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Antimicrobial Properties of *Pliek U* as Traditional Acehnese Fermented Spices

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ABSTRACT

The high prevalence of antimicrobial resistance globally has increased the burden of disease. The phenomenon demands urgency to explore new antimicrobial drugs, one of which is sourced from natural ingredient, spices. *Pliek U*, as one of the spices traditionally processed by Acehnese, has attracted researchers to explore its potential source for antimicrobial substances. The objective of this literature review is to explore the characteristic, production, chemical composition, and antimicrobial mechanism of *Pliek U*. A literature search was carried out on PubMed, Science Direct, Springer Link, and Google Scholar using the keywords "*Pliek U*", "Coconut Flesh", and "Antimicrobial". The articles obtained were then screened based on predetermined criteria. The results of the analysis were displayed in tabular and narrative form. The final result was 8 main articles and 10 additional articles after screening 65 articles obtained from the database. *Pliek U* powder is traditionally produced and has unique characteristics that make it easy to recognize. *Pliek U* mechanism of action based on the chemical composition and the role of lactic acid bacteria in the fermentation process. Analysis shows that *Pliek U* is a promising antimicrobial source, so it needs to be researched and developed further in the pre-clinical to clinical phases.
Keyword: Antimicrobial, Fermentation, *Pliek U*, Spices.

ABSTRAK

Tingginya prevalensi resistensi antimikroba secara global telah meningkatkan beban penyakit. Fenomena tersebut menuntut adanya urgensi untuk mengeksplorasi obat antimikroba baru, yang salah satunya bersumber dari bahan alam, rempah-rempah. *Pliek U* sebagai salah satu rempah yang diolah secara tradisional oleh masyarakat Aceh menarik perhatian para peneliti untuk mengeksplorasi potensi sumber zat antimikroba. Tujuan dari tinjauan literatur ini adalah untuk mengetahui karakteristik, produksi, komposisi kimia, dan mekanisme antimikroba *Pliek U*. Penelusuran literatur dilakukan di PubMed, Science Direct, Springer Link, dan Google Scholar dengan menggunakan kata kunci "*Pliek U*", "Daging Kelapa", dan "Antimikroba", Artikel yang diperoleh kemudian disaring berdasarkan kriteria yang telah ditentukan, Hasil analisis ditampilkan dalam bentuk tabel dan narasi, Hasil akhirnya adalah 8 artikel utama dan 10 artikel tambahan setelah disaring diperoleh 65 artikel dari database. Serbuk *Pliek U* diproduksi secara tradisional dan mempunyai ciri khas yang unik sehingga mudah dikenali. Mekanisme kerja *Pliek U* berdasarkan komposisi kimia dan peran bakteri asam laktat dalam proses fermentasi. Analisis menunjukkan bahwa *Pliek U* merupakan sumber antimikroba yang menjanjikan, sehingga perlu diteliti dan dikembangkan lebih lanjut pada fase praklinis hingga klinis.
Keyword: Aktivitas Makan, *Momordica charantia* L, *Lactuna indica* L., Heliothis armigera.

1. Introduction

Antimicrobial resistance (AMR), a phenomenon of changes in microbes that cause a reduction in the effectiveness of drugs used to treat infectious diseases, has emerged and is one of the biggest public health threats in this century. According to the 2019 Antimicrobial Resistance Collaborators prediction, AMR would be the cause of 4.95 million deaths across 204 countries and territories [1]. As many as 10 million deaths worldwide could result from AMR by 2050, according to a 2016 review of the subject [2]. AMR has a significant disease burden, hence research and development of novel medications must be started right away [1]. Spices are one of the natural products that are rich in active substances and have been widely studied to assess their potential antimicrobial activity in the context of discovering new antimicrobial drugs [3].

Spices hold a crucial place in human food habits and are integrated into the culinary customs of all cultures worldwide [3]. Over time, aromatic herbs and spices have been utilized as natural preservers, flavor boosters, coloring elements, and dietary additions to promote well-being and better health [4]. According to reports, the populations of more than 80% of World Health Organization (WHO) member states use herbal and natural products [5]. Based on their possible health benefits, between 5 and 10% of adult Americans utilize botanical supplements such as spices [4]. In Indonesia, almost 80% of people utilize medicinal herbs to prevent and treat illness [6].

