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RESEARCH ARTICLE

Microencapsulation of *Jeringau Rhizome* essential oils (*Acorus calamus* L.) using β-Cyclodextrin

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Abstract

Background: The way to improve the stability of *jeringau rhizome* essential oils is microencapsulation using β-cyclodextrin. **Aim**: To determine the efficiency of coating the jeringau rhizome essential oil with β-cyclodextrin and examine its thermostability. **Method:** The microencapsulation method used was freeze-drying with a ratio of 1:20 and 1:30. **Results:** The microcapsule efficiency at the ratio of 1:20 and 1:30 was 81.67% and 60.70%, respectively. The thermostability test results showed that the degradation constant of 1:20 microcapsule at 50°C and ambient temperature was 0.0054 and 0.0029, respectively, with a half-life of 128.33 hours and 238.97 hours. Meanwhile, the degradation constant of 1:30 microcapsule was 0.0182 and 0.0080, with a half-life of 38.07 hours and 86.63 hours. **Conclusion:** The highest efficiency is in the ratio of 1:20 with a percentage of 81.67%. In the thermostability test, the 1:20 microcapsule was better protected and had a longer half-life than the 1:30 microcapsule.

Introduction

Indonesia is a tropical country with the highest biodiversity in the world (Murdopo, 2014), most of which are medicinal plants. There are 30,000 medicinal plants grown in Indonesia, but only 1,200 have been used as raw materials for herbal medicines (Salim & Munadi, 2017). The jeringau plant (*Acorus calamus* L.) is one of the medicinal plants that can be used in herbal medicine. It has many properties, and its rhizome is rich in essential oils.

The *Jeringau rhizome* essential oil contains active ingredients β-asarone (82%), colamenole (5%), colamen (4%), colameone (1%), methyl eugenol (1%), and eugenol (1%) (Kementan, 2012). The essential oil of *Jeringau rhizome* produces pharmacological effects and is used as a raw material for the cosmetic, food, and pharmaceutical industries. Huge benefits of *Jeringau rhizome* essential oil indicate that the *Jeringau rhizome* essential oil has the potential to be developed as a raw material with high selling power. However, this oil has not been entirely utilised because it has shortcomings, such as easy evaporation at room temperature, easy oxidisation, insolubility in water, and instability to environmental influences of oxygen, sunlight, and heat (Capelezzo *et al*., 2018).

The method used to improve the stability of essential oils is microencapsulation. Microencapsulation is a technique in confining a material using a particular coating material to protect the core material. The objective of microencapsulation is to protect the core material from environmental influences, improve the physicochemical properties of the core material, and maintain the stability of the core material in storage. The polymer used as a coating was β-cyclodextrin. Microencapsulation in β-cyclodextrins is an effective method for protecting active compounds against oxidation, heat degradation, and evaporation (Mahmudah, 2015).

According to Martin and authors in 2010, the best microencapsulation method of essential oil is freeze-drying. Freeze-drying is a method for volatile materials

due to the lower operating temperature, slow drying rate, and vacuum use (Martin *et al*., 2010). Microencapsulation prevents fungal and bacterial contamination of the core material, which is protected by the capsule wall; it also preserves flavour more and increases the added value of spices (Champagne & Fustier, 2007).

This study focused on the microencapsulation of jeringau rhizome essential oil using β-cyclodextrin coating. It is based on the previous research conducted by Cakrawati and the authors in 2018, where limonene was microencapsulated by the freeze-drying method using the β-cyclodextrin coating. Limonene microencapsulation helps mask the bitterness of the bioactive compound and protects against damage caused by oxygen, heat, or light. The bioavailability of bioactive compounds and organoleptic characteristics in microencapsulated products by freeze-drying is better due to the minimum use of heating. The results revealed that microencapsulation with a ratio of 1:20 had an efficiency of 80.52% (Cakrawati *et al*., 2018). A study by Ponce and the authors in 2010 indicated that the inclusion of complex thymol-β-cyclodextrin and cinnamaldehyde-β-cyclodextrin remained stable up to 75% during long storage time (Ponce Cevallos *et al*., 2010). Microencapsulation of the *Jeringau rhizome* essential oil with the β-cyclodextrin coating is expected to increase the development and use of Indonesian spices as a higher quality raw material.

