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Original Article

ANTIBACTERIAL ACTIVITY OF LIQUID CRYSTAL NANOPARTICLES GEL OF BINJAI LEAVES METHANOL EXTRACT (*MANGIFERA CAESIA* JACK. EX. WALL.) AGAINST *PROPIONIBACTERIUM ACNES*

HAFIZ RAMADHAN, DYERA FORESTRYANA*, WAHYUDIN BIN JAMALUDIN, SATRIYO KRISNA PALGUNO

Department of Pharmacy, University of Borneo Lestari, Jl. Kelapa Sawit 8 Bumi Berkat Kel. Sungai Besar Banjarbaru 70714, South Kalimantan, Indonesia

***Email: dyeraforestryana21@gmail.com**

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ABSTRACT

Objective: This study aims to compare the anti-*Propionibacterium acnes* activity between the optimum formula of gel liquid crystal nanoparticles of Binjai leaves methanol extract and the gel of extract without the liquid crystal nanoparticles system.

Methods: Preparation of liquid crystal nanoparticles using top-down methods with 6% Capmul-GMO 90 and 5% Plantacare 818. Preparation of the optimum gel formula using 7% Viscolam Mac 10 as a gelling agent. The antibacterial activity was evaluated by cup plate technique and clindamycin was used as a positive control.

Results: The results obtained are liquid crystal nanoparticles based gel of methanol extract of Binjai leaves produces a strong category as anti-*Propionibacterium acnes* with an average inhibition of 15.33±1.2413 mm and the gel of extract without the liquid crystal nanoparticles system only produces 13.53±1.241 mm.

Conclusion: The gel of Binjai leaves extract with a liquid crystal nanoparticles system has a higher antibacterial effect on *Propionibacterium acnes* than the gel of extract without the liquid crystal nanoparticles system.

Keywords: Gel, Liquid crystal nanoparticles, Binjai leaves, *Propionibacterium acnes*

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INTRODUCTION

Acne is one of the most common skin disorders in adolescents and persists into adulthood [1]. The presence of blackheads and whiteheads (known as comedones), scaly red skin (known as seborrhea), pinheads, papules, and pimples on the neck, face, and back region are the most general symptoms of acne. Most of the symptoms are caused by bacteria *Propionibacterium acnes* as the main pathogen microorganism [2, 3]. Treatment of acne in each patient depends on several factors. These factors include the clinical grade of acne, the size and number of lesions, the severity of inflammation, the oil content of the skin, the presence of hyperandrogenism in women, and the presence of scar tissue [4]. Besides cosmetic purposes, the advantage of topical use for the treatment of various skin disorders is to bypass hepatic first-pass effects and the systemic availability of many drugs is limited to skin organelles such as hair follicles, thereby avoiding unwanted adverse reactions and enhancing local therapeutic effects. Topical acne can be treated using various antibiotics, benzoyl peroxide, and retinoids [5]. Clindamycin and erythromycin antibiotics effectively use to treat pustular and inflammatory acne. However, acne treatment used is synthetic antibiotics which in their use often cause side effects and can also lead to bacterial resistance [3, 6].

Nowadays, the application of herbal extracts in modern cosmetics became very trendy. The Market share cannot deny that people require cosmetics that include mixtures of natural origin [7, 8]. A lot of plant extracts have found wide applications to treat acne. One of the alternative antibacterial to treat acne can come from natural ingredients. The Binjai plant with Latin name *Mangifera caesia* Jack. Ex. Wall) is one of the natural ingredients of the Mangifera species that has antibacterial properties. Binjai has a typical compound from the xanthone group called mangiferin which has antibacterial activity against gram-negative and positive bacteria [9].

