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Antifungal activity of microcapsule propolis from *Tetragonula spp.* to *Candida albicans*

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Abstract

Propolis is a mixture of resin and saliva of *Tetragonula spp*. that have an antifungal activity. The purpose of this study was to develop spray-dried microcapsule propolis (SDMP) and to analyze its antifungal activity to *Candida albicans*. The SDMP was obtained using maltodextrin and gum Arabic coating with a spray drying method. The antifungal activity of SDMP of rough propolis (taken from the outside beehive) and smooth propolis (taken from the inside beehive) was analyzed. The macroscopic characterization showed that SDMP had a brownish-yellow powder form and a spherical uniform particle with the size of 9.32 - 14.61 µm. The encapsulation efficiency of SDMP of smooth and rough type was 81.22% and 83.04%; moisture content of 5.58% and 5.84%; water solubility of 98.19% and 98.31%, respectively. The diameter of microbial inhibitory to *C. albicans* was in the range of 6.33±1.5 to 10±2.5 mm. SDMP displayed remarkable activity in the assays against *C. albicans.*

Keywords: Candida albicans; microencapsulation, propolis; spray drying

1. Introduction

Candidiasis is one of the most common fungal infections which are caused by *Candida sp.* Candidiasis infection is one of the existing diseases in Indonesia ranging from superficial (oral cavity, esophagus, intestine, vagina, and other epidermal and mucosal surfaces) to deep infection (brain, eye, kidney, liver, heart, and other major organ tissues) [1]. Here propolis is known to have antifungal properties to *Candida sp* [1].

Propolis is a mixture of resin and bee saliva collected by honeybees from plant buds, leaves, and stems with various physical properties [2]. It has various chemical constituents affected by location, vegetation, and time [3] with the main ingredients of flavonoids, tannins, and phenolic acids [4]. Indonesian propolis genereally has a number of pharmacological properties as the antioxidant, anti-inflammatory, antibacterial, anticancer, anti-angiogenic, xanthine oxidase inhibitory, and antifungal [5–9].

Observations performed by Sahlan reported the ability of ethanol extract of Indonesian propolis (EEP) to inhibit the growth of *Candida albicans*. Thus, propolis is potential to be developed into a pharmaceutical preparation for treating candidiasis [9]. Indonesian propolis was tested with LC-MS/MS methods, which resulted in the founding of three anti candidiasis compounds: adhyperforin, kurarinone, and deoxypodophyllotoxin [9]. The chemical compounds contained in Indonesian propolis differ from other propolis in accordance with its vegetation source; the bee that produces it, and its geographic origin environment, also differentiating its antifungal potential.

However, the application of propolis in the manufacture of pharmaceutical preparations is still limited in view of some characteristic properties of propolis including its low solubility in water, having sticky thick liquid, gummy, and blackish-brown form with strong taste and aroma [10]. Propolis microencapsulation using the spray drying method could be employed to overcome propolis handling properties' problematic obstacles. The microencapsulation with spray drying is not only low cost but also effective method to turn suspensions into the powdered microparticles, which comprise one or more wall materials and a core. The microencapsulation process protects the bioactive compounds from any adverse environmental conditions [8].

The purpose of this study was to develop spray-dried microcapsule propolis (SDMP) and to analyze its antifungal activity to *Candida albicans.* The determination of Indonesian propolis, specifically from *Tetragonula spp*. bee as an antifungal agent, revealed its ability as candidiasis drug.

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2. Materials and Methods

2.1. Samples

Ethanol extract propolis (EEP) was obtained from the RIN Biotek Indonesia Company. It was taken from North Luwu - South Sulawesi. There were two types of propolis: rough propolis (taken from the outside beehive) and smooth propolis (taken from the inside beehive).

2.2. Microencapsulation of Propolis by Spray Drying

Rough and smooth EEP was extracted from *Tetragonula spp* beehive with a method as described by Sahlan et al. [11]. The encapsulant material was prepared from maltodextrin (MD) and gum arabic (GA) 10:1 as described by DK Pratami et al. [12]. Maltodextrin DE 18 (dextrose equivalent) and gum arabic were purchased from Bratachem Co. (Jakarta, Indonesia). The ratio between the coating material and EEP was 1:1. The coating material solution was prepared by stirring 10 g of MD, 1.0 g of GA in 100 mL of distilled water at 6000 rpm for 30 minutes. Then the coating material solution was homogenized by an Ultra-Turrax T18 (IKA, Königswinter, Germany) for two minutes at 15 000 rpm. Then, the 300 ml of EEP (37.067 mg/mL solids) was added gradually and homogenized in the Ultra-Turrax T18 fortwo minutes more. The mini spray dryer (Büchi B290, Flawil, Switzerland) was employed to the obtained SDMP. The operational conditions of the spray dryer included nozzle diameter of 1.5 mm; aspirator 100%; flow rate at 8 mL/min; spray gas of 600 L/min, inlet temperature at 110ºC; and outlet temperatures between 65ºC and 73ºC.

