ORIGINAL ARTICLE

Antimicrobial Activity of Potassium Aluminum Sulfate and *Phyllanthus emblica* Leaves Extract against Common Skin Pathogens, In vitro Study

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Background: Because of the growing use of antibiotics worldwide, the expansion of antibiotic-resistant bacteria becomes increasingly threatening to medical treatment. Many natural substances have been reported to have antimicrobial properties.

Objective: To evaluate the antimicrobial property and the synergistic interaction between potassium aluminum sulfate and *Phyllanthus emblica* leaves against common skin pathogens, including antibiotic-resistant *Cutibacterium acnes*, antibiotic-susceptible *C. acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Materials and Methods: The present study was an experimental, cross-sectional study. The microorganisms were tested using the agar well diffusion method and the broth microdilution method to determine the minimum inhibitory zone (MIZ) and minimum inhibitory concentration (MIC), respectively. Finally, the synergistic interaction was evaluated by checkerboard assay to determine the fractional inhibitory concentration (FIC) index.

Results: The highest inhibition zone, at 21 mm, was observed on *P. aeruginosa* with the alum concentration of 20% w/v. The lowest inhibition zone, at 6 mm, was noticed on *S. aureus* with 2.5% concentration of alum. *P. emblica* extract at a concentration of 5 mg/mL and 25 mg/mL demonstrated antimicrobial activity against *S. aureus*, *S. epidermidis*, *M. luteus*, and *C. acnes*. The lowest MIC of alum solution (0.0195% w/v) was seen on *S. epidermidis*. The lowest MIC of *P. emblica* extract, at 0.097 mg/mL, was spotted on *C. acnes*. The highest FIC index, of 0.421, was observed on *M. luteus* and the lowest FIC index, at 0.046, was seen on *S. aureus*.

Conclusion: Both alum and *P. emblica* leaves exhibited substantial antimicrobial activity and can be combined for enhanced efficacy.

Keywords: Alum; *Phyllanthus emblica* leaves; Antibiotic-resistant *Cutibacterium acnes*; Antimicrobial activity; Synergistic interaction

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Various skin microorganisms can cause or be associated with cutaneous diseases. For instance, *Cutibacterium acnes* is correlated with acne vulgaris, while *Staphylococcus aureus* is responsible for the majority of skin and soft tissue infections, including impetigo, cellulitis, and furuncles[1-3]. *Staphylococcus epidermidis* is recognized as an important opportunistic pathogen and the most common cause of infections related to indwelling medical devices[4]. *Micrococcus luteus* is also an opportunistic pathogen, reported to cause localized cutaneous infections in immunocompromised patients, particularly those with HIV-1 infection[5].

Pseudomonas aeruginosa, is a Gram-negative bacilli, can infect skin and soft tissues, with infections characterized by greenish discharge and a fruity odor, notably in conditions like ecthyma gangrenosum and green nail syndrome[6]. *Candida albicans* commonly reside in moist areas such as vagina, mouth, and skin folds, with Candidiasis occurring when host conditions change[7].

Currently, antibiotics or antifungals are used to treat both skin infections and certain non-infectious skin diseases. However, antibiotic-resistant bacteria and antifungal-resistant fungi are rapidly increasing, reducing the effectiveness of these drugs[8]. Many natural products have demonstrated antimicrobial properties in various recent studies.

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Potassium aluminum sulfate [$KAl(SO_4)_2$] is an odorless, colorless crystalline solid widely found in parts of Asia. It has been used for a long time in various industries, including as a food preservative, water purifier, and deodorant[9]. Multiple studies have shown that potassium aluminum sulfate exhibits antimicrobial properties against bacterial and fungal species, including *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, and *S. aureus*[10].

