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# Extraction of Flavonoids from *Merremia mammosa* Using Ethanol Solvent in a Fixed-Bed Column

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**Abstract.** This research aims to investigate the best operating condition for the extraction process of flavonoids from *Merremia mammosa* root by determining the mass transfer coefficient ($K_e$). The root was prepared by drying, crushing, and sieving into a homogeneous size and then extracted in a fixed-bed column using 70 wt% of ethanol as a solvent for 2 hours. The obtained samples were then analyzed every 30 min using high-performance liquid chromatography. The parameters investigated in this research were particle size of 1.275, 1.85, and 4.01 mm and solvent flow rate of 3, 6, and 9 mL/s. Based on the experimental data, the value of $K_e$ was calculated using the Hooke-Jeeves numerical method of optimization. The results showed that the decrease in particle size and the increase in solvent flow rate could increase the $K_e$ values, leading to the high concentration of flavonoids extracted using the solvent. The $K_e$ values obtained in this research ranged from 0.3145 m/s to 0.7880 m/s. The empirical equation that shows the correlation between $K_e$ and the parameters can be expressed as $Sh = 1.10 \times 10^{14}\, Re^{0.0564}\, (1 - \varepsilon)^{0.8718}$ with a relative error of 6.13% compared with the experimental data (*Sh* is the Sherwood number, *Re* is the Reynolds number, and $\varepsilon$ is the porosity of the fixed-bed column).

**Keywords:** Extraction, Fixed-bed column, Flavonoids, Mass transfer, *Merremia mammosa*

## INTRODUCTION

Indonesia, as a tropical country, has notable biodiversity. About 40,000 endemic plants grow in the fields, and 7,000 of them have been used as medicine by the local cultures (Marchianti *et al.* 2021). One of the potential plants used for biomedical application is *Merremia mammosa*. This plant grows well in tropical lowlands, particularly inside the forests, which vines with a length of 3–6 meters. On the dry land, *M. mammosa* can weigh up to 5 kg or more and contains approximately 5.92% of an active compound called flavonoids at 40 °C drying temperature (Marchianti *et al.* 2019; Wahjuningsih *et al.* 2019). Flavonoids are widely used in biomedical applications as an anti-inflammatory agent, analgesic, wound healer, and medicine to treat various diseases, such as cancer, typhoid fever, and diabetes (Hidayat *et al.* 2015; Marchianti *et al.* 2018).

In addition, these natural products also act as antioxidants, as revealed by various *in vitro* models from previous research (Sowndhararajan *et al.* 2010). Those benefits,

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as mentioned earlier, of flavonoids bring to the rising demand for *M. mammosa* extract for pharmaceutical products.

Previous research on the extraction of flavonoids from *M. mammosa* has been successfully conducted. The flavonoids in *M. mammosa* roots were extracted using 96 wt% ethanol and ultrasonic agitation (Sakinah *et al.* 2018). The obtained flavonoids were then analyzed using the aluminum chloride colorimetric method, resulting in a mass fraction of 0.17%. Another research used a solvent extraction method followed by vacuum drying to extract the flavonoids from *M. mammosa* leaves (Ratnadewi *et al.* 2018). This method obtained 449.46 mg of quercetin equivalent/g extract using methanol as a solvent. Arunachalam and Parimelazhagan (2012) have also successfully extracted flavonoids from the same plant genus as *M. mammosa* but in different species using distilled water at 25°C through shaking for 48 hours (Arunachalam and Parimelazhagan 2012). Their results showed that the solvent extraction method that was being used could produce flavonoids extracted from *M. mammosa,* which reduces the elevated blood glucose level and lipid profile of diabetic rats and has no effect on normal rats.

All those previous works show that the utilization of solvent extraction methods to obtain flavonoids from *M. mammosa* is subtle and practical. However, the optimization of the extraction process needs to be investigated to determine the optimum conditions. The optimum parameters could be used to reach the high yield of extract from the extraction process. In addition, higher stability of the extract could also be obtained at the optimum conditions (Putri *et al.* 2019).

This research aims to explore the best operating condition for the flavonoid extractions process from *M. mammosa* root powder in a fixed-bed column. The parameters investigated in the present work are the diameter size of raw materials and solvent flow rate. The mass transfer process during extraction was investigated by obtaining the overall mass transfer coefficient ($K_e$) of flavonoids from *M. mammosa* root using 70 wt% ethanol as solvent. Then, the impact of these parameters on $K_e$ was expressed as an empirical equation (Sari 2016; Yuzki 2016).

