Molecular encapsulation of bioactive molecules of Ruku-Ruku leaves (*Ocimum tenuiflorum* Linnen) as a preliminary stability study

H Parbuntari*, S B Etika¹, and E Delvia¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Padang Utara, Padang 25132 West Sumatera, Indonesia

*hesty5193@fmipa.unp.ac.id

Abstract. Ruku-ruku (RK) leaves in West Sumatra contain various essential oil which is known as secondary metabolites. They could influence the use of these bioactive properties as medicinal compounds. Essential oils have unstable properties to oxidation, evaporation, or chemical reaction. One method for maintaining compound stability is the encapsulation method. This study performs the encapsulation of RK in β-CD. By using the co-precipitation method, this study produces pure crystal with 62.5% of the efficiency of encapsulation. The diffraction pattern of β-CD: RK identified the crystals pattern with a sharp peak of diffraction and most of these peaks showed patterns of diffraction in β-CD. The absorption intensity of the pure CD was only 2.5 but after inserting the RK in its cavity the intensity increased to 2.7. It shows that there is an interaction between the non-covalent part of β-CD and the essential oil molecules. Furthermore, the inclusion complex β-CD: RK is more stable as the increase in the boiling point of the ICs in the range of 282-289°C.

1. Introduction

Plants with a distinctive aroma are often used to add flavor to food or eliminate fishy smells. Ruku-ruku (*Ocimum tenuiflorum* L.) leaves have a similar morphology to basil leaves which are often used as fresh vegetables. The leaves of ruku-ruku (RK) are usually only used when making fish curry, especially by the people of West Sumatra. The similarity in morphology and the distinctive odor of basil and RK leaves gives the assumption that RK leaves also contain essential oils like basil leaves. The biological activity of basil leaves is an antioxidant, anticancer, antihypertensive, anti-inflammatory, etc [1].

RK is widely available in tropical climates such as West Sumatra. The leaves of RK in West Sumatra are only allowed to grow wild, whereas in other countries many RK leaves are cultivated [2]. People in West Sumatra only use RK leaves to get rid of the fishy smell of fish. The fishy odor can be lost due to the presence of essential oils in the leaves of RK, which are secondary metabolites. Besides, secondary metabolite compounds contained in RK leaves can have biological activities such as antibacterial, antioxidant, antidepressive, etc [3].

The content of secondary metabolites from one region to another can be different. Soil chemistry which includes pH, CEC (Cation Exchange Capacity), and nutrient content can affect soil fertility. The
difference in nutrient content in the soil can affect the production of secondary metabolites [4]. This difference causes a reduction or increase in the biological activity of RK leaves.

The activity of secondary metabolites can also be influenced by the nature of the compound itself. Secondary metabolite compounds are unstable against several factors such as oxidation, evaporation, or chemical interactions. Therefore, in order to maximize the specific function of secondary metabolites, current research focuses on developing methods for maintaining the stability of compounds.

One method to maintain the stability of compounds is the molecular encapsulation method. Encapsulation is the entrapment of important compounds into a hollow compound or matrix so that the properties of essential oils can be maintained [5]. Currently, there are many coating compounds and encapsulation methods used in research. Therefore, the selection of the trapping compound and the appropriate encapsulation method must be taken into account in order to maximize the molecular entrapment process.

A trapping compound that is often used in medicinal research is β-cyclodextrin (β-CD). β-CD is the least soluble but, at the same time, possesses the most suitable cavity size for complex formation with many drugs [6]. In addition, many studies have carried out the synthesis of both precursors and β-CD itself [7]. A fairly simple method for trapping the secondary metabolites of RK leaves is kneading [8]. However, this method can only be used if the inclusions you want to make are only in solid form. Therefore, this study uses a co-precipitation method which is also simple and has high solubility to polar solvents such as water.

2. Experimental

2.1. Materials

The chemicals used in this study were RK leaves, β-cyclodextrin (β-CD) with 99% purity, ethanol (CH$_3$CH$_2$OH) with pro-analysis quality (Merck), n-hexane, magnesium sulfate anhydrous and ethyl acetate (EtOAc) purchased in Sigma Aldrich and Merck. During the preparation process, double deionized water was needed to dissolve the CD.

2.2 Instrumentation

Equipment used for the isolation of bioactive molecules and for the encapsulation of β-CD essential oils are vials, beakers, dropper pipettes, stirring rods, magnetic stirrers, ovens, measuring cups, measuring pipettes, dropper pipettes, thermometers, Buchner funnels, vacuum pumps, spray bottle, spatula, mortar pestle, distillation set, and melting point apparatus.

