Encapsulated Lime Peel Essential Oil (*Citrus hystrix*) Into Chitosan Nanoparticle: New Entity to Enhanced Effectivity Against Propionilbacterium Acne in Vitro

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Encapsulated Lime Peel Essential Oil (*Citrus hystrix*) Into Chitosan Nanoparticle: New Entity to Enhanced Effectivity Against Propionilbacterium Acne in Vitro

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Abstract. Acne Vulgaris (AV) is a chronic inflammatory disease that affects 85% of the population at least once throughout their lives. The pathogenesis of AV including hypercolonization in pilosebaseous glands by *P. acnes*. Effective and efficient acne therapeutics including topical acne medications remain a crucial aspect of acne research. The lime peel essential oil contains triterpenoid steroid and has antibacterial effect against *P. acnes*. Chitosan is a natural material produced by deacetylyation of chitin from crustacean exoskeleton. Being biocompatible, biodegradable, and affordable, chitosan is widely explored in biomedical field as active agent or drug carrier. In this study, the lime peel essential oil was encapsulated into chitosan nanoparticle through ionotropic gelation technique to be formulated into topical acne cream preparation. The aim of this study was to evaluate and to compare the efficacy of encapsulated and non encapsulated lime peel essential oil into chitosan nanoparticle in inhibiting *P. acnes*. The inhibition zone of *P. acnes* which known as non-growth area after exposure to lime peel essential oil encapsulated into chitosan nanoparticle was calculated. The result showed chitosan nanoparticle formula led to an inhibitory zone of 20,61 mm in diameter, while non-encapsulated formula only led to 10-20 mm. Result led to conclusion that chitosan nanoparticle encapsulation formulation led to improved
P. acnes inhibitory activity of lime peel essential oil.

1. Introduction

Acne Vulgaris (AV) is a chronic inflammatory skin disease that affects approximately 85% of young adolescents' population, at least once throughout their lives.[1] *P. acnes* is known as one of the important of responsible agent in AV pathogenesis.[2] *P. acnes* is able to create a biofilm made of extracellular polysaccharides. This biological glue increases the adherence of *P. acnes* to follicular walls, favouring the modulation of integrins. Furthermore, it regulates the bacterial growth and metabolism, induces the development of *P. acnes* colonies and confers resistance to antimicrobial agents and to host inflammatory cells, resulting in a second mechanism of bacterial resistance[3]. Inflammatory Acne Vulgaris needs a good management of acne especially with the recent rise in antibiotic-resistant microbes.[4-6] Rebalancing the natural microbiome of the skin by restoring the natural skin barrier, limiting the proliferation of P. acnes on the skin by using topical antibacterials and natural ingredients which do not cause resistance and regulating quantity and quality of sebum will be the main acne treatment challenges in the future.[3]

Until today, there are numerous cosmetic products available in the market to deal with acne problems, including benzoyl peroxide antibiotics, azelaic acid, and retinoids.[7-11] However, these anti-acne drugs have their individual side effects.[8] This motivates us to find an alternative treatment derived from natural ingredients that have minimal side effects. One plant ingredient that is empirically and scientifically shown to be efficacious towards anti-acne is the essential oil of citrus fruits (*Citrus hystrix*). The essential oil of lime peel contains terpenoids and linoleic acid which can help in preventing and eliminating acne. Terpenoid functions by disrupting and destroying *P. acnes* cell membranes, while linoleic acid works as anti-inflammatory agent, to

minimize the inflammation caused by acne. Conventionally, essential oil of lime (*Citrus hystrix)* was adopted for acne treatment by direct topical application. Such usage can cause patient discomfort, and is impractical due to stability issue. Thus, it is necessary to stabilize the lime peel essential oil in nanocapsule form for topical application.