## EXPERIMENTAL AND MODELING

### Materials

Fresh *M. mammosa* known as *bidara* in Indonesia was obtained from Bantul, Yogyakarta, Indonesia. The technical grade of ethanol (70%, General Labora) was used as a solvent in the extraction process.

### Research procedures

The experiment was conducted in three steps, including the preparation of *M. mammosa* root, the extraction process, and the analysis of the samples. First, *M. mammosa* root was sliced into small pieces and dried at room temperature. The dried particles were then crushed using mortar and pestle and then sieved into three diameter particles to obtain a homogeneous size. The particle size varied at 1.275, 1.85, and 4.01 mm. The crushed particles were placed in a fixed-bed column. Two liters of 70 wt% ethanol was prepared in a stirred tank, which was connected to the fixed-bed column as shown in Figure 1. The length of the glass column is 30 cm, with an inner diameter of 3 cm. The volume of the plastic stirred tank is 8 L. The extraction process was done in 2 hours with solvent flow rate varied at 3 mL/s, 6 mL/s,

and 9 mL/s. During the extraction process, samples were analyzed every 30 minutes using high-performance liquid chromatography (HPLC). As a standard solution, quercetin was used to quantify the concentration of flavonoids.

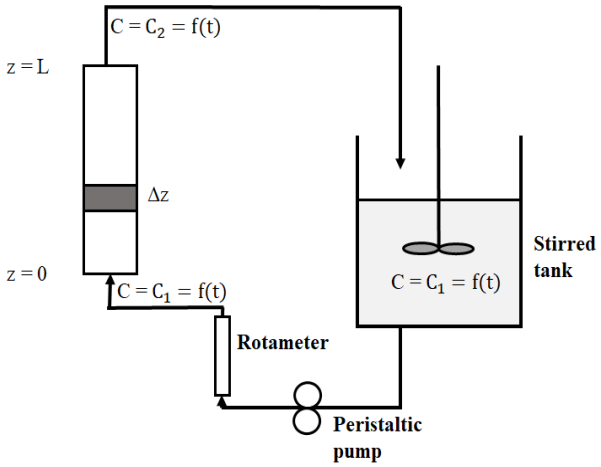


**Fig. 1:** Schematic diagram of the experimental apparatus.

## Modeling

Based on the experimental data, the mass transfer coefficient ($K_e$) was calculated. The following assumptions were used to calculate the $K_e$ value:

1. The mixing process in the stirred tank resulted in a homogenous solution.
2. The process was isothermal.
3. The particle size was fixed.
4. The particle distribution inside the column was homogenous.
5. The flavonoids concentration at the solid surface was saturated.

Based on those assumptions, the mass balance of total flavonoids in the aqueous phase at the element volume in the fixed bed column can be expressed as follows:

$$\left[\left(A.v.\varepsilon.C_A\big|_z\right)+\left(-D_{AB}.A.\varepsilon.\frac{\partial C_A}{\partial z}\bigg|_z\right)+K_{e,a}.a.A\Delta z.(1-\varepsilon).(C_A^{\ *}-C_A)\right]$$
$$-\left[\left(A.v.\varepsilon.C_A\big|_{z+\Delta z}\right)+\left(-D_{AB}.A.\varepsilon.\frac{\partial C_A}{\partial z}\bigg|_{z+\Delta z}\right)\right]=A.\Delta z.\varepsilon.\frac{\partial C_A}{\partial t} \quad (1)$$

Eq. (1) can be simplified into Eq. (2) below:

$$-v.\frac{\partial C_A}{\partial z}+D_{AB}.\frac{\partial^2 C_A}{\partial z^2}+K_e a.\frac{(1-\varepsilon)}{\varepsilon}.(C_A^{\ *}-C_A)=\frac{\partial C_A}{\partial t} \quad (2)$$

In addition, the value of $D_{AB}$ is neglected due to the diffusivity inside the pore being small and can be eliminated. So, Eq. (2) can be presented as below:

$$-v.\frac{\partial C_A}{\partial z}+K_e a.\frac{(1-\varepsilon)}{\varepsilon}.(C_A^{\ *}-C_A)=\frac{\partial C_A}{\partial t} \quad (3)$$

where the value of $C_A^*$ is 87.7343 mg/L. The dependent variable $C_A$ is a function of time ($t$) and axial position ($z$). Therefore, it can be expressed as $C_A(z,t)$ to state all of the boundary conditions in this system as follows:

1. Initial condition, $C_A(z = 0, t = 0) = 0$;
2. Boundary condition I, $C_A(z = 0,t) = C_1$ (finite);
3. Boundary condition II, $C_A(z = L,t) = C_2$ (finite).

