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Original Research Article

Advancements in topical antifungal treatment: Cream and hydrogel nanosponge integration loaded with Palmarosa essential oil

Iwan Setiawan[1]*, Diska Ayu Febrilyana Wandani[1], Ernawati[2], Fadli Asmani[3], Eka Wisnu Kusuma[4], Kurniawan[5]

[1]Department of Pharmaceuticals and Pharmaceutical Technology, Sekolah Tinggi Ilmu Kesehatan Nasional, Baki, Kewarasan, Sukoharjo, Center of Java, Indonesia, 57552, [2]Department of Formulation of Natural Material Preparation Technology, Sekolah Tinggi Ilmu Kesehatan Nasional, Baki, Kewarasan, Sukoharjo, Center of Java, Indonesia, 57552, [3]School of Pharmacy, Management and Science University, Malaysia, [4]Department of Pharmacology and Clinical Pharmacy, Sekolah Tinggi Ilmu Kesehatan Nasional, Baki, Kewarasan, Sukoharjo, Center of Java, Indonesia, 57552, [5]Pharmacy Department, Faculty of Health Sciences, Universitas Darussalam Gontor, Ponorogo Indonesia

For correspondence:* ***Email:* *iwan.setiawan02@stikesnas.ac.id*

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Abstract

Purpose: *To optimize the preparation of hydrogel and creams integrated using nanosponge loaded with palmarosa essential oil.*
Method: *Palmarosa nanosponge essential oil was formulated by oil-in-water (o/w) emulsion solvent diffusion method using various ratios of drug to polymer; ethylcellulose (EC). Polyvinyl alcohol (PVA) and dichloromethane were used to create the aqueous and dispersed phases, respectively. Furthermore, nanosponge (NS) was studied for particle size and zeta potential, followed by integration into cream and hydrogel preparations. The preparations were evaluated for physicochemical characteristics and antifungal activity in vivo in animals induced by Candida albicans.*
Results: *Results showed that the physical testing for optimum preparation of hydrogel had a pH of 6.9 ± 0.10, adhesion of 1.43 ± 0.40 seconds, spreadability of 5.43 ± 0.55 cm and viscosity of 276 ± 25.16 dPa.s. Meanwhile, for cream preparation, pH, adhesion, spreadability and viscosity values were 6.6 ± 0.2, 2.25 ± 0.37 seconds, 7 ± 0.70 cm, and 1500 ± 5 dPa.s, respectively. The in vivo test for antifungal activity in test animals induced with Candida albicans showed that treatment had a significantly different effect on the healing process of candidiasis infection.*
Conclusion: *This study offers new knowledge regarding the development of hydrogel preparations and nanosponge integration cream containing palmarosa essential oil. The preparations had the advantages of efficacious ingredients, with good drug release and spreadability profile through the skin.*

Keywords: *Nanosponge, Hydrogel, Cream, Optimization, Palmarosa Essential Oil, Antifungal, Candida albicans*

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INTRODUCTION

Candidiasis is a disease caused by *Candida albicans* associated with the appearance of a red rash on the skin. The areas most frequently infected are the groin, between the toes and the armpits [1]. Recent studies have uncovered a novel aspect of *Candida albicans*, namely the ability to survive and resist antibacterial drugs through the formation of biofilms [2,3]. The formation of biofilms is associated with several factors, specifically the physical and chemical properties.

The extracellular matrix contains various components such as carbohydrates, proteins, hexosamine, phosphorus and uronic acid. The chemical composition of this matrix is able to protect *Candida albicans*, hindering antibacterial drugs from effectively reaching and eradicating the infection. Biofilm produce a matrix containing certain polysaccharides that are resistant to enzymes. For example, *Candida tropicalis* biofilm contains significant amounts of hexosamine in the form of chitinase-resistant polysaccharides. Growth under continuous flow conditions may also increase matrix production, which contributes to resistance to antibacterial drugs such as amphotericin [4]. Therefore, it is necessary to explore the use of traditional medicinal plants capable of addressing the limitations of antibacterial or antifungal drug resistance. There is also a need to develop dosage forms that overcome the *Candida* biofilm matrix.