Equipments used in this research were set of flasks, Erlenmeyer, test tubes, heater stirrer, magnetic stirrer, thermometer, oven, electronic scale, set of pipette volume, vacuum filter, Büchner filter, funnel, spatula, distillation set, and rotary evaporator.

The instrument used in this research are Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared (FT-IR Perkin Elmer Frontier), UltraViolet-Visible (UV-Vis) (Specord 210 Plus) and X-Ray Diffraction (XRD PANalytical X’Pert Pro).

2.3 Procedures

2.3.1 Distillation and Characterization of Essential Oils. The distillation method used in this experiment is a simple distillation method. The leaves of the young RK are cut into small pieces and dried. After that, 15 g of dried RK leaves are added about 50 ml of water and followed by steam distillation for 4-5 hours. The distillate was extracted with n-hexane three times and anhydrous sodium sulfate was added to remove the remaining water, then heated to 68°C. The essential oil is stored in a dark glass vial at 40 C for further analysis. The essential oil obtained in the RK leaves extraction method will be characterized using Gas Chromatography-mass spectrometry (GC-MS).
2.3.2 Preparation of β-CD: RK Inclusion Complex. 1.5 grams of β-CD was dissolved in 60 mL of a mixture of ethanol and deionized water (DW) (20: 80 v/v) at 65°C for 45 minutes. The essential oil from the leaves of ruku-ruku (*Ocimum tenuiflorum* Linnen) was added with a volume of 0.5 mL, 0.75 mL, and 1.0 mL, respectively, into the β-CD solution. The mixture was stirred on a magnetic stirrer with a speed of 1400 rpm for 3 hours at 65°C and left for 1 hour at room temperature. The inclusion complexes are then vacuumed with a vacuum pump until the complexes dry, the inclusion complexes are stored for further characterization.

2.3.3 Characterization of β-CD: RK Inclusion Complex. The inclusion complexes of β-CD: RK were characterized by Fourier Transform Infrared (FT-IR) spectrophotometer, Ultra Violet-Visible (UV-Vis), X-Ray Diffraction (XRD), and melting point test.

2.3.4 Determination of Essential Oil Encapsulation Efficiency. In determining the efficiency of the research procedure encapsulation refers to the research procedure that has been modified according to the previous research, the total essential oil content in the inclusion complex represents the amount of oil included in the β-CD cavity plus the amount of oil adsorbed on the surface of the β-CD molecule. To determine the essential oil content in the encapsulation product (inclusion complex), 0.5 g of the inclusion complex was suspended in 5.0 mL of deionized water (DW) and 15 mL of n-hexane followed by low-speed stirring using a magnetic stirrer. The organic phase containing the free oil is separated. The aqueous phase was extracted with 5 mL n-hexane three times. The extract was dried over anhydrous magnesium sulfate. After the vacuum evaporation of the solvent using a rotary evaporator, the oily residue was weighed and expressed as \(m_1\). To determine the adsorbed oil on the surface of the β-CD molecule, 20 mL n-hexane was added to 0.5 g of the inclusion complex powder and then stirred using a magnetic stirrer at 400 rpm at room temperature for 30 minutes. The mixture is filtered and the powder is washed with 10 mL n-hexane. The organic filtrate is dried over anhydrous magnesium sulfate. Magnesium sulfate was decanted, the final extract was vacuumed using a rotary evaporator and the oily residue was weighed and expressed as \(m_2\).

The Encapsulation Efficiency (% EE) is calculated by the equation:

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

With: \(m_{\text{inclusion complex}} = \frac{m_{\text{total oil}} + m_{\text{surface oil}}}{2}\)

Total oil: the total mass of oil trapped in the β-cyclodextrin cavity
Surface oil: the total mass of oil adsorbed on the β-cyclodextrin surface

3. Results and Discussion
Identification of essential oils using gas chromatography-mass spectrophotometry was used to determine the number of compounds contained in RK in West Sumatra. The essential oil of RK leaves is automatically injected into a column with a temperature of 280°C which has been flowed with helium gas. The essential oil is in the form of gas together with helium gas (mobile phase) through the 30 meters Thermo TG-5MS capillary column. In this column, there is a separation of the components of the compounds contained in the essential oil.

The results of the identification of GC-MS of RK have 3 main components, namely D-Limonene, 6-Octenal, 3,7-Dimethyl- (R), 2, 6-Octadien-1-ol, 3,7-dimethyl. Based on the literature, the *Ocimum tenuiflorum* L. plant has different chemical contents caused by several factors such as soil quality or the environment in which the plant grows [9].