Inside the stirred tank, the simplified mass balance can be expressed as Eq. (4):

$$\frac{Fv}{V}.(C_2-C_1)=\frac{dC_1}{dt} \quad (4)$$

where the term $\frac{Fv}{V}$ can be expressed as the residence time distribution ($\tau$), resulting in the following equation:

$$\frac{(C_2-C_1)}{\tau}=\frac{dC_1}{dt} \quad (5)$$

The equation can be solved numerically using a combination of the finite difference equation and Runge–Kutta methods. The value of $K_e a$ can be estimated using the Hooke–Jeeves numerical method of optimization. The optimized variable of $K_e a$ and the sum of squares for error of $C_A$ were the objective functions. Then, the correlation between the influencing parameters and the value of $K_e a$ can be expressed as the following empirical equations (Perry *et al.* 1997):

$$Sh = a_0 . \mathrm{Re}^{b_1} . Sc^{b_2} . (1-\varepsilon)^{b_3} \quad (6)$$

$$\frac{K_e . d}{D_{AB}} = a_0 . \left( \frac{\rho . v . d}{\mu} \right)^{b_1} . \left( \frac{\mu}{D_{AB} . \rho} \right)^{b_2} . (1-\varepsilon)^{b_3} \quad (7)$$

Given that the same solvent was used, the second dimensionless group can be merged with $a_0$ to form the constant $a_1$, resulting in the following expression:

$$\frac{K_e . d}{D_{AB}} = a_1 . \left( \frac{\rho . v . d}{\mu} \right)^{b_1} . (1-\varepsilon)^{b_3} \quad (8)$$

Then, $a_1$, $b_1$, and $b_3$ were evaluated using the multilinear equation numerical method.

## RESULTS AND DISCUSSION

In the present study, flavonoids from *Merremia mammosa* were extracted using ethanol as a solvent in a fixed bed column. Ethanol was used as a solvent because it has adequate polarity so that it can act as a good extracting solvent for polyphenol (Borja *et al.* 2014). The experimental data of the flavonoid concentration extracted using different solvent flow rates (i.e., 3, 6, and 9 mL/s) as a function of time for the particle diameter of 4.01 mm are presented in Figure 2.

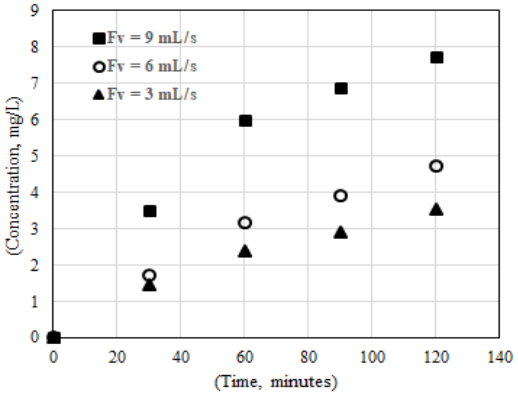


**Fig. 2:** The concentration of flavonoids extracted using different solvent flow rates as a function of time for the particle diameter of 4.01 mm.

The results showed that at the same diameter of the particle, the concentration of flavonoids increased with the increase of solvent flow rate. That means the increase in solvent flow rate could increase the turbulence of fluid, which tends to increase the mass transfer area *(Mulyono et al.* 2013). The increasing solvent flow rate also tends to decrease the film resistance, which contributes to the acceleration of the mass transfer process.

Figure 3 presents the flavonoid concentration in the stirred tank as a function of time and particle diameter (i.e., 1.275, 1.85, and 4.01 mm) using the same solvent flow rate of 9 mL/s. As shown in the graph, different particle sizes affect the concentration of obtained flavonoids. With smaller particle sizes, the concentration of flavonoids extracted using the solvent tends to increase. That is because the smaller the particle size, the higher the total contact surface area of the particle and solvent. The mass transfer rate of flavonoids from the particle to the solvent also tends to increase.

Moreover, the smaller the particle size is, the shorter the diffusion path of the particle from inside the bulk to the surface.