Method

Essential oil distillation

The distillation was performed by steam and water method. A total of 2,000 g of the *Jeringau rhizome* was placed into a kettle/distillation pan filled with water. The distillation process was completed for four hours at a temperature of ± 100°C. The essential oil obtained was stored in a tightly closed bottle and protected from light.

Microencapsulation of the Jeringau rhizome essential oil

The microencapsulation method used was freeze-drying. The microencapsulation of *jeringau rhizome* essential oil using β-cyclodextrins was prepared in a ratio of 1:20 and 1:30. The microencapsulation was prepared by mixing ten grams β-cyclodextrin with 100 mL 70% ethanol using a magnetic stirrer at a speed of 500 rpm and a temperature of 40°C for 15 minutes. The mixture was taken as much as 20g and 30g. In each comparison, one gram of the *Jeringau rhizome* essential oil was added and continued stirring for four hours. Then, it was dried using a freeze dryer at a temperature of -80°C for 24 hours. The obtained microcapsules were weighed and stored in a brown vial bottle (Cakrawati *et al*., 2018). The results of microencapsulation and β-cyclodextrin were characterized using the Scanning Electron Microscope Hitachi TM-3000.

Determination of the efficiency of Jeringau Rhizome essential oil

The first step applied to determine the efficiency of the *Jeringau rhizome* essential oil microencapsulation was to make a standard curve using a UV-Vis spectrophotometer at a wavelength of 245 nm. The second step was to analyse the content of total oil in the microcapsule, i.e., the oil contained in the coating and on the surface of the microcapsule (Jayanudin *et al*., 2017). A total of 20 mg of 1:20 and 1:30 microcapsules were dissolved in 70% ethanol. Moreover, the absorbance was measured using a UV Vis spectrophotometer at a wavelength of 245 (Masrukan & Santoso, 2019). The third step was to analyse the surface oil in microcapsules. Surface oil is the oil on the surface of the microcapsule; its amount affects the efficiency value of the microcapsule. The more the surface oil, the less the microcapsule efficiency (Jayanudin *et al*., 2017). A total of 20 mg microencapsulated essential oil of Jeringau rhizome was dissolved in 5 mL of n-hexane. Then, it was shaken and filtered using filter paper. The absorbance of microcapsule filtrate was measured using a UV-Vis spectrophotometer at a wavelength of 245 nm (Masrukan & Santoso, 2019). The efficiency was calculated from the difference between the total essential oil content and the microencapsulated content surface oil. The efficiency of the microencapsulate was calculated by the formula (Handayani *et al*., 2018):

$$\% \text{ Efficiency} = \frac{\textit{Total Oil-Surface Oil}}{\textit{Total Oil}} \times 100\%$$

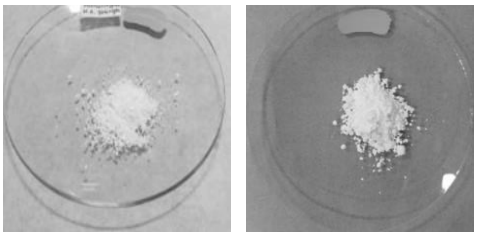
Microcapsule thermostability test

In each ratio, as much as 20 mg of microcapsule was put in 5 closed glass brown vial bottles. The thermostability test was administered at 50°C and ambient temperature. The absorbance measurements of essential oils were performed at 0, 24, 48, 72, and 96 hours with a concentration of 200 ppm, and the absorbance was measured at a wavelength of 254 nm (Wanda *et al*., 2017).