2.3. Physical Characterization of SDMP

One gram of each SDMP sample was determined for the moisture content (MC) measurement by moisture balance analyzer AMB (Adam, USA) at the temperature of 105°C. The solubility of SMDP in water was determined based upon the gravimetric analysis method. For that, one gram of SDMP was added in 100 mL distilled water under magnetic stirring for 5 minutes. The solution was filtered through a filter paper weighed on an analytical balance before used. After filtration, filter paper and residue were dried in the oven for 1 hour at the temperature of 105 °C. The solubility of SDMP in water (%) was calculated by using the following Eq. (1).

$$\%\,Solubility = \left[1 - \left(\frac{P_c - P_b}{\frac{100 - \%MC \times P_a}{100}}\right)\right] \times 100\% \quad (1)$$

where Pc (g) refers to the weight of the filter paper plus residue after dried; Pb (g) is the weight of the filter paper weighed before used; Pa (g) is the weight of the SDMP; and MC (%) is the moisture content of the SDMP.

The morphology of SDMP was observed by scanning electron microscope (JSM6400; JEOL, Tokyo, Japan) at the Fire and Safety Engineering Laboratory, Faculty of Engineering, State Jakarta University. SEM analyses were performed at room temperature, and all samples were coated with a layer of gold in a vacuum before observing microscopy. The size of SDMP was determined by Beckman Coulter Delsa Nano Particle Size Analyzer (PSA) in Physics Research Institute LIPI, Serpong.

2.4. Chemical Characterization of SDMP

The chemical quantification of SDMP was determined as total phenolic content (TPC) and total flavonoid content (TFC) described by Diva et al. with modification [13]. Folin-Ciocalteau method with gallic acid as its standard was used to estimate the TPC. Total phenolic levels were estimated by measuring gallic acid and described in mg GAE/g extract content. This method was based on the color change of Folin-Ciocalteau reagent that was reduced by sodium carbonate in the presence of phenolic substances. The absorbances of gallic acid with concentrations of 0 to 300 µg/mL were used to create the standard calibration curve. 50 mg of SDMP was weighed and dissolved in methanol to 200 µg/mL concentration, where this content was stirred using a vortex for 5 minutes. Then, a 5 mL Folin-Ciocalteau reagent and 0.5 mL of diluted samples were mixed in a tube. After leaving the tube in a dark environment for 5 minutes, 4 mL sodium carbonate solution was added into the tube. The absorbance was measured with spectrophotometer UV-Vis at 745 nm only after 15 minutes of reaction time in a dark room.

The determination of TFC was carried out using the $AlCl_3$ method with quercetin as its standard. Total flavonoids levels were estimated by measuring quercetin and described in mg QE/g extract content. The absorbances of quercetin with the concentrations 0 to 200 µg/mL were used to create the regression linear calibration. The 50 mg SDMP was made diluted with methanol to a concentration of 200 µg/mL. The sample solution was taken 0.5 mL into the test tube, and then was added with 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$ solution, 2.8 mL aquadest, and 0.1 mL KCH_3COO 1 M. The result of the mixture was vortexed and let standing for 30 minutes. The absorbance subsequently was measured at λ 415 nm with spectrophotometer UV-Vis.

2.5. Microencapsulation Yields (MY) and Efficiency (ME)

The MY was determinated by calculating the mass lost during the spray drying process. The MY (%) was calculated as the ratio of the SDMP collected after the spray drying experiment to the total initial amount of EEP and encapsulant. The MY (%) was calculated using Eq. (2).

$$MY = \frac{\text{Mass of the SDMP after drying}}{\text{Theoretical Mass}} \times 100 \quad (2)$$

The microencapsulation efficiency (ME) was performed to determine the ability of maltodextrins – gum arab coating of active compounds from propolis extract. The ME (%) process was calculated based on Eq. (3).

$$ME = \frac{A - B}{A} \times 100 \quad (3)$$

where, A is the total phenolics added initially to the solution entering the spray dryer (mg GAE/g) and B is the total phenolics unencapsulated in the SDMP (mg GAE/g).