Plant extracts are mixtures consisting of many constituents of different chemical structures, coexisting and displaying a very broad spectrum of chemical and biological activity; when applied directly to the skin, the extract has a low ability to penetrate the absorption barrier, resulting in a low activity [8, 10]. The skin has a multi-layered structure consisting of the epidermis, dermis, and hypodermis. The epidermis is also divided into different layers including stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum which are the strongest barrier against penetration of drugs. The scientific approaches indicate that a large number of different factors influence the permeation process. It has been proven that the progress through the horny layer depends on the delivery system, lipophilicity, molecular size, concentration, the structure of applied compounds, and many other factors [11].

Topical drug delivery through the skin has its drawbacks and limited drug absorption, as it must pass through the skin barrier. However, skin penetration enhancement of the drug can be provided by nanotechnology. In recent years, nanoparticles have been highly regarded as a permeation enhancement strategy to overcome the barrier characteristics of various skin layers. Nanotechnological carriers enhance the topical delivery of various anti-acne bioactive molecules through dermal localization along with a reduction in their side effects. Biodegradation and controlled release of drugs from nanotechnology carriers play an important role in the success of anti-acne therapy [12-14].

The carrier properties of nanoparticles have various advantages such as increasing the absorption effect, increasing the penetration of the active substance and being released in control. One of the lipid nanoparticle systems is liquid crystal nanoparticles (LCNPs). LCNPs appear to be favored because of their similarity to the lipid structure of the skin. The highly ordered structure and possible chemical modifications make it possible to obtain a better clinical response. The application of the system will be facilitated if it is made in preparation. The preparation will assist in the application of the compound and aid in better effectiveness for the bioavailability of the active compound. The gel preparation has high water content, so it can reduce the risk of further inflammation due to the placement of oil in the pores so it is suitable for use as a topical preparation. Skin with an oily texture is highly suitable for using gels preparation

as they have the capability to reduce oil production of the skin and protect the skin from dryness [15, 16]. This study aims to compare the anti-*Propionibacterium acnes* activity between the optimum formula of gel liquid crystal nanoparticles of Binjai leaves methanol extract and the gel of extract without the liquid crystal nanoparticles system.

MATERIALS AND METHODS

Materials

All materials, including ingredients of the liquid crystal nanoparticles system and gel formula, solvents, reagents, and medium of the antibacterial assay, were obtained from Merck KGaA. *Propionibacterium acnes* ATCC 11827 and clindamycin 2 µg/disc (Oxoid, Indonesia) were used for the antibacterial assay. The mature leaves were collected and powdered according to Ramadhan *et al.* [7] in November 2021 from Guntung Manggis Village, Banjarbaru, South Kalimantan, and Binjai plant specimens were identified with the Latin name *Mangifera caesia* Jack. Ex. Wall. in the Basic Laboratory, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

Preparation of extract

The leaf powders was extracted with methanol as a solvent (1: 5) by using the Soxhlet apparatus. The liquid extracts were filtered through Whatman No. 1 and evaporated using a rotary evaporator and dried with a water bath at 50 °C [17].

Preparation of liquid crystal nanoparticles system (LCNPs) and gel formula

Liquid crystal nanoparticles system (LCNPs) made using Top-down method. Lipids are heated on a hotplate magnetic stirrer at a temperature of 60 °C. The lipids were added to the extract and then stirred with a stir bar at 150 rpm to obtain the oil phase (Binjai leaves extract and Capmul-GMO 90). For the aqueous phase, the weighed surfactant (Plantacare 818) was added with hot deionized aqua and stirred with a stir bar until dissolved. The water phase was added to the oil phase and homogenized with a homogenizer at a speed of 2000 rpm for 5 min and stirred with a vortex for 5 min [16, 18]. The LCNPs compositions are given in table 1.

Table 1: Preparation of LCNPs of binjai leaves methanol extract

Materials	Composition
Binjai leaves extract	0.5%
Capmul-GMO 90	6%
Plantacare 818	5%
deionized aqua	til 100 ml

The gel base was made with Viscolam MAC 10 7%, then water was added and TEA was added until a gel mass was formed. Methylparaben and propylene glycol were dissolved in water and stirred until homogeneous. The mixture was put into a gel base and a LCNPs of binjai leaves methanol extract was added and then stirred consistently until homogeneous [19]. The optimum formula of gel is given in table 2.