Phyllanthus emblica Linn. (or *Emblica officinalis* Gaertn.) is commonly found throughout tropical Southeast Asia[11]. Its leaves contain compounds such as gallic acid, chebulic acid, ellagic acid, chebulinic acid, chebulagic acid, amlic acid, and alkaloids like phyllantine and phyllantidine, which possess antimicrobial and anti-inflammatory properties[12]. Therefore, the present study aimed to evaluate the antimicrobial properties and synergistic interaction of potassium aluminum sulfate and *P. emblica* against common skin pathogens.

Materials and Methods

Preparation of tested microorganisms

All microorganisms were acquired from the Department of Microbiology, Faculty of Medicine, Srinakharinwirot University. Bacteria used in the present study included clinical isolates of *C. acnes*, standard strains of *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *M. luteus* (ATCC 10240), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 10231).

C. acnes strains used in the present study were clinically isolated in a previous study[13]. Three antibiotic-resistant strains, which were *C. acnes* no. 8, which is clindamycin and erythromycin resistant, *C. acnes* no. 23, which is clindamycin and erythromycin resistant, and *C. acnes* no. 41, which is erythromycin resistant, and three antibiotic-susceptible strains, including *C. acnes* no. 50, 69, and 74 that were cultured under ananaerobic conditions.

Preparation of potassium aluminum sulfate solutions

Potassium aluminum sulfate was purchased from Merck (Darmstadt, Germany). Fifty grams of potassium aluminum sulfate were dissolved in 100 mL distilled water to obtain 50% concentration. Later, a 50% potassium aluminum sulfate solution was diluted into four concentrations 2.5, 5, 10, 20% w/v, which is a slight modification from the previous studies[14,15].

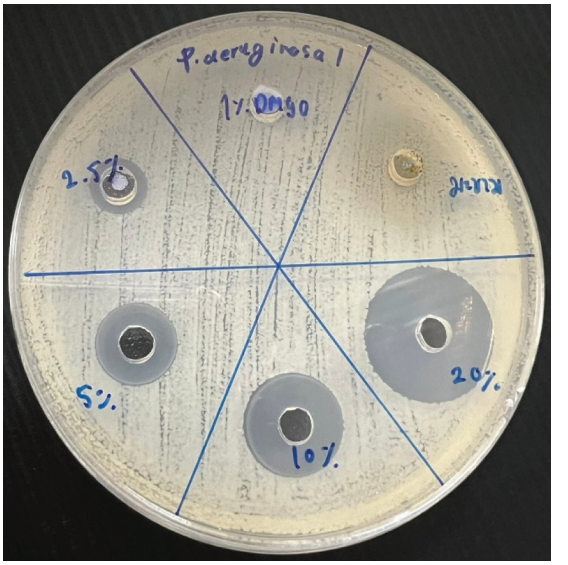

Figure 1. Agar well dilution method.

Preparation of *P. emblica* leaf extracts

P. emblica leaf powder was acquired from the Department of Microbiology, Faculty of Medicine, Srinakharinwirot University. The plant was extracted with 95% ethanol, by maceration method. Then, the *P. emblica* leaf extract was dissolved in dimethyl sulfoxide (DMSO) (Amresco, Ohio, USA) to create two concentrations at 5 mg/mL and 25 mg/mL.

In vitro antibacterial activity testing of alum and *P. emblica* extract

Antimicrobial activity of potassium aluminum sulfate and *P. emblica* extract was evaluated using the agar well diffusion method (Figure 1). *C. acnes* was cultured in Brain-Heart infusion medium supplemented with 10% horse serum. The other tested bacteria were cultured in Mueller-Hinton medium. *C. acnes* was adjusted to 1×10^8 CFU/mL. While other tested bacteria were adjusted to 1×10^7 CFU/mL. *C. albicans* was cultured on Sabouraud dextrose agar (SDA) and adjusted to 1×10^8 CFU/mL suspensions compared to 0.5 McFarland standard.