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

The sample was SDMP, propolis powder without encapsulation, and the encapsulant (maltodextrin - arabic gum). Samples were dropped onto a thin layer of KBr for 1 drop, and characterized using an FTIR analyzer Shimadzu IR Prestige21/FTIR-8400S. FTIR spectra were read on infrared waves from 4000 to 500 cm^{-1}. Tests were carried out at the Laboratory of the Department of Chemical Engineering, Universitas Indonesia.

2.7. Antifungal Assays

The stock ATCC culture *C. albicans* was obtained from the Department of Parasitology, Faculty of Medicine, Universitas Indonesia. The stock culture used was in a minimum age of 48 hours. The test was done with the disc diffusion method as described by Silici et al. and Pereira et al. with slight modifications [14,15]. The diffusion method using paper discs with a diameter of 6 mm. All tools, before being used were sterilized in the autoclave for 15 minutes at 121°C. The *C. albicans* inoculum was prepared to the Muller Hinton medium for later inoculation in the petri dish. The preparation was done to obtain McFarland 0.5 standard turbidity equal to the concentration of 1.5 x 10^8 / ml cell density. The prepared *C. albicans* colonies were then transferred using the counter strike method to its medium. Paper discs that have been immersed in a sample solution for 3 minutes were placed on the surface of the agar media that has been inoculated with the test fungus using sterile tweezers, and then incubated for 48 hour at room temperature in a reverse manner. Fluconazole and nystatin were used as a positive control. Blank discs were later soaked in SDMP at 3 different concentrations: 1; 3; and 5% (%w/v) before being used. After 48 hours of incubation at room temperature, the diameter of microbial inhibition was measured using a vernier caliper.

3. Results and Discussion

3.1. The Physical Characterization of SDMP

In this research, the SDMP was obtained by the spray drying process using maltodextrin-arabic gum as an encapsulant. The liquid EEP could conserve into a powder with a very homogenous small size. The macroscopic of SDMP was in the form of a brownish-yellow powder with a distinctive smell of propolis, and bitter taste (Fig. 1).

The MY of spray-dried EEP without microencapsulation (Fig. 1a) was only 22% indicating 78% lost during the spray dry process, and stuck to the spray drying chamber glassware. The SDMP from rough and smooth propolis type is shown in Fig. 1c and Fig. 1d. MY value was 67.27± 6.75% and 67.72% ± 6.75%, respectively. From the MY value, it can be seen that microencapsulation could improve the yield of the spray drying process. The MY of SDMP microcapsules had a good value. It valued higher than SDMP as obtained by Marquiafável et al. using gum Arabic - silicon dioxide (1:1) encapsulant, resulted in MY between 31.85 and 67.60% [16].

Fig.1. Spray drying powder results: (a) Propolis without encapsulation; (b) maltodextrin-gum Arabic; (c) SDMP rough type; and (d) SDMP smooth type

In this research, as shown in Table 1 the moisture content of SDMP for both smooth and rough type was 5.84% and 5.58% the water solubility was 98.19% and 98.31%, respectively.

Table 1. The characterization of SDMP

Parameters	Spray-dried EEP without encapsulation	SDMP smooth type	SDMP rough type
MY (%)	22.00 ± 6.75	67.72% ± 6.75	67.27 ± 6.75
Moisture content (%)	2.04 ± 6.75	5.84 ± 0.01	5.58 ± 0.05
Water solubility (%)	74.01 ± 0.02	98.19 ± 0.02	98.31± 0.02
Particle size (µm)	-	9.32 µm	14.61 µm

Note: The data was given in mean + SD, n = 3 experiment. EEP, ethanolic extract propolis, MY, microencapsulation yield; and SDMP, spray drying microcapsule propolis

The results of this study showed that the solubility of SDMP in the water was affected by maltodextrin in which when more maltodextrins were added, the solubility of propolis powders was higher and the content of moisture came to be lower [12]. Maltodextrin successfully increased the solubility and decreased the moisture content of propolis. The problem of low solubility in water was solved by microencapsulation. Some studies have explored the use of maltodextrin with dextrose equivalent (DE) value ranging from 10 to 20 to protect bioactive compounds, such as phenolic compounds [8]. The microencapsulation using the spray drying method could be employed to overcome propolis handling properties' problematic obstacles.

The microencapsulation could increase the value of solubility in water, the moisture content, and MY. The higher encapsulant concentration in the SDMP could increase the percentage of moisture content, water solubility, and MY [12]. The moisture content of SDMP was below 10%, the maximal limitation of powder moisture content. The measurement of moisture content below 10% indicated the high percentage of the core material and encapsulant in spray-dried powder. The minimal moisture content value could prevent decompositiondue to chemical degradation or microbial

contamination [17]. The EEP without encapsulated had lower moisture content compared to the SDMP, due to its lipophilic characteristic. The maltodextrin encapsulant had a hygroscopic characteristic that increased the moisture content value.