Table 2: Preparation of gel formula

Materials	Composition (%) of	
	extract gel	LCNPs gel
Binjai leaves extract	0.5	-
LCNPs of Binjai leaves extract (ml)*	-	50
Viscolam Mac 10	7	7
Propylene glycol	15	15
TEA	0.5	0.5
Methylparaben	0.18	0.18
Aquadest (ml)	til 100	til 100

*50 ml≈0.5% of Binjai leaves methanol extract

Antibacterial activity assay

The antibacterial activity of plant extracts and liquid crystal nanoparticles based gel of Binjai was evaluated by cup plate technique. *Propionibacterium acnes* suspension that was made according to Ramadhan *et al.* [20] was inoculated and spread exactly 100 µl on six plates of Mueller Hinton Agar (MHA) media which were Binjai leaves methanol extract, LCNPs of extract, gel of extract, gel of extract LCNPs, negative control used gel base, and positive control using clindamycin. Each plate is given one hole, which will be filled with samples test, positive control, and control negative. All cultures were diffused at 2-8 °C for 14-18 h and then incubated at 37 °C for 24 h. After incubation, Furthermore, observations were made by measuring the diameter of the clear zone, which showed a zone of inhibition of the growth of *P. acnes* bacteria. All treatments were carried out in triplicate independently [21].

RESULTS AND DISCUSSION

Preparation of extract

Mangifera caesia Jack. ex. Wall or Binjai is an endemic plant originating from Borneo island and has been shown to have many properties, even antibacterial activity [22, 23]. Based on these activities, this research was carried out with the aim of exploring the antibacterial activity of Binjai leaves that were extracted by the Soxhlet method with methanol as solvent and to formulate liquid crystal nanoparticles based on a gel of methanol extract of Binjai leaves and to determine the antibacterial activity of gel liquid crystal nanoparticles of methanolic extract of Binjai leaves. The Soxhlet process was carried out until 33 cycles. The Soxhlet extraction was chosen based on the research of Norliyanti *et al.* [24], which states that Soxhlet can extract total flavonoids more optimally and produce a powerful antioxidant activity than maceration on Binjai leaves. The Soxhlet extraction method has the principle of heating and immersing the sample. This method causes a pressure difference between the inside and outside of the cell, resulting in the breakdown of cell walls and membranes. Heating can help in opening plant tissues so that they can attract some of the active substances of secondary metabolites that cannot be released only at room temperature. An increase in temperature will cause the diffusion process to be greater, so the extraction process will also run faster [25].

In the Soxhlet method, the extraction process runs continuously and the sample is extracted by condensed pure solvent so that the yield is higher than in the maceration method [26]. In addition, the use of methanol in the Soxhlet process can extract more phytochemical compounds, which is proof that the yield (34.61%) is greater than the research of Khairiah *et al.* [27] (13.01%) who performed soxhlet extraction on Binjai leaves with 96% ethanol as solvent. Adham *et al.* [28] stated that the total flavonoid content in Binjai leaves was directly proportional to the polarity of the solvent. The higher the polarity of a solvent, the higher the flavonoid can be extracted. The study also showed that the highest total flavonoids were found in the extract using methanol, followed by ethanol and the lowest was n-hexane. The high extract yield indicates that the number of compounds extracted from a sample is higher [29].

Preparation of liquid crystal nanoparticles system (LCNPs) and gel formula

Liquid crystal (LC), also called mesophase/mesomorphic phase, is a special state of matter which has both solid crystalline order and liquid flow characteristics. Lyotropic LCs consist of oil, surfactant, and water, and in general, the lamellar liquid crystal (LLC) phase of the cosurfactant (tidy phase) consists of amphiphilic molecules, which are arranged as bilayers separated by layers of water. The lamellar liquid crystal system

has certain advantages as a topical drug delivery system. It is possible to obtain a controlled release for hydrophilic and hydrophobic drugs, increase drug solubility, provide long-term hydration to the skin, and also drug transport across the skin can be modified by changing parameters such as temperature and release pH. medium, and the amount of substance in the formulation. Therefore, this study aims to prepare a lamellar/lyotropic liquid crystal formulation containing methanol extract of Binjai in gel form [14].