Result

Simplicia of the *Jeringau rhizome* was 2,000 g and yielded 12.5 mL of essential oil (6250%). The results of *Jeringau rhizome* essential oils microencapsulation using the freeze-drying method are presented in Figure 1. The

physical appearance of the 1:20 and 1:30 microcapsules is a white powder with a distinctive aromatic smell like the aroma of the essential oil. The 1:30 microcapsule powder has finer particles than the 1:20 microcapsule. The image of β-cyclodextrin SEM results (Figure 2) shows that the particles are irregular rectangular, with rough surface area and uneven size. It shows the morphological differences in shapes: β-cyclodextrin particles are much larger than the microcapsule particles.



(a) (b)

Figure 1: Microencapsulate 1:20 (a) and Microencapsulate 1:30 (b)

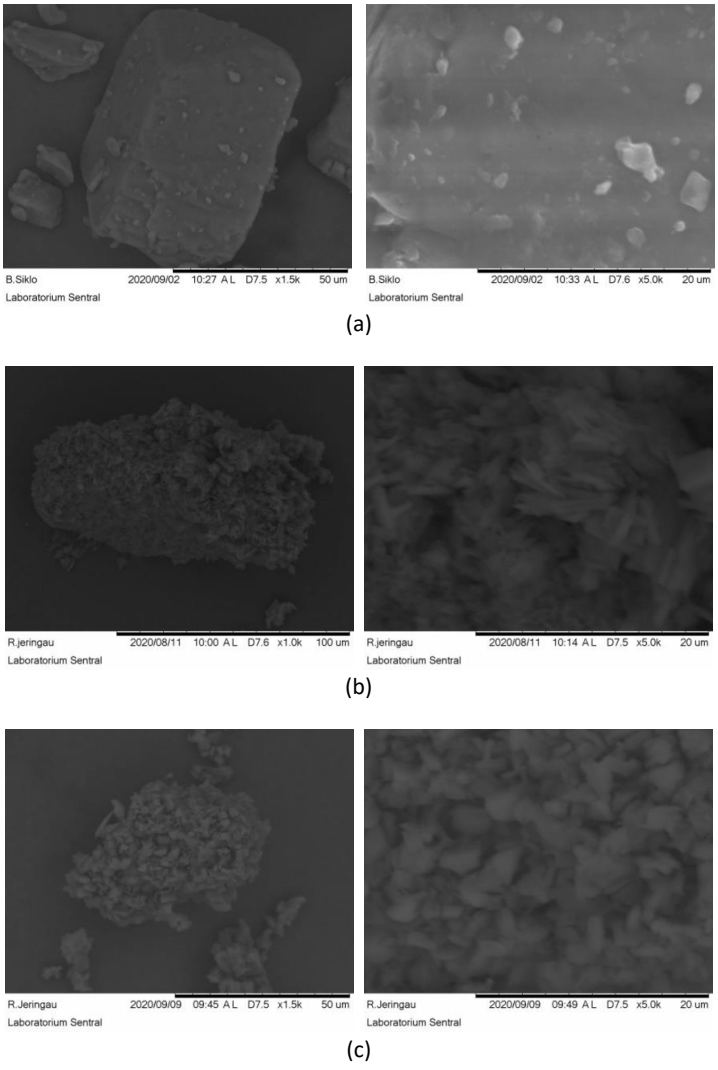


Figure 2: β-cyclodextrin (a), Microencapsulate 1:20 (b), Microencapsulate 1:30 (c)

The determination of *Jeringau rhizome* essential oil efficiency was performed to determine the percentage of essential oil in microcapsules. The result of the manufacture calibration curve showed a linear relationship between concentration and absorbance coefficient r^2=0.9993 and the linear regression

equation y = 0.0378x + 0.0204. Then, the absorbance value was checked at a wavelength of 245 nm to determine the concentration of total oil and surface oil for 1:20 and 1:30 microcapsules. The absorbance value obtained was plotted on the calibration curve until the concentration of total oil and surface oil in the microcapsule was obtained. The result of the microcapsule efficiency is shown in Table I.