Plants are the most abundant natural resource for the search and development of new drugs with various effects, especially to overcome resistance [5]. Essential oils are part of herbal plants that have antifungal activity. As an antifungal, essential oils which are hydrophobic, work by disrupting the enzymatic and cellular respiration system via penetration of the mitochondrial membrane and causing damage to fungi cells [6].

Several drugs have been used in the treatment of *Candida albicans* infection. Ketoconazole ointment (2 %) is an imidazole antifungal agent that works by inhibiting fungal activity through the inhibition of ergosterol biosynthesis and formation of cell membranes and enzymes. It has a broad-spectrum antifungal effect and is highly effective in inhibiting demethylation of lanosterol to ergosterol. The mechanism of action against *Candida albicans* is by stimulating phagocytosis and inhibiting filament growth. The active side of ketoconazole inhibits the respiratory system by blocking NADH oxidase activity at the mitochondrial level, thereby causing direct membrane damage [7].

Palmarosa plant (*Cymbopogon martini*: Poaceae) is used for making cosmetic products, namely perfume, soap and skincare, as well as in botanical pesticides, including anti-mosquitoes and fungicides. The plant contains active compounds such as tannins, flavonoids and phenolic compounds known to act as antioxidants [8]. One of the active ingredients contained in essential oil is geraniol, which has great potential as an antifungal agent. Geranniol acts by interfering with ergosterol biosynthesis, causing lesions or changes in membrane permeability and interrupting of cell cycle [9]. However, the major limitation of palmarosa essential oil is its low solubility in water solvents. To overcome this deficiency, it is necessary to select a dosage form capable of increasing solubility and ultimately enhancing effectiveness [9]. Consequently, this study aimed to develop palmarosa essential oil preparation integrated into nanosponge, which has the advantage of increasing the water solubility of drugs due to the small pore size of 0.25 µm (equivalent to 250 nm).

EXPERIMENTAL

Preparation of nanosponge using emulsion solvent diffusion method

Palmarosa essential oil (PT. Rumah Essential Tawang mangu, Karanganyar, Indonesia) containing Tween 80 surfactant was dispersed in a solution of dichloromethane (10 mL) and ethylcellulose (10 mg). The prepared dispersion phase was dropped slowly into 15 mL of an aqueous solution containing Polivinil alcohol (PVA), which had been previously developed in hot water. This was followed by constant stirring for 30 minutes and warming to a temperature of 400 ^{o}C. The sample was then dissolved in 50 mL of water and the sonication process continued for 2 hours to obtain nanosponge preparation in liquid form.

Table 1: Nanosponge preparation loaded with palmarosa essential oil

Material	Function	Quantity
Palmarosa Essential Oil	Active substance	5 mL
Ethyl cellulose (EC)	Copolymer	10 mg
Polivinil Alcohol (PVA)	Copolymer	3 g
Dichloromethane	Solvent	10 mL
Tween 80	Surfactant	1 mL
Water	Solvent	50 mL

Table 2: Preparation of nanosponge cream loaded with palmarosa essential oil

Material	Run							
	1	2	3	4	5	6	7	8
Nanosponge	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Setil alcohol	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Stearic acid	0.25	0.50	0.00	0.75	0.50	1.00	1.00	0.00
Triethanolamine	0.75	0.50	1.00	0.25	0.50	0.00	0.00	1.00
Glycerin	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Propylene glycol	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Methyl paraben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Water	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 3: Preparation of nanosponge hydrogel preparation loaded with palmarosa essential oil

Material	Run							
	1	2	3	4	5	6	7	8
Nanosponge	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Carbopol	0.00	1.00	0.75	0.00	1.00	0.50	0.50	0.25
Triethanolamine	1.00	0.00	0.25	1.00	0.00	0.50	0.50	0.75
Metil paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propylene glycol	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Water	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Particle size analysis and zeta potential measurement