Antibacterial activity assay

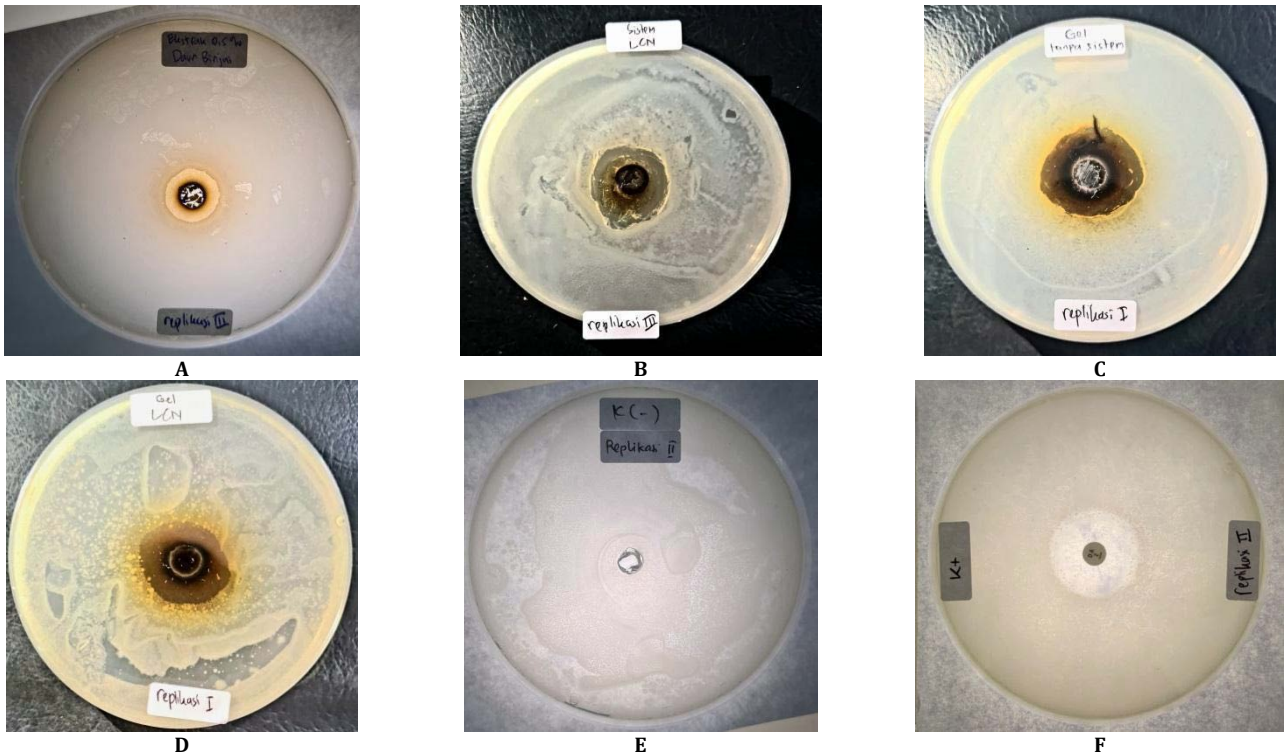
In the antibacterial activity test using, the well diffusion method/cup plate technique with a 6 mm diameter of the reservoir. This method was chosen because it is able to obtain a larger diameter of the inhibition zone than the disc diffusion method. The well diffusion method has a higher osmolarity to penetrate the concentration of active compounds of the extract into the media than the disk diffusion method because the disc diffusion test sometimes gives inaccurate results due to some limitations such as the ability of the extract to pass through the disc pores and the inability of hydrophobic compounds to diffuse into the agar medium. In the well method, each hole is filled with extract concentration so that osmolarity occurs more thoroughly and is more homogeneous, and the resulting extract concentration is higher and stronger to inhibit bacterial growth [30].