Pliek U (Acehnese language) is one of the spices whose health benefits have been extensively studied. A traditional spice blend known as "*Pliek U*" is used in Acehnese cooking, an Indonesian culinary style exclusive to the Aceh area of northern Sumatra. *Pliek U* is made by fermenting coconut meat (Cocos nucifera L.) for a few days [7-10]. Acehnese have traditionally utilized *Pliek U* and its oil for cooking (rujak seasoning, chili sauce, vegetables) and medicinal purposes (reduces fever, promotes wound healing, eases joint stiffness, eases stomach pain, and allays headaches) [8]-[12].

Previous preclinical studies have demonstrated *Pliek U*'s antimicrobial properties, including its ability to operate as an antibacterial [8], antifungal [13], and antiviral [14,15]. Additionally, several earlier researchers created the antimicrobial preparation *Pliek U* as a functional food [8,13] and topical ointment [16,17]. Although many studies have examined the antimicrobial effects of *Pliek U*, there is a lack of comprehensive information on the characteristics of *Pliek U* and how it is produced, its chemical composition, and its mechanism of action as an antimicrobial. Therefore, this literature review aims to explore the characteristics, production, chemical composition, and antimicrobial mechanisms of *Pliek U*, to provide a foundation for future *Pliek U*-related research and development.

2. Methods

The literature search was carried out using PubMed, Science Direct, Springer Link, and Google Scholar databases in January 2024 using the keywords "*Pliek U*", "Coconut Flesh", and "Antimicrobial". The inclusion criteria used in this study were articles discussing the antimicrobial activity of *Pliek U*, published in the last 10 years. Meanwhile, the exclusion criteria were articles that were incomplete and did not use Indonesian or English. Researchers then carried out screening and overall reading of articles. The results of the main article analysis obtained are displayed in tabular form. Other information related to the topic is presented in narrative form.

3. Result and Discussion

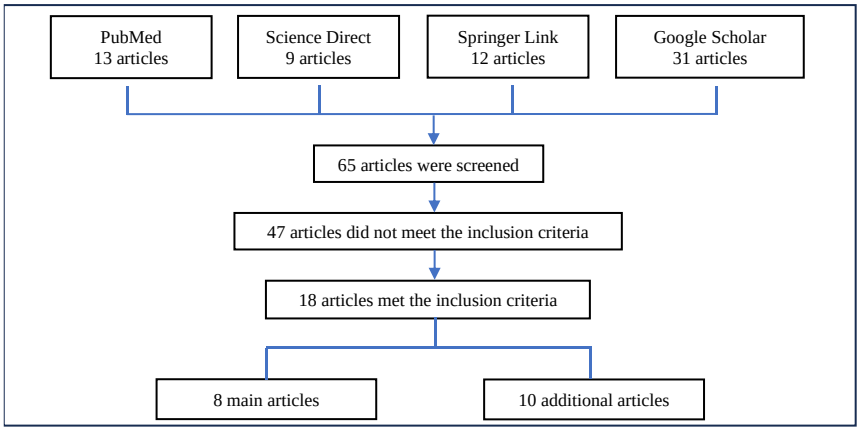

Figure 1. Article Screening Flow

The literature search process resulted in 65 articles. Based on the final results of screening articles according to inclusion and exclusion criteria, 8 main articles and 10 additional articles were obtained. The article screening flow can be seen in Figure 1. The results of the main article analysis can be seen in Table 1, while the analysis of additional articles is described in narrative form in several subheadings below.

3.1 Pliek U Characteristics

Pliek U is a common powder from Aceh Province, Indonesia, used in traditional recipes. It may be kept for a long time before use and is prepared from dried, fermented coconut (Cocos nucifera L.) endosperm [18]. As a traditional dish blended with the main meal, *Pliek U* is quite popular among the Acehnese [9,19]. "Patarana" is an additional term for *Pliek U* that is used [20]. This is easily recognizable because of its dark color, strong scent, rancid smell, slight sour, and taste [13,21]. Figure 2 shows the shape of the *Pliek U*.