Table I: Efficiency *Jeringau Rhizome* essential oils

Ratio	Total microencap-sulate (mg)	Total oil (mg)	Surface oil (mg)	Efficiency (%)
1:20	400.2	138.83	25.44	81.67
1:30	1371.6	99.53	39.12	60.70

The purpose of the thermostability test is to determine the degradation constant and half-life on the effect of 50°C and ambient temperature. This test was performed on microencapsulated and non-microencapsulated essential oils. The thermostability test results can be seen in Table II.

Table II: Results of degradation constants and half-life at 50°C and ambient temperature

Ratio	Degradation constants		$t_{½}$ (half-life)	
	50°C	Ambient	50°C	Ambient
Microencapsu-late 1:20	0.0054	0.0029	128.33 hours	238.97 hours
Microencapsu-late 1:30	0.0182	0.0080	38.08 hours	86.63 hours

Discussion

In this study, the essential oil yield of *Jeringau rhizome* was 0.63%, slightly different from previous findings, where Raina and the authors obtained 0.9% (Raina *et al*., 2003). The low yield of *Jeringau rhizome* essential oil in this study was due to several factors, including the place of growth, treatment or sample conditions, climate, light intensity, type of plant, and, most importantly, the distillation tool. If the device has a steam leak, the essential oil evaporates, thereby reducing its yield value. The *Jeringau rhizome* essential oil was brownish-yellow and had a distinctive odour according to the producing plants. In 2017, Rita and the authors stated that the physical appearance of the *Jeringau rhizome* essential oil in their research was also brownish-yellow and had a very sharp aroma (Rita *et al*., 2017).

The microencapsulation of *Jeringau rhizome* essential oils was performed using the freeze-drying method. This activity consists of two steps, i.e., the homogenisation process and freeze-drying. The homogenisation process was applied using two variations in the ratio of the coating material, i.e., β-cyclodextrin: 1:20 and 1:30. This coating could form inclusion complexes by introducing more hydrophobic compounds into the central cavity of the cyclodextrin molecule. In the process of forming the inclusion complex, there is an interaction between the functional groups of the essential oil compounds and the groups located in the cavities in cyclodextrin (Bestari, 2014).

During the homogenisation process, the stirring speed was 500 rpm, referring to the research of Pujiastuti and the authors in 2017, which explained that the stirring speed affects the particles' size. The greater the speed, the smaller the resulting particle size will be (Pujiastuti et al., 2017). However, Sirojuddin and the authors in 2015 argued that increasing the stirring speed increases the strength and frequency of collisions between particles, causing the breakdown of the coating material and the release of the core substance into the solvent (Sirojuddin et al., 2015). The second step was freeze-drying. It began with a freezing process and was continued with a drying process by sublimation. This mechanism is different from the usual drying process. Drying usually occurs through evaporation at high temperatures so that the dry part of the product forms a crust on the surface, which creates an obstacle for the diffusion of steam from the wet part to the environmental air. As a result of the normal drying process, the product has a dry crust outside and a wet centre. Freeze-drying is a sublimation mechanism at cold temperatures. Water vapour diffuses from the wet parts into the ambient air, forming a product that dries well and has finer particles (Hariyadi, 2013).

The results of the freeze-drying microencapsulation show that the 1:30 microcapsule powder has finer particles than the 1:20 microcapsules, consistent with the nature of β-cyclodextrins which can form hydrogen bonds with the surrounding –OH groups (Bestari, 2014). The more coating material was used, the more water molecules were bound, resulting in a powder with finer particles.

Li and the authors and Cakrawati and the authors stated that the particle morphology of the 1:20 and 1:30 microcapsules was irregular because of thermal expansion during drying and thermal stress (Cakrawati *et al*., 2018; Li *et al*., 2018). The morphological differences in the shape show that β-cyclodextrin particles are much larger than the microcapsule particles (Rakmai *et al*., 2018). The formation of

inclusion complexes between β-cyclodextrin and essential oils produces smaller and relatively finer microcapsule particles (Li *et al*., 2018). The change in particle morphology during the encapsulation process reveals the interaction between β-cyclodextrin and essential oils (Cakrawati *et al*., 2018).