Table 3: Diameter of the inhibition zone of anti-*Propionibacterium acnes* assay

Samples	Means (mm)±SD (n=4)	Category
Binjai Leaves Extract	6.95±0.529	Middle
LCNPs of Binjai Leaves Extract	14.33±1.28	Strong
Gel of Binjai Leaves Extract	13.53±1.241	Strong
Gel of Binjai Leaves Extract LCNPs	15.33±1.2413	Strong
Control (+)	14.76±0.416	Strong
Control (-)	0±0	Weak

*The diameter of the reservoir was 6 mm; values represent mean±SD, n=4

Based on the research results in table 3 showed the gel of LCNPs containing 0.5% methanol extract of Binjai leaves had the highest inhibitory power of 15.33±1.2413 mm. Meanwhile, the gel of Binjai leaves methanol extract with the same concentration had a lower inhibitory power that was included in the strong category (13.53±1.241 mm) than LCNPs of extract (14.33±1.28 mm), but the 0.5% methanol extract of Binjai leaves showed the lowest inhibition zone of 6.95±0.529 mm which was included in the medium category according to antibacterial classification category by Ramadhan [21]. The results show that the application of the liquid crystal nanoparticles system into the gel improves the physical properties of an active substance and improves the release of the active compound so that it has a higher antibacterial effect on *Propionibacterium acnes* than gels without the liquid crystal nanoparticles system. The antibacterial activity of methanol extract binjai leaves loaded gel was significantly higher when compared to the extract. The inhibitory of the gel of extract, the gel of Binjai leaves extract LCNPs, and LCNPs of Binjai leaves extract were equivalent to the clindamycin as the positive control, with an inhibitory power of 14.76±0.416 mm, which was shown in (fig. 4). The results gave promises that the LCNPs as a delivery system can increase drug penetration to the skin especially Binjai leaves as a traditional drug from South Kalimantan that has potential as anti-acne for cosmetic ingredients [31].



A B C

D E F

Fig. 1: The inhibition zone of (A) the extract, (B) the LCNPs of extract, (C) the gel of extract, (D) the gel of extract LCNPs, (E) negative control, and (F) the clindamycin as the positive control against *Propionibacterium acnes*

P. acnes has been recognized as a prime target for the medical treatment of acne because this bacterium promotes acne inflammation by inducing increased production of pro-inflammatory cytokines, resulting in the accumulation of neutrophils and oxygen-free radicals produced by neutrophils in acne lesions. Acne topical treatments are associated with various side effects like skin irritation, dryness, flaking, itching, etc. New drug delivery systems have been used to reduce the side effects of drugs used topically. Topical acne treatment makes direct contact with the target site before entering the systemic circulation, which reduces systemic side effects from parenteral or oral administration of drugs [32]. The application of the LCPNs can be a solution for an alternative delivery system for topical use, especially for a natural ingredient such as Binjai as an alternative anti-acne treatment. Each part of the Binjai plant has antibacterial properties, but the leaf of Binjai has a typical compound from the xanthone group called mangiferin which has antibacterial properties against gram-negative and gram-positive bacteria [7, 33]. Mangiferin is a xanthone glicoside found in the genus mangifera. Mangiferin has been reported in various parts of the leaves, fruits, stem bark, heartwood, and roots of the Mangifera species. Mangiferin has attracted a lot of interest to study because it presents many pharmacological activities, including antibacterial activity [9].

CONCLUSION

The conclusion of the study is the gel of Binjai leaves extract with a liquid crystal nanoparticles system has a higher antibacterial effect on *P. acnes* than the gel of extract without the liquid crystal nanoparticles system. The application of the liquid crystal nanoparticles system into the gel improves the physical properties of an active substance and improves the release of the active compound so that it has a higher antibacterial effect on *P. acnes* than gels without the liquid crystal nanoparticles system.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

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