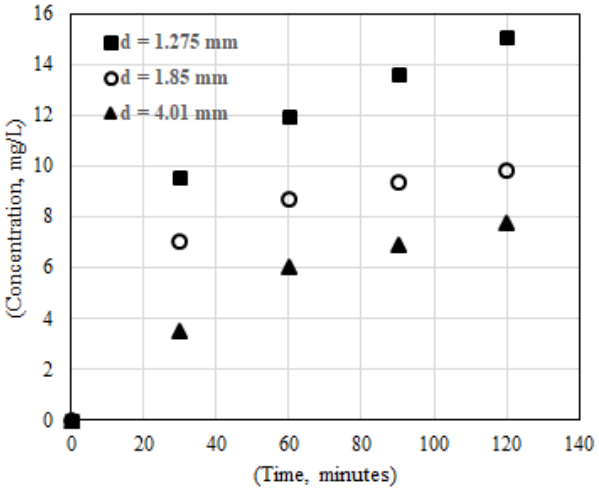


**Fig. 3:** The concentration of flavonoids at different particle sizes as a function of time for the solvent flow rate of 9 mL/s.

The rate of extraction was evaluated by neglecting the diffusivity of flavonoids $D_{AB}$. Then, the concentration of flavonoids in the stirred thank was calculated using Eqs. (3) and (5). The calculated result was compared with the concentration of flavonoids derived from experimental data. Figure 4 shows the different flavonoids concentration extracted using different solvent flow rates based on the calculation and experimental data for the particle diameter of 4.01 mm. Notably, the tendency of flavonoids extracted using different solvent flow rates based on the calculation and experimental data is consistent.

Meanwhile, the concentration of flavonoids as a function of time and particle size based on the calculation and experimental data is shown in Figure 5 for the solvent flow rate of 9 mL/s. Figure 5 shows that the increasing trend of the concentration of flavonoids based on the calculation is similar to that based on the experimental data.

The similarity of the trend of flavonoid concentration as a function of time-based on the calculation and experimental data indicates that the proposed mathematical model used for the calculation fits the experimental data.

The empirical equation, which shows the correlation between Sherwood number, Reynolds number, and porosity, was calculated using Eq. (8). The obtained empirical equation is $Sh = 1.10 \times 10^{14} \cdot Re^{0.0564} \cdot (1 - \varepsilon)^{0.8718}$, with a 6.13% relative error compared to the experimental data. This equation is effective for the extraction of flavonoids from *Merremia mammosa* in a fixed bed column at solvent flow rates ranging from 3 mL/s to 9 mL/s and particle size diameters of raw material ranging from 1.275 mm to 4.01 mm. The correlation between Sherwood number, Reynolds number, and porosity is depicted in Figure 6.

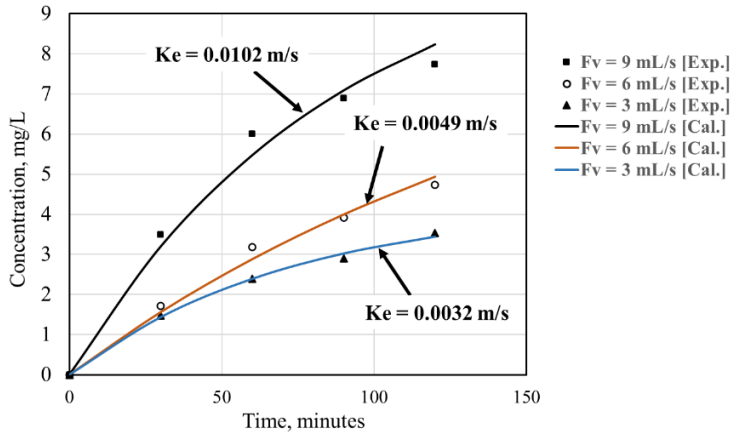


**Fig. 4:** Concentration of flavonoids as a function of time and solvent flow rate based on the calculation and experimental data for the particle diameter of 4.01 mm.

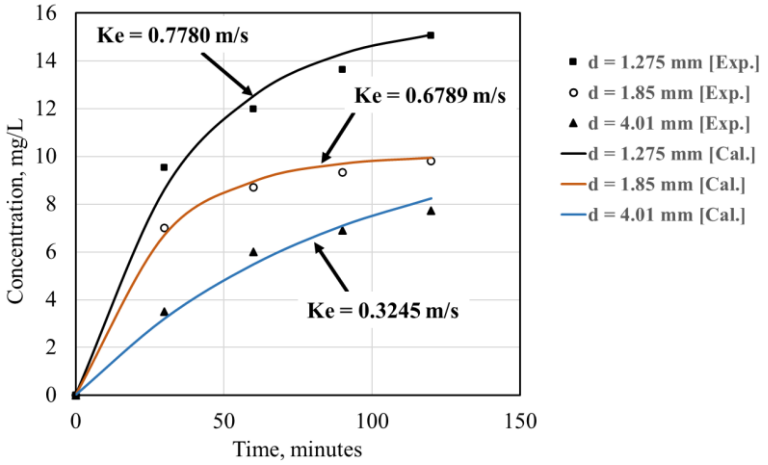


**Fig. 5:** The concentration of flavonoids as a function of time and particle size based on the calculation and experimental data for the solvent flow rate of 9 mL/s.