Essential oil is a secondary metabolite found only in certain organisms. The content of secondary metabolites found in the leaves of ruku-ruku in five regions in West Sumatra has different chemical content. By conducting a phytochemical test, it was found that the identification results of flavonoids

3
and steroids or terpenoids were different. The differences in the identification results were caused by several factors, namely temperature, humidity, exposure to light, and salinity [4].

In the process of its formation, the essential oil of RK which is more non-polar will enter the β-CD cavity to replace ethanol that was previously trapped in the β-CD cavity. This occurs because the β-CD cavity is hydrophobic so that if a more non-polar molecule enters the β-CD cavity, the trapped more polar molecule will be replaced by the non-polar molecule. The inclusion complexes formed will be characterized using FT-IR, UV-Vis, XRD, and melting point determination.

**Figure 1.** The FT-IR spectrum (a) β-CD (b) RK 1 mL (c) RK 0.75 mL (d) 0.5 mL

The FT-IR spectrum of the preparation of β-CD-RK using the coprecipitation method has a spectrum almost similar to pure β-CD which is the main character in the process of forming a host-guest inclusion complex. The formation of this inclusion complex can be clearly observed at the wavenumber 2800 cm⁻¹ to 3600 cm⁻¹ in each additional variation of the volume of essential oil, which indicates a shift in the wavenumber of the inclusion complex spectrum towards a higher wavenumber. This indicates that there has been an interaction between β-CD and the essential oil molecule of RK (an inclusion complex has been formed) [10].

The three tables show some increasing and decreasing changes in wave number (Δδ). This increase is due to the insertion of the essential oil molecule of RK into a cavity rich in β-CD and will increase the density of the electron cloud, which will lead to an increase in the frequency of the wavenumber. Whereas the decrease in frequency between the inclusion complex and its constituent molecules is due to changes in the microenvironment that lead to the formation of hydrogen bonds and the presence of Van der Waals forces during interactions to form inclusion complexes.

The UV-Vis spectroscopy of β-CD showed absorption with a maximum absorbance of 2.4976 A. The complexation process causes changes in the absorption spectrum of guest molecules. During the spectral change, the guest molecular chromophore (RK) is transferred from polar to non-polar β-CD. These changes are caused by a disruption in the electronic energy levels of the guest molecule (RK) caused by direct interaction with β-cyclodextrins.

The UV-Vis results showed an increase in the absorptivity of each inclusion complex that was produced. The interaction between guest molecules (RK) and β-CD molecules plays an important role in the explanation of this phenomenon. In the β-CD cavity, the guest molecules will form various types of interactions with β-CD molecules, such as hydrogen bonds, dipole-dipole interactions, dispersion forces, which will make different contributions to the conformational variations of the guest molecules and immobilization of guests.
The results of the characterization of the inclusion complex using UV-Vis spectroscopy were observed to have increased and decreased absorbance. An increase in absorbance occurred at 1 mL inclusion complexes and a decrease occurred at 0.5 mL inclusion complexes. This difference can be considered as evidence of an interaction between β-CD and guest molecules in the process of forming inclusion complexes. In this process the hydrogen bonds formed can be considered as the main force in the formation of inclusion complexes, because the hydrogen bonds can reduce the energy of the 'n' orbitals so that a hypochromic shift or a blue shift can be observed. In this hypochromic, the increasing polarity of the solvent at the peak shape transition will shift to a shorter wavelength, this is due to the increase in the solvation of the electron pair so that the energy decreases.

When compared the diffraction patterns between the three inclusion complexes, there was a decrease in the intensity of each inclusion complex. The intensities of the 0.5 mL inclusion complexes were generally higher than the 0.75 mL and 1.0 mL inclusion complexes. These results prove that the
more ruku-ruku leaf essential oil is added, the lower the intensity of the inclusion complex and the degree of crystallinity of an inclusion complex.

The melting point test of the inclusion complex is one of the stability tests of RK. The use of β-CD as an essential oil coating material can increase the stability of RK, both from volatility, oxidation, dehydration, hydrolysis, and thermal decomposition because what is formed is a solid or crystalline inclusion complex that makes the melting point of the β-CD: RK is almost like the melting point of pure β-cyclodextrin, namely (> 290°C).

Table 1. The difference of melting points of RK: β-CD

| Volume of RK | The melting point |
|--------------|------------------|
| 0.5 mL       | 289°C            |
| 0.75 mL      | 287°C            |
| 1 mL         | 282°C            |

The difference in melting points of the three inclusion complexes is related to the number of molecules of RK trapped in the β-CD cavity. The melting point of the inclusion complex of 1.0 mL was lower than that of the inclusion complex of 0.75 mL and 0.5 mL, this indicates that the number of ruku-ruku leaves essential oil molecules was trapped more than the inclusion complexes of 0.75 mL and 0.5 mL and also this melting point value is also influenced by the bonding during the formation of the inclusion complex. This result can be proven by data on encapsulation efficiency and also data contained in the FT-IR spectrum, which shows a significant shift in the wavenumber when compared to the β-cyclodextrin wavenumber.

