin was dissolved in 25 mL of methanol and obtained the standard solution (1000 ppm), then made a series of concentrations in 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 125 ppm, then they were pipetted and put into a volumetric flask and diluted with 5 mL of methanol (MeOH). To determine the maximum wavelength, the standard solution concentration of 10 ppm was taken and added 3 mL of MeOH, 0.2 mL of 10% AlCl_3 , 0.2 mL of 1 M CH_3COOK and aqua dest until reached 10 mL of the flask and then shaken until homogeneous. Perform scanning at a wavelength of 400-500 nm. Then the curve of the relationship between wavelength and absorbance was observed to determine the maximum wavelength from the obtained spectrogram. The remaining solution was kept and used to determine the operating time. Quercetin wavelength was measured from 0-40 minutes. Then the relationship between absorbance and time was observed until the operating time could be determined. For the measurement of total flavonoids, 0.5 mL of the test sample and standard solution were taken, and then added 3 mL of MeOH, 0.2 mL of 10% AlCl_3 , 0.2 mL of CH_3COOK , and added to 10 mL of distilled water. It was incubated for 30 minutes followed by the measurement of absorbance by UV-Vis spectrophotometry at 439.5 nm. This measurement was carried out in triples. In addition, before the measurement, optimization is carried out to find the maximum operating time and wavelength.

Inclusion Complex Synthesis and Characterization (G- β -CD)

Inclusion complex synthesis was carried out using two methods to compare which method was more effective in trapping flavonoid compounds. The first method is co-precipitation. A mixture of ethanol and aqua dest (1:4) of as much as 60 mL was added to dissolve 1 g of β -CD. It was stirred for 45 minutes at 65°C and saved this mixture for further encapsulation. It was called the β -CD solution. The coprecipitation method was started by dissolving of β -CD solution with three different volumes of the extract, which were 1 mL, 0.5 mL, and 0.75 mL. They were stirred for 30 minutes and allowed to stand for about 1 hour. Then,

they were saved for further characterization, namely UV-VIS, and FTIR. On the other hand, the encapsulation in the kneading method was carried out with a similar method from coprecipitation but the β -CD used is pure. They were sonicated separately for 30 minutes and saved for 1 hour before they were left in the oven to become powder at 70°C. Then, they were saved for further characterization, namely UV-VIS, and FTIR.

Calculation of Encapsulation Efficiency

There are two positions of flavonoids after encapsulation. They are trapped inside the cavity or adsorbed on the surface of the microcapsules of the G- β -CD. Therefore, to calculate the all flavonoids in the G- β -CD, these two probabilities should be included. Firstly, approximately 0.5 g of the G- β -CD was mixed in 15 mL n-hexane and 50 mL deionized water then it is stirred at low-speed. The non-polar and polar phases were separated and checked for the presence of flavonoids. The same procedure was replayed until there was only one phase that shows a positive result in the Shinoda test. The extract was dried with anhydrous MgSO_4 followed by evaporation utilizing a rotary evaporator. Then, a label of m_1 was given as the number mass of the thick residue. To calculate the flavonoids adsorbed on the surface of the G- β -CD, 20 mL of n-hexane was added to 0.5 g of the G- β -CD while it is stirred at 400 rpm at 25°C for 30 minutes. It was filtered and washed with about 10 mL of n-hexane. The extract was dried with anhydrous MgSO_4 and decanted. After that, it was vacuumed using a rotary evaporator. In the end, the oily residues were weighed and expressed as m_2 .¹²

The efficiency of encapsulation (%EE) is determined by the equation:

$$\%EE = \frac{m_1 - m_2}{m_{\text{inclusion complex}}}$$

Where : $m_{\text{inclusion complex}} = \frac{m_{\text{total flavonoid}} + m_{\text{surface flavonoid}}}{2}$

Total flavonoid = total number of flavonoids trapped in the cavity of G- β -CD (g)

Surface flavonoid = total number of flavonoids adsorbed on the surface of G- β -CD (g)