Figure 2. *Pliek U* [21]

3.2 Pliek U Production

To prepare *Pliek U*, old or semi-old coconuts must be split (not open) and incubated for four to five days at room temperature (29 to 36 ºC). Then, the shredded coconut meat must be incubated for four to five days in a closed container without light exposure. The meat then be dried in the sun and pressed for more than five days using a special hydraulic pressure tool called “Peneurah” that separates the oil from the meat. The final products are *Pliek U* oil (also known as Minyeuk Simplah or Minyeuk Reutek) and coarse *Pliek U* powder. The *Pliek U* processing procedure generally takes 15 to 20 days [15,19,22]. This food has undergone spontaneous (accidental) fermentation or is preserved without the use of a starting culture [7,8,9,10,13]. Different modifications that arise throughout the fermentation process lead to different metabolites that are created from the original components or as a result of fermentation and may be discovered in the final product [10]. Figure 3 shows the production scheme for *Pliek U*.

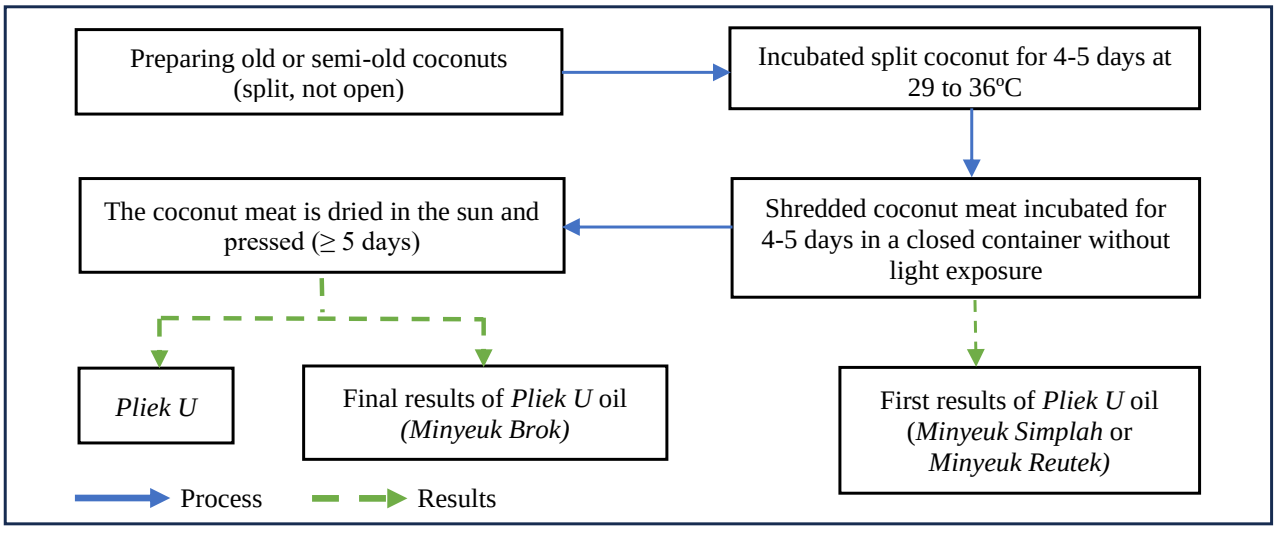

Figure 3. *Pliek U* Production Scheme.

3.3 Pliek U Chemical Composition

Pliek U has the potential to be exploited as a therapeutic source due to its unique chemical composition [14]. One of natural substance that serves as a source of fatty acids is *Pliek U*. Gas Chromatography-Mass Spectrometry (GC-MS) study was performed on the *Pliek U* extract to ascertain the composition of different fatty acids. These include lauric acid (C12), capric acid (C10), caprylic acid (C8),

palmitic acid (C16), myristic acid (C14), oleic acid (C18), palmitoleic acid (C16), linoleic acid (C18), tetradecanedioic acid, 7-10-13-hexadecanedioic acid, 9-12-15-octadecanedioic acid, along with esters and alcohols. Among these, lauric acid is the most prevalent [9,14,23]. According to Arpi in [15], each of them belongs to the medium-chain fatty acids (MCFA) category.

As an antibacterial, antifungal, antiprotozoal, and antiviral, lauric acid is significant [14,16]. Because it may be changed into monolaurate in the bodies of humans and other animals, lauric acid (C12), an MCFA, has a crucial purpose. Antiviral, antibacterial, and antiprotozoal properties are shared by monolaurate. This substrate is essential for the production of monoglycerides as antibacterials due to its extremely high amount of medium- and short-chain saturated fatty acids [10,16].