Bacteria were spread on the surface of the appropriate medium. Subsequently, 4-mm diameter wells were created in the agar using a sterile cork borer. Different concentrations of potassium aluminum sulfate solutions and *P. emblica* extracts were filled in each well at 50 µl each. DMSO was used as negative controls. *C. acnes* was incubated at 37°C for 72 hours under anaerobic conditions with 80% nitrogen, 10% carbon dioxide, and 10% hydrogen. Other tested bacteria were incubated at

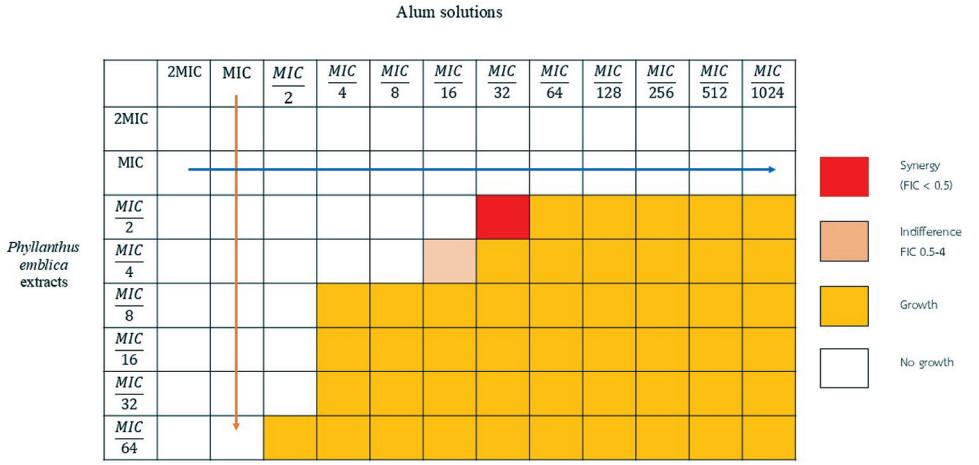

Figure 2. Checkerboard analysis setup.

37°C for 24 hours. *C. albicans* was incubated at 30°C for 72 hours. Later, the diameter of inhibition zones around the well were measured and recorded in millimeters.

Broth microdilution method was used to determine the minimum inhibitory concentration (MIC), a serial two-fold dilution of potassium aluminum sulfate solutions and *P. emblica* extracts were produced in 96-well microtiter plates in an initial concentration of the MIC acquired from agar well diffusion method of 50 µl. Then 50 µl of suspension for each bacterium and fungus (1×10^6 CFU/mL) was added in each well. Also 30 µl of 0.02% resazurin was added to each well to evaluate the growth of microorganisms. If the color of resazurin did not turn from blue to red, then the growth of microorganisms was inhibited. Both antibacterial activity testing methods were performed in two separate experiments.

Synergy test

Synergistic interaction between potassium aluminum sulfate and *P. emblica* extract was evaluated by checkerboard analysis[16] (Figure 2). The 96-well microtiter plates were used in the method. Eight concentrations of *P. emblica* extract and twelve concentrations of potassium aluminum sulfate were added to each well. The concentrations were calculated from the MIC of each tested solution[17]. The concentrations were prepared in 2-fold serial dilutions, the initial concentration was twice the MIC of *P. emblica* extract and twice of the potassium aluminum sulfate solution. The fractional inhibitory concentration (FIC) index was the sum of the FIC values of the tested compound. The FIC of each tested solution was calculated by dividing the concentration of the substance in that well by the MIC of that substance alone[18]. Synergy was identified when the FIC index was less than or equal to 0.5. No interaction was determined when the FIC index is between 0.5 and 4. Antagonism was interpreted when the FIC index was more than 4[17].

Statistical analysis

Statistic and data analysis used the Stata Statistical Software, version 16 (StataCorp LLC, College Station, TX, USA) to perform statistical analysis in the present study. Descriptive statistics were used in the present study. Minimum inhibitory zone (MIZ) and MIC were reported as the mean of the two separate experiments.