Nanosponge size measurements were carried out using the Particle Size Analyzer (PSA) test which is based on the Electrophoretic Light Scattering method on colloidal or liquid samples. Nanometer particle sizes range from 0.6 nm - 300 nm, and the sample must be transparent (clear), stable, not agglomerate and does not settle or react during testing. Zeta Potential, which shows the repulsive force between particles, was used to characterize the surface charge properties of nanoparticles. The zeta potential is good if the value is greater than +30 mV or less than -30 mV, signifying a high degree of stability. A large zeta potential value, either positive or negative, will cause repulsive forces between particles. Meanwhile, low zeta potential value will lead to clumping on the surface or aggregation.

Infection of test animals with *Candida albicans*

The mice used as test animals were induced with *Candida albicans* suspension to achieve fungal infection. The induction was carried out by subcutaneously injecting each mouse with 100 µL of the fungi dissolved in 0.9 % NaCl. Before induction, mice were allowed to acclimatize to the new environment for one week. This study was approved by the Health Research Ethics Committee at Dr. Moewardi General Hospital (Protocol No. 1.505 / XI / HREC / 2023).

In vivo antifungal activity of nanosponge loaded with Palmarosa essential oil in *Candida albicans*-induced mice

The *in vivo* antifungal test aimed to determine whether topical preparations of hydrogel and nanosponge integrated cream containing palmarosa essential oil had a fungi healing effect. Candidiasis in mice was observed and determined daily from the first day to the 10th day. Sampling was conducted on the 10th day because the positive control could provide a healing effect on this day. Data on healing wounds from *Candida albicans* infections were determined using calipers.

Statistical analysis

The data are presented as mean ± standard deviation (SD). For all tested parameters, a one-way analysis of variance followed by Tukey's *post-hoc* test was performed. The condition for data to be tested using ANOVA is that the normality and homogeneity test results must be > 0.05.

RESULTS

Particle size analysis and zeta potential measurement of nanosponge-integrated hydrogel topical preparation containing palmarosa essential oil

Figure 1 shows the results of particle size testing using PSA (Particle Size Analyzer). Data obtained showed that topical preparation increases the solubility of palmarosa. Nanosponge was formulated using different

polymer ratios, Ethylcellulose, PVA and dichloromethane in a ratio of 1:3:1.5, while size reduction to nano levels was confirmed through particle size analysis. The preparation had a particle size of 113.0 nm and the zeta potential values were -27.8 mV which means having a sufficiently high negative charge on the nanosponge surface, to produce a stable nanosponge with minimal agglomeration. Based on previous reports, nanosponge preparations must have a small pore size ≤ 250 nm. This suggests that the nanosponge sample meets the criteria as the particle size was below 250 nm.

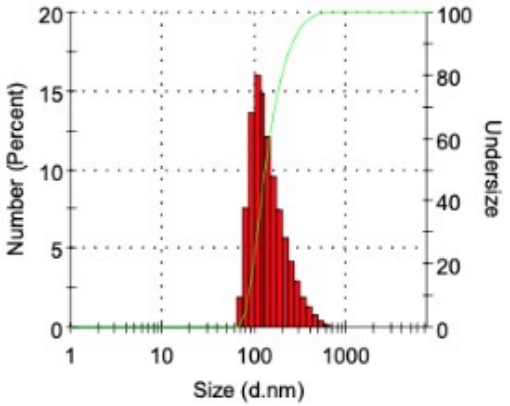

Figure 1: Results of testing the particle size of nanosponge integrated hydrogel topical preparation containing palmarosa essential oil