Several things contribute to *Pliek U*'s high fatty acid content, one of which is the method of processing it—breaking the coconut apart during the fermenting process. The more coconut is opened, the more it is exposed to contamination from microorganisms, particularly mold, which produces high levels of free fatty acids, and from drying it in the sun or other light source, which starts the oxidation process that produces short-chain free fatty acids and ketones. A hydrolysis process also occurs in tandem with this event. The breakdown of fat into glycerol and free fatty acids can be sped up by light [15].

Many of the health advantages associated with coconut eating are assumed to be attributable to phytochemical components found in meat and coconut oil extracts, which were tested for phytochemical compounds. These chemicals included flavonoids, carotenoids, phenolic acids, saponins, glycosides, tannins, alkaloids, glycosides, and terpenoids [24].

3.4 Lactic Acid Bacteria In Pliek U Fermentation

Lactic acid bacteria (LAB) are a group of microbes that play an important role in the fermentation process of various types of traditional food [8]. During the fermentation, LAB produces metabolites including organic acids, diacetyl, hydrogen peroxide, and bacteriocin which will cause changes in taste and shape and can inhibit destructive bacteria and pathogens [8,13].

3.5 Pliek U Antimicrobial Activity

A compilation of previous preclinical studies evidence on the topic of *Pliek U*'s antimicrobial activity can be seen in Table 1 as follows.

Table 1. *Pliek U*'s Antimicrobial Properties Evidence

No	Author	Intervention	Method	Microbe	Control	Results
1	[7]	LAB isolated from *Pliek U* (*Lactobacillus plantarum*) with a colony count of 6.8 x 10^8 CFU/g	Identification of bacterial species by 16SrRNA gene amplification, antimicrobial activity using disc diffusion method *(in vitro*	*Escherichia coli* (*E. coli*) dan *Staphylococcus aureus (S. aureus*	Positive: Ciprofloxacin, Negative: None	a. *E. coli* isolate: E1, E2, E3, E4, E5 show inhibition zone of 7,5 mm, 8 mm, 7,2 mm, 7 mm, 6,8 mm. b. *S. aureus* isolate: E1, E2, E3, E4, E5 with inhibition zone 13 mm, 13,3 mm, 10 mm, 8,6 mm, 8,4 mm.
2	[14]	Fatty acids (lauric, capric, linoleic, caprylic, myristic, palmitic, oleic)	Molecular docking	Sars Cov-2 protein and Enzym	None	For 3CLpro (6LU7), E Protein (5X29), Plpro (6WX4), Spike Ectodomain Structure (6VYB), RdRp (6M71), and Plpro (6VXX), linoleic acid has the greatest interaction with the receptors with the lowest binding affinity of -4,9; -4,7; -5,8; -5,3; -4,3 and -5,5 kcal/mol.
3	[8]	LAB isolated from *Pliek U*	Diffusion method *(in vitro)*	*Candida albicans (C. albicans), S. Aureus, E. coli*	None	a. *S. aureus* isolates: SP1, SP2, SP3, SP4, SP5 show inhibition zone of none, 7 mm, 7.7 mm, 0.25 mm, 0.25 mm b. *E. coli* isolates: SP1, SP2, SP3, SP4, SP5 with an inhibition zone of 2.5 mm, 1.5 mm, none, none, 28 mm.