Chitosan is a marine product derived from the shells of crustacean, including shrimps. Chitosan was produced by deacetylation of chitin, which is the structural element of the crustacean exoskeleton and the cell walls of fungi.[13] Chitosan is a polysaccharide, which consist of acetylated and deacetylated units. The acetylated units are formed by N-acetyl-D-glucosamine while the deacetylated units are composed by β-(1,4)-D-glucosamine. Chitosan is hypoallergenic and has natural antibacterial properties, which qualities make it useful as wound healing agent.[14] Chitosan is biocompatible and biodegradable and its low cost suggest the biomedical applications in wound healing.[15]

Nanoparticle, as one of nanotechnology products, have unique physical and chemical properties maximized for therapeutic effect, including for wound healing.[16] Therefore, this study based on chitosan copolymer nanoparticle as an active aging for enhancing skin repair (wound healing). Nanocarrier systems as an innovative cosmetic delivery technology have been used widely. Most drug or cosmetic delivery particle technology was based on lipid carriers, such as liposomes and solid lipid nanoparticles of 100-300 nm diameter. Nanoparticles have unique physical properties making them ideal for usage I various skin care products currently on the market.[16]

In this study, we chose to encapsulated the lime peel essential oil into chitosan nanoparticle, given its biocompatibility, biodegradability and minimal toxicity. In addition to improving the essence stability, the polymeric nanocapsule also facilitates penetration of the skin barrier. In particular, the chitosan NP-formulation is compared to its non-encapsulated form in terms of inhibiting *P. acnes* growth *in vitro*

2. Materials and Methods

2.1 Materials

Essential oil of lime peel, chitosan, sodium tripoliphospate, chlorinated, tween 80, propylene glycol, glyceryl monostearate, sodium lauryl sulfate, methyl paraben, propyl paraben, acetyl alcohol, paraffin liquid, alfa tocopherol, distilled water, buffer solution, DMSO, peptone broth, methylene blue, nutrient agar, diluent NaCl solution, culture of *P. acnes* bacteria.

2.2 Methods Synthesis of lime peel essential oil encapsulated into chitosan nanoparticle

Created 1% chitosan solution by dispersing chitosan powder in 1% glacial acetic acid solution, laying overnight and filtering, chitosan solution in beaker glass, then homogenizing the solution using magnetic stirrer with 450^{o}C temperature. Next, mix the tween 80 1% into chitosan solution, homogenized for 30 minutes, then weighed 2.5 grams of lime peel essential oil, obtained from the MIC test result and then added as a solvent, drop the oil solution bit by bit into the solution chitosan to form emulsion. The next stage of the emulsion is stirred at high speed, using ultaturax at a speed of 13000 rpm, for 30 minutes to form a color change, from cloudy to clear yellow. Then a 0.1% sodium tripolyphosphate dose of 68 ml was carried out at a rate of 1t / 3 sec, with stirring using a magnetic stirrer at 300 rpm, if the increased addition time would cause precipitation of the chitosan in the nanocapsule. The formation of nanocapsule is characterized by turbidity and the color changes to a clear yellow. Composition of chitosan : lime peel essential oil (*Citrus hytrix)* (1:0,8)

2.3. The determination of Minimum Inhibitory Concentration (MIC) of lime peel essential oil.

Created the mother liquor essential oil of lime peel a concentration of 2,5%. From the mother liquor, made the test solution essential oil of lime peel with a concentration of 2.0%; 1.5%; 1.0%; 0.5%; 0.25%; 0.125%. Into a tube containing 5 mL of test solution at each concentration, added 0.5 mL bacterial suspension, divortex, the incubated at 37ºC for 24 hours. Obtained the lowest concentration that can inhibit microbes, the Minimum Inhibitory Concentration (MIC) to be used as a dose of lime peel oil in the cream dosage formulations. The best concentration of lime peel essential oil that used in this study was 2,5%.

2.4. Examination of lime peel essential oil encapsulated into chitosan nanoparticle

Suspension of nanocapsule essential oil of kaffir lime peel in evaluation of particle size distribution and zeta potential test by using particle sizer analyzer, and nanocapsule morphology test with scanning electromicroscopy (SEM). Drying of nitrocapsule lime peel essential oil. Drying is done by freeze drying method, which is done at -43^{0}C or lower temperature at a pressure of 16.5 Pa. The working principle of freeze drying method is based on the lyophilized process where the nanocapsule solution of the essential oil of lime peel is frozen until it becomes solid ice/ phase then converted gas phase (steam).

