Treatment of Subcutaneous Infections With Plu Kao Extract in Nanogel

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Abstract
Plu Kao (Houttuynia cordata) extracts have well known antibacterial and anti-inflammatory effects. The potential for using Plu Kao extract to treat subcutaneous bacterial infections is investigated here. Because the crude extract is insoluble in water, subcutaneous penetration is enhanced by encasing it in a nanogel. It is found that applications of nanogel containing 10% and 15% Plu Kao extract are an effective way to enhance the penetration of Plu Kao extract through pig skin. It is also shown that nanogel containing 10% and 15% Plu Kao extract are both effective inhibitors of Staphylococcus aureus, indicating that this may be an effective method for treating subcutaneous infections in humans.

Keywords: *Plu Kao, Houttuynia cordata, nanogel, subcutaneous skin inflammation*

I. INTRODUCTION

Cellulitis is a skin condition that presents as swelling, redness and pain at the site of an infection. The most common cause is bacterial infection that may enter the body through wounds, cracks, or damaged skin. The most common bacterial species involved is Staphylococcus aureus. (*See a Doctor*, 2018)

Tinida Inta et al. (2019) studied the inhibition of *S. aureus* with a crude extract of Cockscomb (*Celosia argentea*). They identified alkaloids, tannins, flavonoids, and saponins in the extract. One or more of these compounds may be the reason that extract of Guava leaf also inhibits this bacterium. (Inta et al., 2019). There are many other herbal extracts known to have the same effect, including *Houttuynia cordata*, known in Thailand as Plu Kao.

Plu Kao, known also as Fish mint, is a well-known herb that contains the flavonoid Quercetin that has antibacterial properties (Bureau of Information, 2020). Because crude Plu Kao extract is largely insoluble in water, poor penetration is a major limitation in its use on the skin. An effective method of delivery is required to treat subcutaneous skin infection.

A hyaluronic acid-grafted polymer was synthesized by Luckanagul et al (2021) that self assembles into nano particles forming a nanogel that can be used to deliver biocompatible metabolites containing curcumin.

A nanogel delivery system is a nanoparticle size carrier system that is a hydrophilic polymer lattice structure. (Kasa et al., 2019). Biomedical scientists often create nanogels from natural polysaccharides like hyaluronic acid because they have outstanding water-holding properties, low toxicity and are biodegradable (Thai Rath, 2020).

Hyaluronic acid can be chemically modified to become polyN-isopropylacrylamide, known as HA-pNIPAM, for use as a trans-dermal delivery system (Luckanagul et al., 2021). Luckanagul et al. (2021) found that nanogel transdermal delivery systems have the potential to improve drug absorption and mobility, which suggests that a nanogel could be used to deliver Plu Kao extract to the subcutaneous layers of the skin to control bacteria induced inflammation.

II. METHODS

Preparation of nanogel HA-pNIPAM and Plu Kao extract

Freshly picked leaves from healthy disease-free Plu Kao plants, were washed and allowed to dry in air for 20 minutes. The leaves were then placed in a 40 °C oven until completely dried, then ground thoroughly to a powder. Two hundred grams of leaf powder was soaked in 400 ml of 95 percent ethanol for 72 hours. The solution was filtered with Whatman No.1 paper, and the residue evaporated in a rotary evaporator at 40 °C until a viscous extract was obtained.

Nanogel, HA-pNIPAM, obtained from Nabsolute Co. Ltd., was prepared at 0.005% w/v in a buffer solution that was agitated by sonication for fifteen minutes. The resulting suspension was left to precipitate overnight at 4°C, then centrifuged at 3,000 g for five minutes. Plu Kao extract was added dropwise to the 0.005% HA-pNIPAM to obtain the following ratios: (100:0 95:5, 90:10 and 85:15) %v/v. The suspensions were centrifuged at 500 rpm. The four samples containing Plu Kao extract were then incubated in the dark at 4°C for 48 h. The preparations incorporating Plu Kao extract were designated as PK-HApNIPAM.

Particle Size Mean and Distribution

Samples containing the three different concentrations of Plu Kao extract, (0%, 5%, 10% and 15%) v/v were placed in a Zetasizer Nano ZS 90 nanoparticle size analyzer to measure particle size and distribution by dynamic light scattering, DLS. Values of mean particle diameter, DLSx, and Polydispersity index, PDI, were obtained.