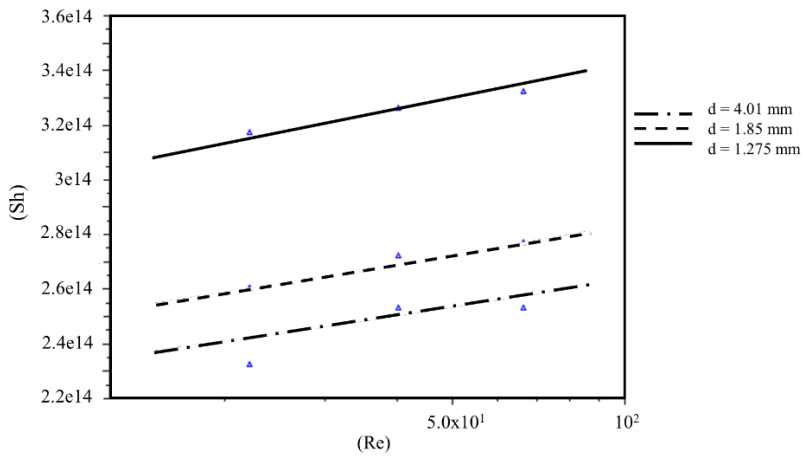


**Fig. 6:** Correlation between Sherwood number, Reynolds number, and porosity.

The obtained empirical equation shows that the rise of solvent flow rate could increase the Reynolds number. That is, the Reynolds number shows the turbulence of fluid through the particle. Thus, the higher fluid turbulence is, the better mass transfer of the flavonoids from the particle to the solvent. Meanwhile, the particle size affects the porosity inside the fixed-bed column. The smaller the particle size is, the lower the porosity. That is, the porosity inside the fixed-bed column will decrease, and the turbulence of fluid will increase. Thus, the contact area and overall mass transfer coefficient will increase.

## CONCLUSIONS

Flavonoids from *M. mammosa* root were successfully extracted using 70 wt% ethanol as a solvent in the fixed-bed column. Then, the optimization of the extraction process was analyzed by varying the solvent flow rate and particle size of the raw material. The $K_e$ value was calculated to determine the effect of those parameters on the mass transfer process. The results showed that the increase in solvent flow rate and the decrease in particle size could increase the $K_e$ values. The $K_e$ values are in the range of 0.3145 m/s to 0.788 m/s. Their correlation is depicted in the empirical equation $Sh = 1.10 \times 10^{14} \cdot Re^{0.0564} \cdot (1 - \varepsilon)^{0.8718}$, with a 6.13% relative error compared with the experimental data. This equation is valid for solvent flow rates ranging from 3 mL/s to 9 mL/s and particle sizes ranging from 1.275 mm to 4.01 mm.

## ACKNOWLEDGEMENT

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## NOMENCLATURE

### Roman letters

$A$ : cross-sectional area of the fixed-bed column [$cm^2$]

$a$ : interfacial mass transfer area per unit volume of the fixed-bed column [$cm^2/cm^3$]

$a_o$, $a_1$, $b_1$, $b_2$, and $b_3$ : constants

$C_A$ : concentration of flavonoids in solvent [$g/cm^3$]

$C_A^*$ : equilibrium concentration of flavonoids in solvent [$g/cm^3$]

$D_t$ : diameter of the fixed-bed column [cm]

$D_{AB}$ : diffusivity of flavonoids to ethanol solvent [$cm^2/s$]

$D$ : particle diameter [cm]

$K_e$ : overall extraction constant [cm/s]

$L$ : column height [cm]

$T$ : time [s]

$Re$ : Reynolds number = $\frac{\rho.v.d}{\mu}$

$Sc$ : Schmidt number = $\frac{\mu}{D_{AB}.\rho}$

$Sh$ : Sherwood number = $\frac{K_e.d}{D_{AB}}$

$V$ : volume of the stirred tank [$cm^3$]

### Greek letters

$v$ : linear velocity of the solvent [cm/s]

$\varepsilon$ : porosity of the fixed-bed column

$\rho$ : solvent density [$g/cm^3$]

$\mu$ : solvent viscosity [g/cm s]

$\tau$ : residence time of mixed-flow solvent in the stirred tank [s]

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