3. Results and Discussion

3.1 Minimum Inhibitory Concentration value of lime peel essential oil

The lime peel essential oil is determined by the value of MIC liquid dilution method.
The value of MIC obtained is 2.5% concentration give the best result.

P. acnes is able to create a biofilm made of extracellular polysaccharides. Furthermore, it regulates the bacterial growth and metabolism, induces the development of *P. acnes* colonies and confers resistance to antimicrobial agents and to host inflammatory cells, resulting in a second mechanism of bacterial resistance. Consequently, the use of topical antibacterial compounds, such as benzoyl peroxide, or botanicals, which do not induce antibacterial resistance, may be a privileged alternative to limit the cutaneous *P. acnes* biofilm.[3]

Fatimah et al, research about new antibacterial compounds is important because resistance of bacteria acne to antibiotics.[17] Some of Essential oils have antibacterial properties. Lime peel essential oil and patchouli essential oil have some terpenoids that act as antibacterial compounds such as Linalool and Seychellene. The purpose of this research was to formulate anti acne serum based on lime peel essential oil and patchouli oil and to determine the zone of inhibition against of *P. acnes*.

3.2 Distribution and size of lime peel essential oil encapsulated into nanoparticles chitosan

The average particle size was 271.7 nm and the index of polydispersity was 0.634. Based on the results of measurements done on nanocapsule particles of lime peel essential oil using Particle Size Analizer (PSA) tool obtained particle size indicating the size of the particle is eligible because it is in the range of nanoparticle size that is 1-1000 nm, with the polydispersity index shows that the nanoparticles are homogeneously distributed. The index value of polydispersity increasingly close to zero indicates that the particle distribution is getting better.

3.3 Zeta potential evaluation results

We measured the zeta potential of lime peel essential oil three times. And the result was 31,2 mV, 45,7 mV, 54,1 mV. And the average was 43,67 mV. Zeta Potential inspection is done by using zetasizer nano series tool with additional disposable capillary device. Particle with a positive zeta value more positive than (+) 30 mV or more negative than (-) 30 mV indicate a stable colloidal system because particles with high potential zeta will have a repulsive force between particles having the same charge, preventing particles to aggregate. From the

measurement results obtained potential mean value of zeta showing nanocapsule particles of lime peel oil has a large repulsive force, so it is expected that the from nanoparticles can remain stable.

3.4 Morphology of particle lime peel essential oil encapsulated in nanoparticles chitosan
Chitosan is not toxic and biodegradable, therefore it can be used as a biomaterial and for the construction of drug delivery system.[15, 18, 19] O-Carboxymethyl chitosan (OCMS) were prepared using ionotropic gelation method used as carrier for tetracycline, this nanoparticles showed sustained release, able to induce cytotoxicity against S. aureus in a targeted manner.[18]

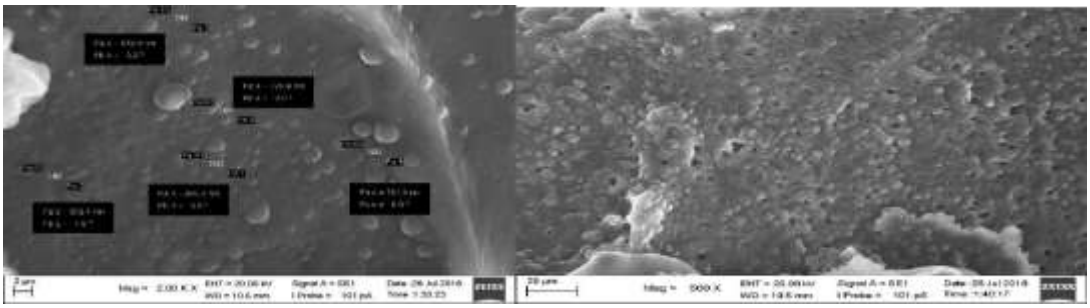

Figure 1. Morphology of particle lime peel essential oil encapsulated in nanoparticles chitosan was taken by TEM