Entrapment Efficiency (%EE) and Drug Loading Efficiency (%DLE)

A UV absorption spectrophotometer at a wavelength of 230 nm was used to measure the density of Plu Kao extract in each of the prepared suspensions. The suspensions were then centrifuged for stratification between the water and the nanogels at 4°C for 15 minutes to find Plu Kao entrapment efficiency in the polymer. The %EE was found as the ratio of the weight of the extract retained in the nanogel and the weight of the total extract. The %DLE was found as the ratio of the weight of the extract contained in the nanogel and the weight of the nanogel without the extract.

Plu Kao Extract Penetration through Pig Skin

The skin from the back of a pig's ear was prepared for permeability testing. A Franz Diffusion Cell was prepared following Luckanagul et al. (2021). The temperature of the receptor was set to 37 °C and the magnetic stirrer was set to 100 rpm. The pig skin was cut to size and sealed at the base of the donor chamber. The acceptor chamber was filled with 12 ml of phosphate buffer, PBS, at a pH of 7.4. One ml of solution was removed and replaced by one ml of new buffer at intervals of 1, 2, 4, 8 and 24 hours. The samples were analyzed with the UV spectrophotometer to find the absorbance at a wavelength of 230 nm and the amount of Plu Kao extract present was found by comparing to the standard curve.

Growth inhibition of Staphylococcus aureus

The agar disc diffusion method was used to test for bacterial inhibition. A petri dish was prepared with Potato Dextrose Agar (PDA). Staphylococcus bacteria were prepared in Mueller Hinton Broth (MB). Sterile spatulas were dipped in the bacterial suspension and then swabbed onto the surface of the agar in the petri dish. Drops of concentrated Plu Kao extract (15% v/v) and nanogels containing the 3 different concentrations of Plu Kao extract were placed on the surface and left to dry. The agar plates were incubated at 37 ºC for 24 hours, then the diameters of the clear inhibition zones were measured across three angles.

III. RESULTS AND DISCUSSION

Particle Size Mean and Distribution

Figure 1 shows the average particle size, DLSx, in each of the four nanogel samples and the size distribution index DLI. The 15% sample of PK-HApNIPAM had the smallest particles (mean 13.1 ± 1.5 nm), while the 5% sample had the largest (280.6 ± 3.3 nm). Supang Khondee (2017) found that insoluble particles larger than 10,000 nm remained on the surface of the skin, particles from

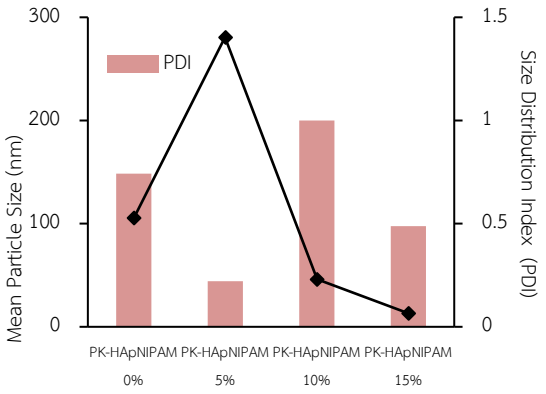


Figure 1. Mean Particle Size and Particle Size Distribution Index (PDI)

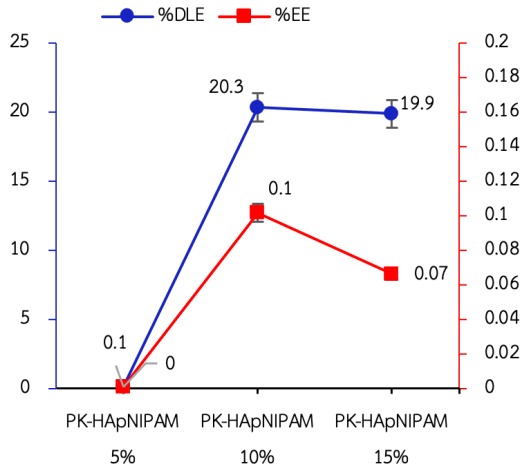


Figure 2. Nanogel Drug Loading Efficiency (%DLE) and Entrapment Efficiency (%EE)

3,000 – 10,000 nm accumulated in hair follicles and particles smaller than 3,000 nm penetrated through the pores in the epidermis. The particle size in all cases here were small enough to penetrate the epidermis.