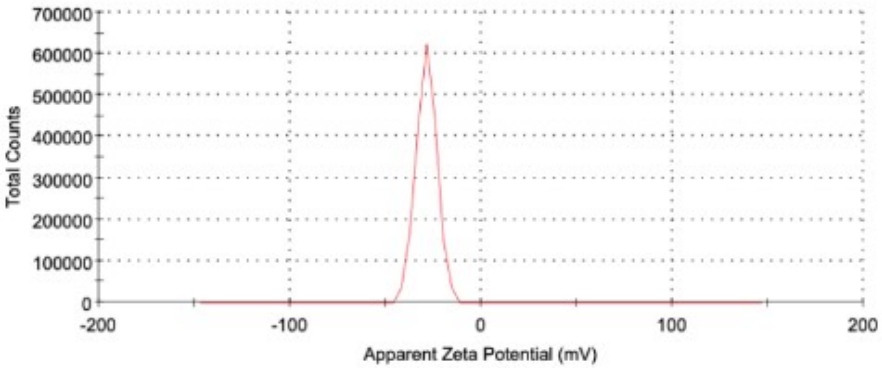

Figure 2: Test results of zeta potential distribution of nanosponge-integrated hydrogel topical preparations containing palmarosa essential oil

The zeta value obtained in this study was -27.8 ± 0.66583 mV (Figure 2), which means having a sufficiently high negative charge on the nanosponge surface, so as to produce a stable nanosponge with minimal agglomeration. The zeta potential boundary value separating stable and unstable dispersion systems is generally at +30 mV or -30 mV. The increase in charge on the particles is directly proportional to the crosslinker ratio used in the formulation. The higher the charge indicates more cations on the particle surface, the higher the stability of the resulting nanosponge.

Physicochemical properties of cream and hydrogel nanosponge-integration loaded with Palmarosa essential oil

The organoleptic test for topical preparations of cream and nanosponge-integrated hydrogel loaded with palmarosa essential oil showed different results. The cream preparation appeared white with a soft texture, while the hydrogel preparation had a clear, transparent appearance with a soft texture. Both preparations met the requirements for good homogeneity, namely the absence of immiscible particles. Furthermore, nanosponge preparation was observed for stability parameters in one month. The results showed that there was no significant change in the physical appearance of the preparation and drug content.

Figure 3 shows that in the cream nanosponge loaded with palmarosa essential oil test, the combination of stearic acid and triethanolamine had a significant effect on increasing the pH. The higher the concentration of stearic acid, the greater the level of acidity, primarily due to the presence of several functional groups. As the amount of stearic acid increased and that of triethanolamine decreased, the resulting spreadability was reduced. Moreover, an increase in stearic acid and triethanolamine concentration was directly proportional to the viscosity and adhesive power.

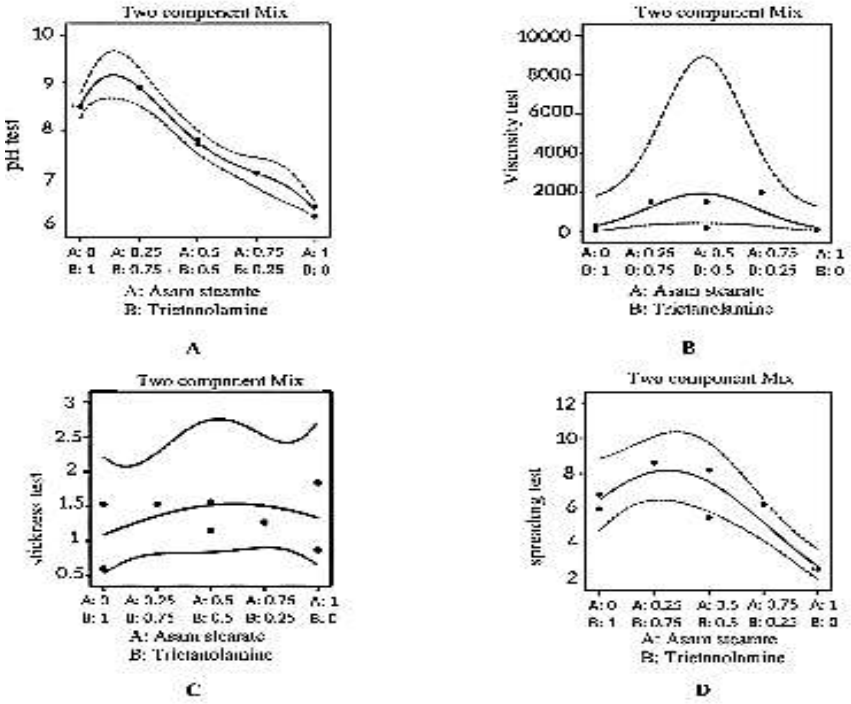

Figure 3: Cream nanosponge loaded with palmarosa essential oil test results (A) pH (B) Viscosity (C) Stickness (D) Spreading test results