Table 1 shows the results of the particle size distribution of SDMP. The particle size on SDMP was influenced by the size of the spray drying nozzle, the thickness of the polymer solution, the dispersion of active substances in polymer solutions, and surface tension.

Fig. 2 shows the micrographs of SDMP. The micrographic of unencapsulated smooth and rough EEP powder as shown in Fig. 2a-b presented the agglomerate particles with heterogeneous shape, uneven surface, and the diameter of 3 to 9 μm. The SEM micrographs of unencapsulated EEP presented in this research were similar to the propolis microscopic analysis as identified by Machado et al. [18]. The micrographics of smooth and rough SDMP shown in Fig. 2c-d presented homogenous spherical particles. The SEM micrographs of SDMP showed the visualization similar as observed by Busch et al. [10]. The micrographic of MD-GA powder as depicted in Fig. 2e as empty encapsulant without any core material inside presented curved surface and vacuole in its core. The spray drying microencapsulation improved the size uniformity and integrity, and showed the better protection of core material microparticles.

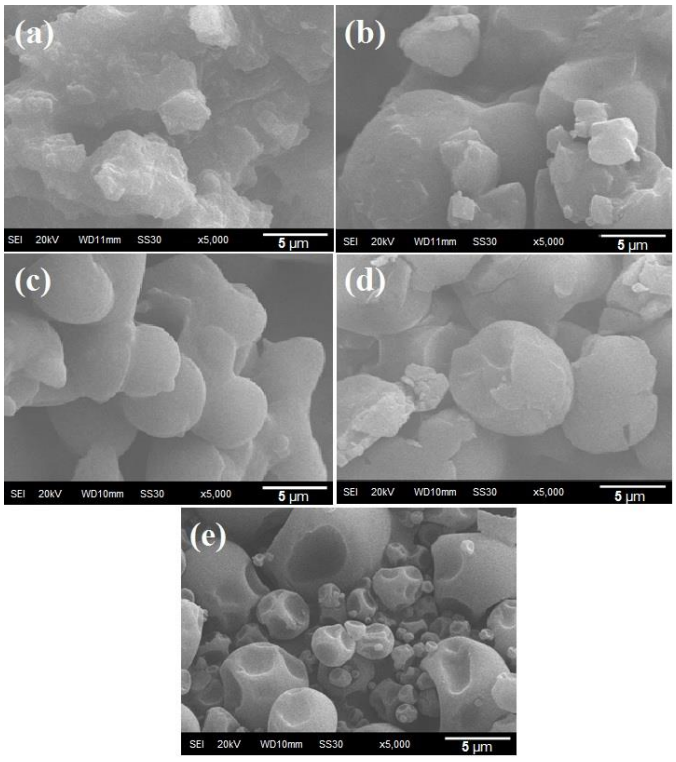

Fig.2. SEM image: (a) Unencapsulated smooth EEP; (b) Unencapsulated rough EEP; (c) SDMP smooth type; (d) SDMP rough type; and (e) MD-GA

3.2. Chemical Characterization of SDMP

Table 2 shows the value of TPC and TFC. Based on a standard curve of gallic acid, we obtained the equation of line $y = 0.0052x + 0.1861$ and $R^2 = 0.99113$ where (Y) refers to absorbance and (X) is concentration. The TPC value in this research was higher than that of research by Marquiafável et al. that achieved TPC of propolis microcapsules in the range of 42.693 to 50.740 mg GAE/gram [16].

In measuring the total flavonoids, quercetin standard was made as a comparison. The value of the total flavonoid content in the sample could be measured from the standard quercetin calibration equation obtained, namely $Y = 0.0067x - 0.0082$, with the relation coefficient value (R^2) = 0.99861.

The EEP smooth type contained a higher TPC and TFC value compared to the rough type. In this case, EEP smooth type, originating from inside the hive, was higher in TPC and TFC than the EEP rough one, originating from outside the hive [9]. Indonesian propolis from *Tetragonula spp.* bee has a TPC and TFC value higher than Malaysian propolis as reported by Rosli et al. [19] in which the TPC value is in the range of 9.1 ± 0.10 μg GAE/mL to 56.9 ± 0.12 μg GAE/mL and the TFC value is in the range of 61.5 ± 0.15 mg QE/mL to 163.9 ± 0.10 mg QE/mL. Meanwhile, the TFC of Indonesian propolis is higher compared to Taiwanese, Brazil, and China propolis in powdery products varied from 2.97 ± 0.05% to 22.73 ± 0.72% [20]. The chemical quantification of propolis even from the same beehive can have different characteristics, content, and properties. The value chemical content in propolis has a difference from its vegetation source, geographic origin, and bee species [21].