The result of the microcapsule efficiency shows that the highest value was 81.67% at a ratio of 1:20, where the β-cyclodextrin coating in the *Jeringau rhizome* essential oil almost reached its optimum point. Asyhari explained that the greater the β-cyclodextrin concentration, the smaller the percentage of compounds absorbed, and the larger the microcapsule wall thickness, making water molecules easier to diffuse through the coating molecule and decreasing microcapsules efficiency (Asyhari, 2013). Table II shows that the higher the essential oil on the surface, the lower the efficiency obtained. The concentration of essential oils on the surface is helpful to see how much essential oil has been covered (Handayani *et al*., 2018). The purpose of microencapsulation is to protect the core material from evaporation and damage. It indicates that the uncovered essential oil will be more easily degraded, evaporated, and oxidised, which reduces its quality.

Thermostability test results demonstrate that the increase in temperature at 50°C resulted in a decrease in the absorbance of the *Jeringau rhizome* essential oil microencapsulate. The pattern of each temperature decreasing absorbance had a difference until the 96th hour, indicating that the increase in temperature causes a higher amount of degraded essential oil. While at ambient temperature, the absorbance value of encapsulated *Jeringau rhizome* essential oil could be read until the 72nd hour, it was not readable at a temperature of 50°C at 48 to 96 hours, indicating that terpenoid compounds in essential oils are not resistant to high-temperature heating. The storage of materials in the open air at high enough temperatures may cause physical and chemical changes to essential oils. One of the changes in the chemical properties of essential oil is through the oxidation process. The oxidation reaction in essential oils mainly occurs in the double bonds in terpenes. The thermolabile terpenoid compound will isomerize. The isomers can change the shape of the configuration or break the double bonds in terpenoid compounds, causing the absorption of light by the chromophore groups to decrease; hence, the absorbance value decreases further. The degradation constant is directly proportional to temperature. The increase in *k* value with increasing temperatures means a faster degradation rate of the essential oil (Sirojuddin *et al*., 2015). The storage temperature of 50°C is associated with the smallest degradation constant value compared to ambient temperature.

Based on Table II, the degradation constant of the essential oil 1:20 is smaller than the degradation constant of 1:30. It proves that the greater the percentage of essential oil absorption efficiency, the smaller the degradation constant value, and the longer the half-life of the essential oil. This result is in accordance with the findings of Mahmudah, showing that microencapsulation with β-cyclodextrins is an effective method to protect active compounds against heat degradation and evaporation (Mahmudah, 2015). The β-cyclodextrin coating used in the microencapsulation of essential oils forms a molecular inclusion complex between the essential oil and the β-cyclodextrin cavity. The inclusion complex can protect the essential oil during a stable storage period at low temperatures (Martin *et al*., 2010).

Further research is necessary for selecting other methods to refine the essential oil (to yield higher quantities), characterise it in microcapsules (mass spectrophotometry, differential scanning calorimetry, or x-ray diffraction), and test its stability (photostability test).

Conclusion

Some conclusions can be drawn from this study. The 1:20 and 1:30 microcapsule efficiency were 81.67% and 60.70%, respectively. The physical appearance of the 1:20 and 1:30 microcapsules is a white powder with a distinctive aromatic smell like the aroma of its essential oil. In the image of β-cyclodextrin SEM results, it is found that the particles are irregular rectangular, with rough surface area and uneven size. The thermostability test result of the *Jeringau rhizome* essential oil microcapsule for the 96th hours indicates that degradation constants of essential oil in the microcapsule 1:20 at a storage temperature of 50°C and ambient temperature were 0.0054 and 0.0029, and a half-life of 128.33 hours and 238.97 hours, respectively, while for the 1:30 microcapsule degradation constants were 0.0182 and 0.0080, and half-lives of 38.07 hours and 86.63 hours, respectively.

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