Particle size distribution also varied, as shown in Figure 1. PK-HApNIPAM 5% had the lowest dispersion with a PDI of 0.22 ± 0.03, followed by PK-HApNIPAM 15% with a PDI of 0.487 ± 0.007, while PK-HApNIPAM 10% had a PDI of 1.00. The PDI value ranges from zero for a perfectly uniform nanogel to 1.0 for highly differentiated nanogels with multiple particle sizes.

A PDI of less than 0.5 is desirable because it is an indicator of the aggregation of smaller particles and uniform particle size leads to more uniform, stable delivery. (Danaei et al., 2018). The most suitable nanogel containing Plu Kao extract is the 15% PK-HApNIPAM with skin permeable mean particle size and a particle size distribution less than 0.5.

Extract Entrapment Efficiency and Drug Loading Efficiency

Extract Entrapment Efficiency (%EE) and Drug Loading Efficiency (%DLE) are shown in figure 2. The results show that the percentage of extract contained in the nanogel is very small. We note that Plu Kao extract is dense and viscous, and when centrifuged is readily precipitated. The percentage of polymer content also affects the retention.

The nanogels were kept for 27 days prior to testing, Table 1 shows that most of the Plu Kao extract content of PK-HApNIPAM 5% and

PK-HApNIPAM	%DLE	%EE	Extract levels before Centrifuging (µg/ml)	Extract levels after Centrifuging (µg/ml)
5%	0.1	0	89.84	0.4
10%	20.3	0.1	195.24	101.7
15%	19.9	0.07	393.18	99.4

Table 1. Nanogel Drug Loading Efficiency (%DLE), Entrapment Efficiency (%EE) and Extract Retention levels.

PK-HApNIPAM 10% is present in the ethanol fraction rather than in the polymer of the nanogel.

It was found that the nanogel containing Plu Kao extract had the ability to retain the extract and extract packaging efficiency at levels suitable for use. PK-HApNIPAM 10% and PK-HApNIPAM 15% had the highest percentage of extract filling and entrapment efficiency.

Further research should be conducted on ways to improve the duration of retention, the %EE, and the %DLE, in order to improve the effectiveness of the Plu Kao nanogel preparations.

Skin penetration

From Figure 3, PK-HApNIPAM 15% has the highest percentage permeability followed by PK-HApNIPAM 10% and PK-HApNIPAM 5%, respectively but the difference between the three concentrations is very small.

The result shows the improved penetration of insoluble extract particles through the pig skin with the nanogel delivery system which is consistent with the research report of Luckanagul et al. (2021).

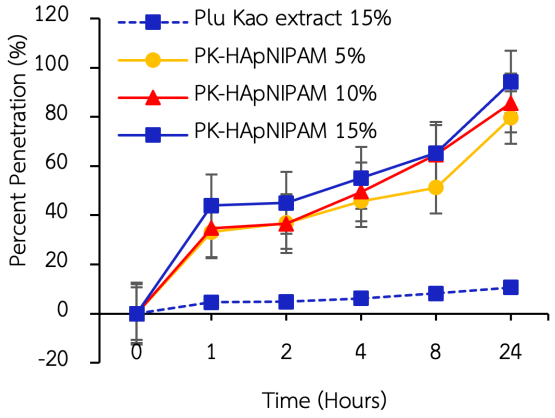
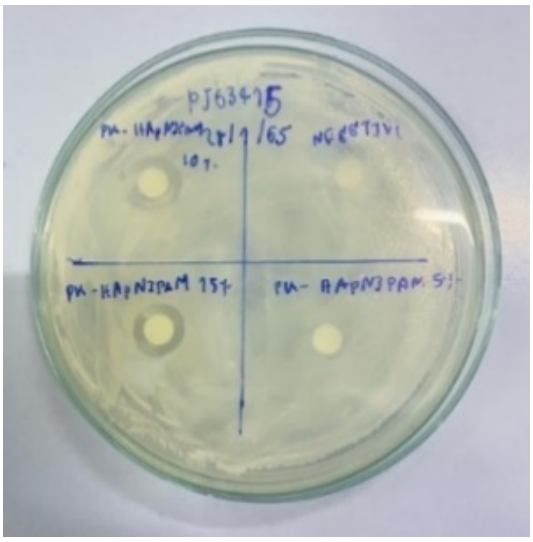


Figure 3. Penetration of extract through pig skin over time.