RESULTS AND DISCUSSION

In this study, two extraction methods have been carried out, namely maceration and MAE. Based on the rendemen, the extraction method with MAE produced more extracts compared to maceration. Within 30 minutes and a solvent of 300 mL, the viscous extract obtained was 3.27%, while maceration only obtained a viscous extract of 2.75% with the amount of solvent doubling MAE in an extraction time of 36 hours. This proves that MAE is a fairly effective method to produce more extracts with less solvent and a short extraction time.¹⁹ A wavelength determination is carried out for further quantitative analysis. The wavelength representing the maximum absorbance was determined to give maximum sensitivity. This study used quercetin as the standard, and the result of the maximum absorbance was 435 nm. Furthermore, operating time measurements were performed which was the time-consuming reaction between flavonoids and AlCl_3 . During the measurement in 60 minutes, it showed the operating time of the quercetin complex in 30 minutes.

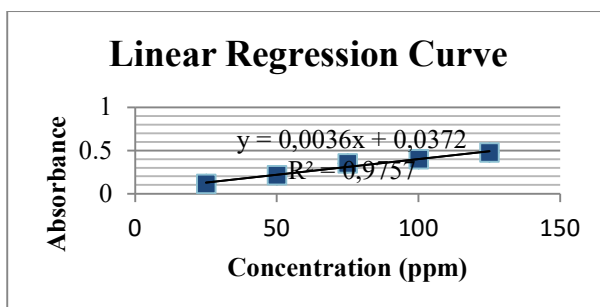
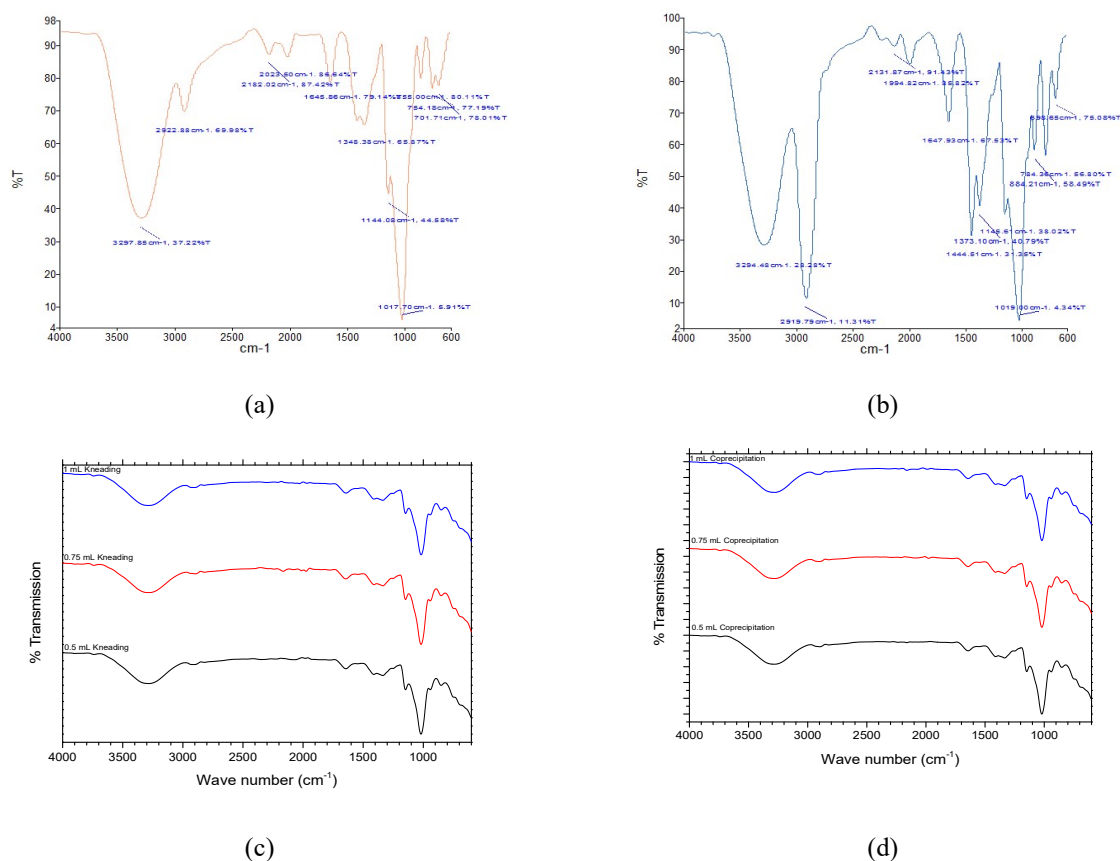