The difference in melting points of the three inclusion complexes is also due to the presence of hydrogen bonds, Van der Waals forces, covalent bonds between β-CD and RK. The endothermic effect in all three variations is related to the loss of the hydroxyl groups in the β-CD: RK obtained as melting points. The type of change in the thermal behavior of the inclusion complex indicates a possible interaction between β-CD and RK leading to the formation of a more stable inclusion complex.

Table 2. The effect of volume RK in encapsulation efficiency

| Volume (mL) | Total Oil (mg) | Surface Oil (mg) | Encapsulation efficiency (%) |
|-------------|----------------|------------------|-----------------------------|
| 0.5         | 8              | 3                | 62.5                        |
| 0.75        | 9              | 5                | 50                          |
| 1           | 15             | 13               | 25                          |

Based on the data on the encapsulation efficiency, there was an increase in the mass of essential oils trapped in the β-CD cavity (Total oil) and an increase in the mass of essential oil which was still adsorbed on the surface of the β-CD cavity (Surface oil). This will result in a decrease in encapsulation efficiency with an increasing volume of RK. These results may be related to the physicochemical properties of β-CD (cavity diameter, inheritance) and guest (geometry, volume, hydrophobic) of RK which plays an important role in the formation of inclusion complexes.

4. Conclusion
The highest number of encapsulation efficiency is 62.5% when adding 0.5 grams of RK. The presence is confirmed by instruments such as UV VIS, FTIR, XRD, and melting point test. The stability of RK increases after encapsulation which is indicated by the formation of the crystalline phase from XRD result and the increase in the melting point of the ICs.
Acknowledgement
This work was supported by a grant from Universitas Negeri Padang, Indonesia.

References
[1] R. P., E. E., E. M. C and K. R., "A review on Krishna Tulsi, Ocimum tenuiflorum Linn," International Journal of Research in Ayurveda and Pharmacy, vol. 3, no. 2, pp. 291-293, 2019.
[2] A. S. Deshmukh, G. B. Deshmukh and P. D. Shirole, "Ocimum Sanctum: A medicinal Gift From Nature," International Journal of Pharmacognosy, vol. 2, no. 12, pp. 550-559, 2015.
[3] S. S. Pingale, N. P. Firke and A. G. Markandeya, "Therapeutic activities of Ocimum tenuiflorum accounted in last decade: A review," Journal of Pharmacy Research, vol. 5, no. 4, pp. 2215-2220, 2012.
[4] H. Parbuntari, S. B. Etika, M. Mulia and E. Delvia, "A preliminary screening of the difference of secondary metabolites Ruku-Ruku Leaves (Ocimum tenuiflorum Linnen) in West Sumatra," Eksakta: Berkala Ilmiah Bidang MIPA, vol. 20, no. 02, pp. 17-24, 2019.
[5] S. S. Sagiri, A. Anis and K. Pal, "Review on encapsulation of vegetable oils: strategies, preparation methods, and applications.," Polymer-plastics technology and engineering, vol. 55, no. 3, pp. 291-311, 2016.
[6] P. Saokham, C. Muankew and P. Jansook, "Solubility of cyclodextrins and drug/ cyclodextrin complexes," Molecules, vol. 23, no. 1161, pp. 1-15, 2018.
[7] H. Parbuntari, N. Sakairi, B. Purwono and R. T. Swasono, "Synthesis and characterisation of a partially methylated dodecyl thiomaltotrioside derivative as a precursor of cyclodextrin analogue," in IOP Conf. Series: Journal of Physics: Conf. Series, Padang, 2018.
[8] F. Setiawan, S. B. Etika and H. Parbuntari, "Pengaruh Waktu Kneading Terhadap Efektifitas Enkapsulasi Molekul Minyak Kemenyan pada β-Siklodekstrin (β-CD)," MENARA Ilmu, vol. XIII, no. 2, pp. 178-185, 2019.
[9] B. Hérault, B. Bachelot, L. Poorter, V. Rossi, F. Bongers, J. Chave, C. E. T. Paine, F. Wagner and C. Baraloto, "Functional traits shape ontogenetic growth trajectories of rain forest tree species," Journal of Ecology, vol. 99, no. 6, pp. 1431-1440, 2011.
[10] M. Kfoury, D. Landy and S. Fourmentin, "Characterization of Cyclodextrin/Volatile Inclusion Complexes: A Review," Molecules, vol. 23, 2018.