3.5 Anti-acne effectiveness of lime peel essential oil encapsulated into nanoparticle chitosan in vitro
Increasing the concentration of lime peel essential oil will increase Inhibitory Diameter Area (IDA) of *P. acnes*. The preparations of the formula I and II non nano encapsulated have strong *P. acnes* inhibitory power (IDA is between 10-20 mm), whereas the Formula III nano encapsulated preparations have very strong *P. acnes* bacterial inhibition area (IDA > 20 mm). This suggests that the resulting cream preparations are effective in inhibiting the *P. acnes.* Fatimah et al, evaluated anti acne serum which had antibacterial inhibitory against *P. acnes* between 20,80-26,12mm, whereas control positive only 12,47mm and control negative 5,78mm.[17]

In our study formula of lime peel essential oil encapsulated in nanoparticle chitosan showed significant higher inhibiting *P. acnes* than the other formula. Leonida et al investigated the use of chitosan for wound healing and as antimicrobial and also has anti aging activity. The authors reported that "nano-sizing" enhanced the activities. Draleos et al also reported that nanotechnology could re-invent the "old" one of active agent in cosmetic dermatology to a "new" great one, and greatly enhance the therapeutic efficacy of the substance currently used.[16] When well-known substances are ground into nanoparticles, they may display completely different properties and can behave in an unpredictable manner. This means that nanoparticles could reinvent the properties of substances currently used in cosmetic dermatology creating different chemical entities from old ones.[16]

Chitosan has found its application in a variety of industries, in cosmetics for drug delivery, for skin hydration, as film forming and to modify viscocity.[20] Chitosan is an effective materials for biomedical application because of their potential agent of their biocompability, biodegradability and non toxicity, apart from their antimicrobial activity and low immunogenicity, also as hydrating agent which clearly points tp an promising potential agent for future development. Biochemical application of chitosan is also based on nanomaterials, Chitosan is an effective material for biomedical wound dressing application and hydrating agent. Chitosan nanoparticles can be used in regenerative medicine, wound healing, cosmetic anti aging, drug delivery.[20]

When well-known substance are ground into nanoparticles, the may display completely different properties and can behave in an unpredictable manner. This means that nanoparticles could re-invent the properties or substances currently used in cosmetic dermatology creating new chemical entities from old ones. Nanoparticle chitosan has been fabricated to maximize its biological activity. Chitosan membrane could be used to fasten wound healing and include cell migration and proliferation due its healing effect and antibacterial activity. Furthermore we note that the development of chitosan nanoparticles could lead to stabilization and will reduce its toxicity and irritation effect, therefore clinical study is needed in the future. The use of chitosan nanoparticles as biochemical materials is promising especially in bone repair and wound healing and many of related studies were conducted in vivo. the research, is relevant to trauma related process and the improvement of wound healing. Jayakumar et al studied the application of chitosan based nanomaterials in the form of hydrogel, fibers, membrane scaffolds and sponge. The other study explored chitosan silver (Ag) nanoparticles and chitosan – zinc oxide (ZnO) nanoparticles as novel chitosan nanoparticles in biochemical application. [15]

Nowadays, nanotechnology has emerged as the third approach, which has opened opportunities for skin drug delivery via nanosystem-like particles, dendrimers, etc. the size of nanosystems for topical and transdermal delivery generally ranges from 1 to 1000nm. They increase skin permeation by enhancing drug solubilization, partitioning of drug into the skin layers, and fluidizing the SC lipids.[21]

4. Conclusion

The encapsulated lime peel essential oil with concentration 2,5% into chitosan nanoparticle by using chitosan-sodium tripolyphosphate as polymer with ionic gelation method and has greater *P. acnes* inhibition activity by forming zone of 20,61 mm inhibition with strong inhibiting classification has stronger inhibiting effect against *P. acnes* compared to non nano encapsulated formula.

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