Nanogel PK-HApNIPAM: a = 5%, b = 10%, c = 15%, d = 0%

Figure 4. Inhibition of S. Aureus

Inhibition of Staphylococcus aureus

Figure 4 and table 2 show the diameters of the inhibition zones of *S. aureus* on the agar plate for the four different extract concentrations in the nanogel.

Significant and similar inhibition is seen for both 10% and 15% HApNIPAM concentrations and no effect is seen for 5%. This finding is consistent with the previous findings that 10% and 15% HApNIPAM concentrations demonstrated significant levels of %EE and %DLE, while 5% showed negligible levels of these measures.

These results show that the 10% HApNIPAM concentration is most effective at inhibiting S. aureus. Further research is recommended into its development as a medical treatment for subcutaneous infections.

IV. CONCLUSION

It was found that the nanogel delivery system HApNIPAM has the ability to deliver an extract of Plu Kao with small particle size (less than 250 micron) with a DPI of less than 0.5 to the subcutaneous layers of pig skin. Most importantly, the nanogel containing the Plu Kao extract is effective in inhibiting Staphylococcus aureus in the laboratory setting. It is hoped that future clinical trials will show that nanogel delivery of a Plu Kao extract is an effective treatment for subcutaneous inflammation caused by the common bacterium Staphylococcus aureus**.**

PK-HApNIPAM	Bacteria Inhibition Area Diameter (mm)			
	1	2	3	Mean
0%	NA	NA	NA	NA
5%	NA	NA	NA	NA
10%	12.5	13.0	12.0	12.5 ± 0.4
15%	11.0	10.5	11.0	10.8± 0.2

Note: NA = No inhibition

Table 2. Bacteria Inhibition Area Diameter for the Nanogel Extract Preparations tested.

V. REFERENCES

Bureau of Information, Ministry of Public Health, (2020). Plu Kao – Kao Tong: Antiviral local herb. Retrieved 11 June 2021, from https://pr.moph.go.th

Danaei, M, Dehghankhold M, Ataei S. Davarani FH, Javanmard R, Dokhani, A Khorasani S, and Mozafari MR (2018). Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. Pharmaceutics 10 (2). DOI: 10.3390/ pharmaceutics10020057

Inta, T., Saelee, A., Samang, P., Srisayaem, M., Phalee, W., Chotinakarin, A. (2019). Staphylococcus aureus and antioxidant activity of crude extracts from cockscomb and guava. Phitsanulok: Faculty of Science and Technology, Phibunsongkhram Rajabhat University.

Kasa P, Farran B, Raju, GS (2019). Are Nanocarriers Effective for the Diagnosis and Treatment of Pancreatic Cancer? In GP Nagaraju (Ed.), *Cancer Sensitizing Agents for Chemotherapy* (pp. 159–174, Ch. 10). Academic Press. DOI.org/10.1016/B978-0-12-817661-0.00010-X

Kondee, Supang (2017). Why is particle size important? Phayao: Faculty of Pharmacy University of Phayao.

Luckanagul, JA., Ratnatilaka Na Bhuket, P., Muangnoi, C., Rojsitthisak, P., Wang, Q., Rojsitthisak P. Self-Assembled Thermo-responsive Nanogel from Grafted Hyaluronic Acid as a Biocompatible Delivery Platform for Curcumin with Enhanced Drug Loading and Biological Activities. Polymers (Basel). 2021 Jan 7; 13 (2):194.

See a Doctor. (2018). Cellulitis (inflamed tissue cells). Retrieved June 4, 2021, from https://www.pobpad.com/cellulitis-Cellulitis.

Thairath. (2020). "Hyalu Ronik", the secret of beauty. Retrieved 11 June 2021, from https://www.thairath.co.th/women/beauty/health/1894288.

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