The ME of spray drying microencapsulation was determined by comparing the TPC or TFC of SDMP value between theoretical TPCs added initially to the solution before entering the spray dryer. The ME of TPC was found higher than that of TFC. Thus, the ME of SDMP was described as the efficiency of microencapsulation to encapsulate the TPC inside wall material. The ME value of SDMP smooth and rough type was 96.53% and 69.40%. The ME value as shown in this study was close to Da Silva et al., obtaining the ME of propolis microparticles 85.1 ± 0.9% [17], Rabia et al. who obtained ME of propolis microencapsulated using complex coacervation 98.77% [22]. A good ME was obtained when the maximum amount of core material was encapsulated inside the encapsulant particles. The ME value was influenced by the spray drying speed and the formation of microcapsules. The TPC of SDMP might increase if the speed was good and fast [23,24].

Table 2. TPC and TFC of EEP and SDMP

Sample	TPC (mg GAE/ g extract)	TFC (mg QE/g extract)
EEP rough type	857.8 ± 3.34	198.93±9.52
EEP smooth type	970.67±6.67	241.56±4.27
SDMP rough type	786.03±9.52	142.53±3.72
SDMP smooth type	744.3±9.60	71.4±7.08

Note: SDMP, spray drying microcapsule propolis; TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalents; and QE, quercetin equivalents

3.3. The FTIR Analysis

As shown in Table 3 the SDMP had the functional group O-H bonds, C = O ester compounds, and C-H compounds. In

the spectra of EEP and encapsulant MD-GA, there was no structural change in SDMP. The spectra of propolis and maltodextrin - gum Arabic as the coating material was maintained after the spray dry process, according to Pratami et al., there may be no chemical reaction between the compounds in propolis with arabic gum – maltodextrin [7].

Table 3. FTIR Spectra Result

Functional Group	EEP	SDMP	Encapsulant MD-GA
O-H	3308 -3450	3303 – 3345	3296 – 3345
C-H	2930 -2936	2929 – 2936	2920 – 2936
C=O	1694 – 1647	1694 – 1647	1647
C-O	1010 - 1150	1010 - 1005	1150 - 1076

3.4. Antifungal Test

The result of the antifungal test was described as the diameter of microbial inhibition (DMI). Table 4 shows the DMI of SDMP rough and smooth type. The DMI value of the SDMP rough type higher than that of the smooth type. According to the value of DMI, SDMP rough type 3% and smooth type 5 % had low sensitivity: while SDMP rough type 5% had medium sensitivity [25].

From all the data above, it can be stated that the difference of SDMP concentration at 1%, 3%, and 5% of both smooth and rough types did not affect the inhibition ability of *Candida albicans*. It might be due to the concentration of SDMP itself was not different. It may be that none of the SDMP concentration was the most effective concentration, thus further research is needed to know the most optimum DMI. When compared between SDMP on most *Candida albicans* rough propolis was found to have a higher diameter of microbial inhibition than smooth type. The rough type, originating from outside the beehive, was higher in DMI than the smooth type, originating from outside the beehive. It was because the bee colony protected the outside nest more from microbial attack than the inside.

Table 4. The diameter of microbial inhibitory of SDMP

Sample	SDMP rough type (mm)	SDMP smooth type (mm)
1 %	4.33 ± 2.5	3.33 ± 1.5
3 %	6.68 ± 1.1	5.33 ± 1.5
5 %	9.67 ± 0.5	7.33 ± 1.7
Flu (K+)	35 ± 1	33.67 ± 2.3
Nys (K+)	21 ± 2.6	19 ± 1.7
K (-)	0	0

Note: K(+), Control positive; Flu, Fluconazol; Nys, Nystatin; K (-), Control negative (Aquadest steril)

4. Conclusion

Propolis microencapsulation using the spray drying method could be employed to overcome propolis handling properties' problematic obstacles. The application of propolis in the manufacture of pharmaceutical, cosmetic, traditional medicine, and food supplement preparations could be done. Propolis has antifungal potency to inhibit the growth of *Candida albicans.* The rough type, originating from outside the beehive, was found higher in DMI compared to the smooth type, originating from outside the beehive.

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