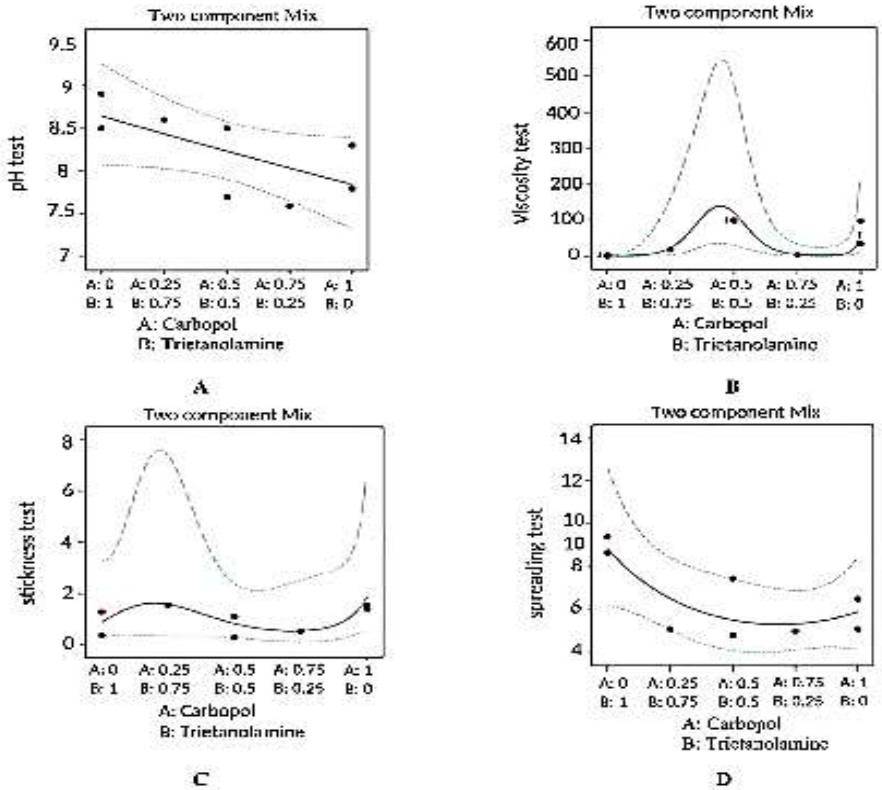

Figure 4: Hydrogel nanosponge loaded with palmarosa essential oil test results (A) pH (B)Viscosity (C) Stickiness (D) Spread power

As shown in Figure 4, carbopol and triethanolamine influenced the pH, adhesion, viscosity and spreading power. Triethanolamine had a more significant effect on increasing the pH of topical preparation compared to carbopol. This pH test was carried out to determine the acidity level of the preparation to prevent skin irritation when used. The difference in the pH of hydrogel was due to the variation in the constituents of each formula. Meanwhile, carbopol had a more significant effect on viscosity and adhesion, enhancing these parameters in topical hydrogel integration nanosponge containing palmarosa essential oil.

The optimum formula for the cream and hydrogel preparations was obtained using the Simplex Lattice Design method. The cream preparation was made with stearic acid and triethanolamine, while hydrogel was prepared using a combination of carbopol and triethanolamine, both with a ratio of 1:1. The quality control optimization test showed that the cream preparation met the requirements with pH of 6.6 ± 0.2, adhesion of 2.25 ± 0.3751 seconds, spreadability at 7.55 ± 0.70534 cm, and viscosity of 1500 ± 5 dPa.s. Similarly, hydrogel preparation met the criteria of good physical test parameters with a pH of 6.9, viscosity of 276 dPa.s, spreadability of 5.43 cm and sticking power of 1.43 seconds.