Fig.-1: Quercetin Linear Regression Curve with linear Regression Formula $y = 0.0036x + 0.0372$

The raw curve concentration series is made with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 125 ppm. If the standard curve series satisfies Lambert-Beer's law, then the absorbance will be directly

proportional to the concentration. This is indicated by a straight-line standard curve with r approaching the number 1 which means that the relationship between the standard curve and absorbance is linear.²⁰ Based on the linear regression formula obtained total flavonoid levels of maceration methods and MAE. In the quantitative test, Gedi leaf steeping obtained flavonoid levels in the total maceration method with an average of 78.028 ppm QE and the total flavonoid content of the MAE method was 180.11 ppm QE. This shows that the total flavonoid levels of the MAE method are higher than the flavonoid levels in the maceration method. It also shows that the MAE method is more effective in extracting Gedi leaf flavonoids. Furthermore, this extract is encapsulated to protect the flavonoid compounds present in these Gedi leaves. The formation of inclusion complexes can be identified using Infrared Spectroscopy as in Fourier transform Infra-Red (FT-IR) spectra. The slight shift or the different intensity of spectra bands in FTIR indicated the presence of guest molecules but the inclining trend would be influenced by the spectrum of the host molecule when the number of the guest molecule was in the range of 5-15% of the mass of the inclusion complex. Nonetheless, current studies were able to prove the existence of G- β -CD marked by a shift in FTIR.²¹ The host (β -CD) and guest (flavonoid molecules) in G- β -CD might interact and lead to a change in the spectrum of FTIR. Differences in the intensity, shape, or shift of the spectra at the peaks of FTIR of Gedi leaf extract and β -CD against their inclusion complexes might prove that an inclusion complex has formed. The IR spectra for inclusion complexes by two methods, i.e., maceration and MAE of Gedi leaf extract, and β -CD are shown in Fig.-2. As seen in Fig.-2c and 2d, the peak shift of the transmittance at a lower frequency indicated that the G- β -CD has formed. The altered peak intensity of absorption indicates that there was a hydrogen bond that was an intramolecular bond in the inclusion complex. This is in line with previous studies that peak shifts and transmittance intensities have proven that such inclusion complexes have formed.¹⁴



If we compare the inclusion complex produced through the kneading method and the coprecipitation method, the percent transmittance of the inclusion complex undergoes quite fluctuating changes. The change in the number can be observed in Table-1 below. Based on these data, the best volume and the best method that can be used is 1 mL by the coprecipitation method.

Table-1: Functional Group Detection in FTIR

Volume (mL)	Functional group	Wavenumber (cm ⁻¹)		% T	
		a	b	a	B
0.50	O-H	3281.19	3283.93	77.13	79.06
	C=C	1643.93	1643.37	88.09	88.74
	C-C	1336.45	1334.56	83.06	83.58
	C-O	1147.90	1148.02	72.54	73.72
		1019.23	1020.36	39.75	43.06
	C=C, C-H in ring	851.10	851.21	75.58	76.78
0.75	O-H	3280.47	3280.27	81.34	77.75
	C=C	1644.31	1643.82	89.98	86.62
	C-C	1336.05	1335.06	85.62	81.48
	C-O	1147.86	1147.96	76.97	71.77
		1018.30	1019.96	48.76	41.66
	C=C, C-H in ring	850.00	851.29	79.41	74.52
1.00	O-H	3282.83	3277.71	76.55	76.05
	C=C	1642.80	1643.24	85.56	86.86
	C-C	1336.73	1334.40	80.31	81.28
	C-O	1147.47	1148.10	70.74	71.06
		1017.85	1019.99	40.70	38.46
	C=C, C-H in ring	850.32	851.22	73.31	74.60