No	Author	Intervention	Method	Microbe	Control	Results
						c. *C. albicans:* no inhibition zone.
4	[9]	*Pliek U* crude extract (Solvent Ethanol, Hexane, Ethanol Residue)	Diffusion method *(in vitro)*	*Salmonella typhi*	Positive: Amoxicillin 10 µg, Negative: Aquades	*Pliek U* ethanol extract (EEP) shows an inhibition zone of 17.2 mm, *Pliek U* hexane extract (EHP) 0 mm, residual ethanol extract (EERP) 17.4 mm, positive control 25.6 mm, negative Control 0 mm
5	[16]	*Pliek U* ethanol extract ointment 5mg/ml	Diffusion method *(in vitro)*	*S. aureus*	Positive: none, Negative: ointment base	The inhibition zone: ointment base (P0) 0 mm, *Pliek U* extract 5mg/ml of 26 mm, *Pliek U* extract ointment 5mg/ml of 15 mm
6	[13]	*Pliek U* crude extract (Solvent hexane, ethanol residue, ethanol)	Diffusion method *(in vitro)*	*B. cereus, B. subtilis, S. aureus, C. albicans*	None	Average Inhibition Zone a. Hexane: *B. cereus* 6.67±0.47 mm; *B. subtilis & S. aureus* none, *C. albicans* 17.33±0.94 mm b. Ethanol residue: *B. cereus* 19.67±0.47 mm; *B. subtilis* 10.33±0.94 mm, *S. aureus* 18.33±0.47, *C. albicans* none. Ethanol: *B. cereus* 20.33±0.47 mm; *B. subtilis* 10.67±0.47 mm, *S. aureus* 19.33±0.47 mm, *C. albicans* 10.67±0.47 mm
7	[17]	*Pliek U* ethanol extract and hexane residue extract, cream formulation	Diffusion method *(in vitro)*	*S. aureus*	Positive: Clyndamicin, Negative: ethanol solvent and cream base	Ethanol *Pliek U* extract with average inhibition zone of 8.66±0.57 mm, Hexane residue extract and cream: none
8	[10]	*Pliek U* ethanol extract cream	Diffusion method *(in vitro)*	*S. aureus*	Positive: Krim Bevalex, Negative: Aquades	The average inhibition zone: F1 (3%) 12.65 mm, F2 (5%) 14.9 mm and F3 (7%) 12.92 mm.

3.6 Pliek U Antimicrobial Mechanism

Some of the antimicrobial mechanisms of *Pliek U* are as follows

3.1.1. Antibacterial

a. Fatty acids

When saturated fatty acids function as antibacterials, they often harm target cell membranes by acting directly on them [16]. Aside from that, suppression of the formation of cell walls is another inhibitory mechanism against bacteria [8].

b. Lactic acids as a result of LAB metabolism

During the LAB metabolic process, lactic acid accumulation of primary metabolites can enter microbial cells and at higher intracellular pH levels, dissociate to produce hydrogen ions. This lowers intracellular pH and interferes with the function of vital metabolites, substrate translocation, and oxidative phosphorylation. By neutralizing or expelling protons, bacterial cells will attempt to

maintain the pH of their internal environment. Bacteria need their growth energy to release protons, therefore this attempt will slow down their growth. The cytoplasmic pH will drop and the cell burden will be high if the extracellular acid concentration is high and the external pH is low. Cells of bacteria will perish. [8,9].

c. Bacteriocin as a LAB metabolic result
The existence of secondary metabolites in the form of bacteriocin compounds, can stop peptidoglycan production in its entirety, weakening the bacterial cell wall and causing lysis in the bacterial cell [8].

d. Compound polarity of the solvent in the extract
The compound's polarity may contribute to its antibacterial action. The active chemical components of *Pliek U* are extracted by Jalma & Zachreini in [9], using an ethanol solvent. The polar active chemicals are supposed to dissolve in the polar solvent like ethanol. Hydrophilic (polar) and lipophilic (non-polar) characteristics determine the polarity of antibacterial substances and influence the hydrophobic (non-polar) balance of bacterial cell walls. Bacterial cells will be impacted by this imbalance in two ways: directly, through membrane damage, and indirectly, through increased membrane permeability.

e. Phytochemical content
The efficiency of several antimicrobial methods of action has been explored, and it is contingent upon the type of phytochemical molecule involved. These mechanisms include blocking the synthesis of nucleic acids by interfering with the activity of bacterial DNA gyrase and topoisomerase IV, blocking the function of the cytoplasmic membrane, blocking the synthesis of cell envelopes, blocking energy metabolism, blocking the synthesis of cell walls, blocking efflux pumps in bacteria, blocking virulence dependent on bacterial enzymes, and blocking membrane-disrupting activities [25].

3.1.2. Antifungal

Sterols bond to antimicrobial agents, causing harm to fungal cell walls. Chitin, cellulose, β-glucan, mannan, chitosan, protein, lipids, and inorganic ions make up the cell walls of Candida albicans. Chitin production is often inhibited by antimicrobial substances that can also affect the development of fungal cell walls. Antimicrobials can also cause damage to microbial cell membranes by denaturing the proteins and lipids that comprise these membranes, which interferes with the germs' ability to exchange essential chemicals. Macrophages and ions exit the cell when the cytoplasmic membrane's integrity function is compromised, resulting in cell injury [8].