Induction of *Candida albicans* suspension in test animals

The mice induced with *Candida albicans* suspension were verified to be infected with candidiasis as shown in Figure 5B. Clinical assessment results showed that inflammation, irritation and redness occurred with the infected area appearing white, erythema or reddish.

In vivo antifungal activity of nanosponge loaded with Palmarosa essential oil on mice induced by *Candida albicans*

As shown in Figure 6, there were differences in wound healing between positive and negative controls, as well as hydrogel and cream preparations. In the positive control, mice experienced healing on days 9 – 10, while negative controls did not experience complete healing. For the optimum cream preparation, mice experienced healing on day 4 and healing was complete on days 9 – 10. The cream preparation produced the highest reduction results followed by positive control and hydrogel.

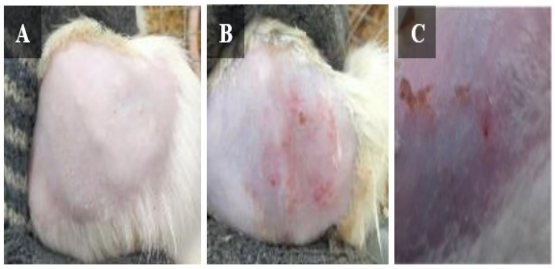

Figure 5: Growth of *Candida albicans* fungi on the back of test mice (A) Before induction of *Candida albicans* Suspension, (B) After induction of *Candida albicans* Suspension (C) References to Fungi in Mice [7]

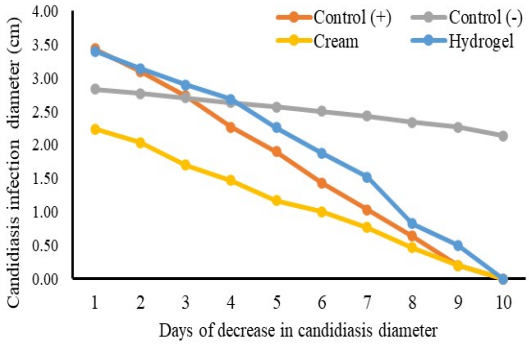

Figure 6: Grouping of treatments in *Candida*-induced animals. Control (+): Cream Ketoconazole (2 %), Control (-) Base, Cream: Nanosponge loaded with palmarose essential oil, Hydrogel: Nanosponge loaded with palmarose essential oil

Table 5: Results for healing fungal wounds treated using nanosponge loaded with palmarosa essential oil cream and hydrogel

Treatment group	Diameter of fungal growth
Control (+)	2.33±0.40
Control (-)	3.23±0.25
Cream	0.73±0.50
Hydrogel	1.90±0.36

The results of the healing of fungal wounds in test animals are shown in Table 5. The untreated group showed the highest diameter of fungal growth infection. The smallest healing value was shown in the palmarose essential oil nanosponge cream treatment group, followed by the palmarose essential oil nanosponge hydrogel and lastly, the control (+) group, which was treated with standard ketoconazole.

DISCUSSION

Fungal infections of the skin have a significant impact on skin health and could worsen if not treated properly. Topical therapy is an attractive option for the treatment of skin infections due to

its various advantages including targeting drugs to the site of infection directly and reducing systemic side effects. In this context, palmarosa is a traditional medicinal plant with antifungal activity [16]. This plant has the potential to be developed into a pharmaceutical product that be used to treat skin diseases caused by fungi including ringworm, tinea pityriasis versicolor, tinea groin and skin disorders in the vital areas of women. In the illustration of Figure 7, *Candida albican* is known to form biofilm, which has been implicated in increased incidence of resistance.