Note: a= coprecipitation, b = kneading

Table-1 provides information that there was a change in transmittance along with a change in the amount of extract given, the more extract given, the lower the transmittance. This suggested that the resulting uptake increases and more molecules are trapped. Therefore, this is in line with other encapsulation studies.²² These FTIR data were also supported by the results of the UV VIS spectra in Fig.-3 which shows that the coprecipitation method has a higher absorbance value, so the probability of the concentration of absorbed extract is much more.

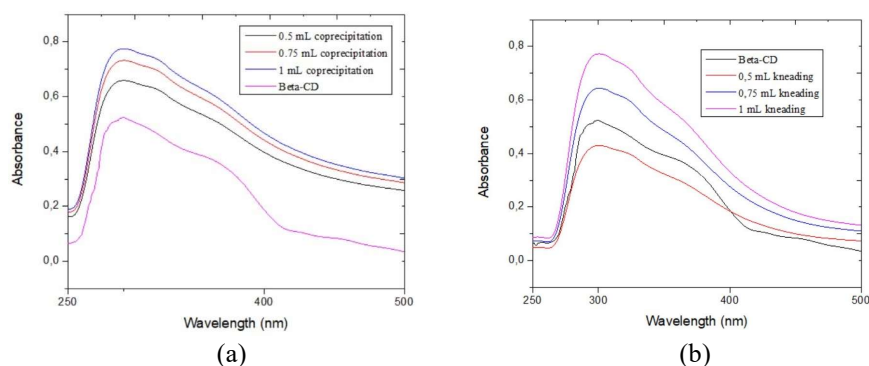


Fig.-3: (a) Combined Data of the Coprecipitation Method, (b) Combined Kneading Method Data

The calculation of the efficiency of the encapsulation of these molecules has been carried out. The total flavonoids are represented by the number of flavonoids trapped in β -CD whereas surface flavonoids are represented by the number of flavonoids located on the surface of the microcapsules, all flavonoids in the inclusion complex greatly affect the efficiency of encapsulation. The number of flavonoids affects the efficiency of encapsulation because the more coating material to be packaged will reduce the number of flavonoids on the surface, consequently, it would decrease the oxidative stability of the microcapsules. By

looking at the %EE data (Table-2), the number of flavonoids inside the cavity of G- β -CD (total flavonoids) and the number of surface flavonoids increased for both methods. The treatment or method of coprecipitation was better because of the help of solvents which will be easily replaced by these flavonoids. These results may be related to guest (geometry, volume, hydrophobicity) flavonoids and the physicochemical properties of β -CDs playing a major role in the formation of G- β -CDs.^{11,12}

Table-2: The Comparison of Encapsulation Efficiency Between Coprecipitation and Kneading

Method	Volume (mL)	Surface oil (mg)	Total oil (mg)	Encapsulation Efficiency (%)
Kneading	0.50	89.9	500	82.02
	0.75	77.5	500	84.50
	1.00	42.5	500	91.50
Coprecipitation	0.50	112.5	500	77.5
	0.75	42.5	500	91.5
	1.00	39.4	500	92.12

CONCLUSION

Based on the linear regression formula obtained total flavonoid levels of maceration methods and MAE, Gedi leaf obtained flavonoid levels in the total maceration method with an average of 78.028 ppm QE and the total flavonoid content of the MAE method was 180.11 ppm QE. This shows that the total flavonoid levels of the MAE method are higher than the flavonoid levels in the maceration method. It also shows that the MAE method is more effective in extracting the flavonoids of Gedi leaf. Furthermore, based on the data of FTIR, UV VIS, and the calculation of efficiency encapsulation, it inclined by adding more extract for both methods. Comparing two different methods in encapsulation, coprecipitation trapped more flavonoids than kneading in every volume of extract.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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