3.1.3. Antivirus

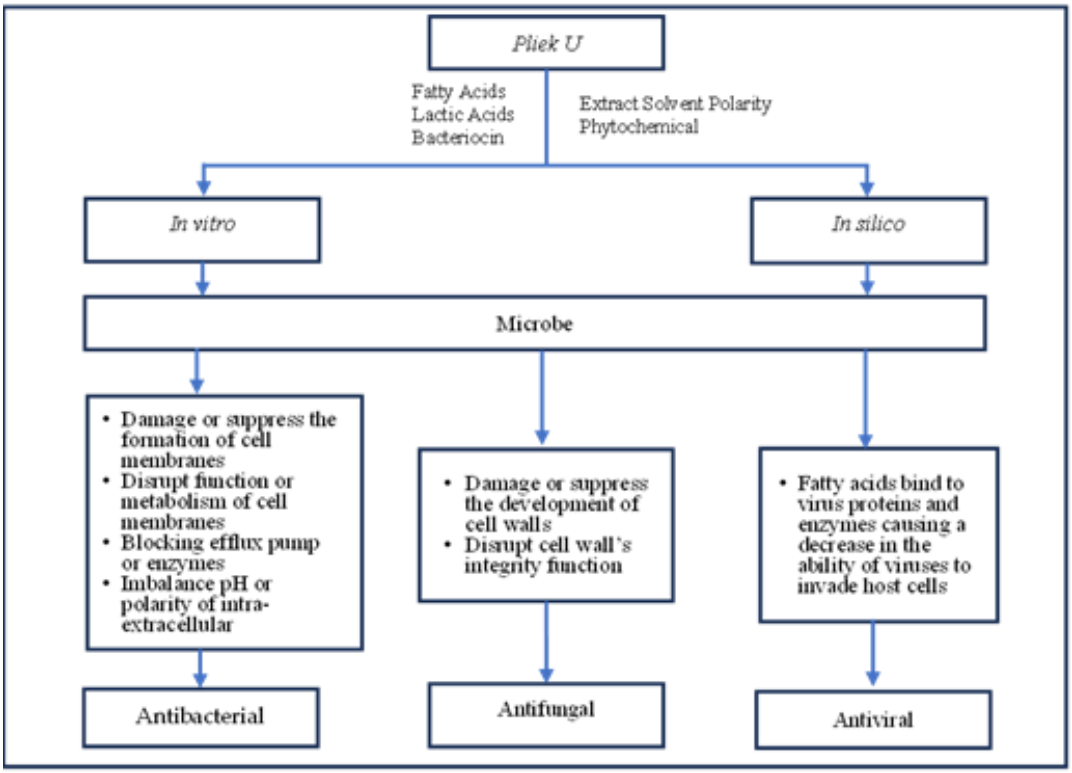

Figure 4. Summary of *Pliek U* Antimicrobial Properties

According to Khairan et al. in [14] molecular docking analysis, linoleic acid exhibits the best interaction with receptors, having the lowest binding affinity towards each of the following: PLpro (6WX4), E protein (5X29), RdRp (6M71), 3CLpro (6LU7) and Spike protein (6VXX). According to these data, linoleic acid binding to SARS-CoV2 proteins and enzymes may interfere with the virus's ability to bind to host cells, decreasing the virus's ability to infect COVID-19 patients. A summary of *Pliek U*'s Antimicrobial Properties can be seen in Figure 4.

4. Conclusion

Pliek U powder is easy to recognize because it has unique characteristics in the form of dark color, strong aroma, rancid odor, slightly sour, and taste after being produced by traditionally fermenting coconut flesh for 15-20 days. This powder is rich in fatty acids, phytochemical compounds, and lactic acid bacteria produced during the fermentation process with its metabolites. Prior studies have proven that *Pliek U* has antibacterial, antifungal, and antiviral effects. The active ingredient works as an antimicrobial by damaging microbial cell walls, changing intra-extracellular pH or polarity, disrupting cell function, and preventing invasion of host cells. Based on the evidence, *Pliek U* can be encouraged as a source of antimicrobials and it needs to continue to be developed and researched from preclinical to clinical trials.

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