The application of nanosponge as a delivery system for encapsulation or accumulation of hydrophilic and lipophilic drugs, by forming a complex, will effectively deliver drugs in a controlled manner at the target site. This system is incorporated into topical preparations, such as lotions, creams, gels, ointments and liquid or powder forms. The advantages of this technology include targeting drugs to specific locations and reducing side effects. Conventional topical systems such as ointments and creams are perceived to be less effective for skin permeation. Therefore, topical nanosponge systems with particulate carriers, such as microspheres and liposomes, are ideal for controlled drug delivery [10].

Development of nanosponge preparations in anti-inflammatory treatment using solvent emulsion diffusion method comprising ethyl cellulose (EC) and polyvinyl alcohol (PVA) as polymers and surfactants [11] and varying the concentration of ethyl cellulose (EC) increases the viscosity and affects the rate of drug release. Another method in making nanosponges with solvent evaporation and ultrasonic-assisted synthesis using Eudragit L100 polymer [7], PVA and Dichloromethane [12]. This method is easy to apply, requires minimal equipment and increase drug solubility but also prolongs drug release. The rate of drug release in nanosponges is influenced by the drug loading capacity [11] and the viscosity of the matrix, which describes the dense polymer network in the nanosponge. A higher viscosity will reduce drug release from the matrix [10]. Excess drug bound to the nanosphere is known to produce higher antifungal efficacy than free drugs [13] and better retention ability and potentially suitable for topical fungal infection treatment, which improves patient compliance [10].

Furthermore, nanosponges increase preparation stability and flexibility of the preparation as well as drug delivery system. The technology has been widely explored for drug delivery of oral and topical administration [14]. Nanosponges accommodate hydrophilic and hydrophobic compounds, while also addressing the problem of poor bioavailability. When applied to the skin, the encapsulated active substance moves freely from the particles into the carrier until saturation and equilibrium are achieved. The flow of active substances from nanosponge particles to the carrier begins in the epidermis until the carrier is absorbed or dried.

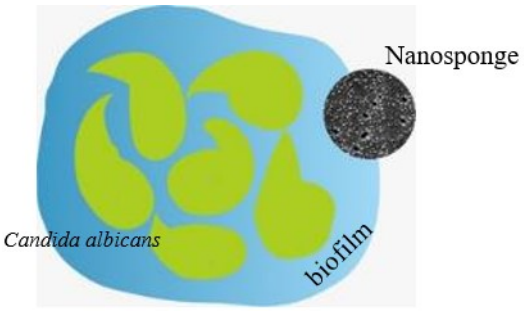

Figure 7: Illustration of cream and hydrogel nanosponge loaded with palmarosa essential oil on *candida albicans* biofilm

Palmarose essential oil as an antifungal compound is formulated into nanosponge preparations of cream and gel. The effect of increasing stearic acid polymers in nanosponge cream preparations loaded with palmarose essential oil and carbopol polymers in nanosponge gels loaded with palmarose essential oil significantly increased the viscosity of the preparation. This increase in viscosity will cause an increase in the density of the polymer network which is related to inhibition of the release of efficacious ingredients in the preparation [15,16]. Analysis using one-way ANOVA for cream and gel preparations compared to the group of test animals that were not given treatment were significantly different (p = 0.003; p = 0.009, respectively). Therefore, cream and gel integrated with palmasora essential oil nanosponge have effectiveness in curing candidiasis. The development of palmarosa essential oil into topical cream and hydrogel preparations integrated with nanosponge increases the solubility of palmarosa, thus solving the problem of bioavailability of this efficacious compound [16]. Based on these results, topical cream and hydrogel preparations integrated with palmarose nanosponge are ideal and effective for use as antifungals.

CONCLUSIONS

The topical nanosponge integrated cream and hydrogel containing palmarosa essential oil developed in this study meets the criteria of

nanoparticles with minimum size (113.0 nm) and the zeta potential value was in -27,8 mV. The hydrogel and cream preparations show great potential as an antifungal agent *in vivo*.

DECLARATIONS

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Ethical approval

Health Research Ethics Committee at Dr. Moewardi General Hospital granted approval for this study (no. 1.505/XI/HREC/2023).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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