Ethical approval

The present study adhered to the principles outlined in the Declaration of Helsinki. However, ethics approval for the present study was not required by Srinakharinwirot University.

Results

In vitro antibacterial activity testing of potassium aluminum sulfate and *P. emblica* extract

In accordance with Davis and Stout's classification, the zones of inhibition could be graded into four groups, which included weak response with a diameter of less than 5 mm, moderate response with a diameter of 5 to 10 mm, strong response with a diameter of 10 to 20 mm, and very strong response with a diameter of more than 20 mm.

Potassium aluminum sulfate solutions of 10% and 20% demonstrated strong inhibitory effects on antibiotic-susceptible *C. acnes* no. 50 and 74.

Table 1. MIZ of potassium aluminium sulfate solutions by agar well diffusion method

Tested organisms	MIZ (mm)			
	Alum solutions (% w/v)			
	2.5%	5%	10%	20%
Antibiotic-resistant strains				
C. acnes (no. 8)	-	-	-	-
C. acnes (no. 23)	-	-	-	-
C. acnes (no. 41)	-	-	-	-
Antibiotic-susceptible strains				
C. acnes (no. 50)	-	-	10	12
C. acnes (no. 69)	-	-	-	-
C. acnes (no. 74)	-	-	11	15
S. aureus	6	11	13.5	23
S. epidermidis	9	12	15.5	24.5
M. luteus	8.5	13	19	26
P. aeruginosa	7.5	12	14.5	21.5
C. albicans	-	-	-	-

MIZ=minimum inhibitory zone; w/v=weight per volume
-, no inhibitory zone

Table 2. MIZ of *P. emblica* extracts by agar well diffusion method

Tested organisms	MIZ (mm)	
	P. emblica extract (5 mg/mL)	*P. emblica* extract (25 mg/mL)
Antibiotic-resistant strains		
C. acnes (no. 8)	-	10
C. acnes (no. 23)	-	24
C. acnes (no. 41)	-	22
Antibiotic-susceptible strains		
C. acnes (no. 50)	-	20
C. acnes (no. 69)	-	26
C. acnes (no. 74)	-	8
S. aureus	10	Not done
S. epidermidis	17	Not done
M. luteus	11.3	Not done
P. aeruginosa	-	-
C. albicans	-	-

MIZ=minimum inhibitory zone
-, no inhibitory zone

Table 3. MIC of potassium aluminium sulfate solutions and *P. emblica* extracts by broth microdilution method

Tested organisms	MIC_{Alum} (% w/v)	$MIC_{P. emblica}$ (mg/mL)
Antibiotic-resistant strains		
C. acnes (no. 8)	-	0.39
C. acnes (no. 23)	-	0.39
C. acnes (no. 41)	-	0.195
Antibiotic-susceptible strains		
C. acnes (no. 50)	0.078	0.097
C. acnes (no. 69)	-	0.097
C. acnes (no. 74)	0.078	0.195
S. aureus	0.156	0.78
S. epidermidis	0.0195	0.39
M. luteus	0.029	0.29
P. aeruginosa	0.156	-
C. albicans	-	-

MIC=minimum inhibitory concentration; MIC_{Alum}=MIC of alum solution; $MIC_{P. emblica}$=MIC of *P. emblica*; w/v=weight per volume
-, not eligible for this process

However, potassium aluminum sulfate solutions failed to inhibit antibiotic-resistant *C. acnes*. The 20% potassium aluminum sulfate showed a very strong inhibitory effect on *S. aureus*, *S. epidermidis*, *M. luteus*, and *P. aeruginosa*. Nevertheless, potassium aluminum sulfate exhibited no antifungal activity against *C. albicans*. *P. emblica* extracts at 25 mg/mL, exhibited very strong antibacterial effects on most of *C. acnes* including both antibiotic-resistant and antibiotic-susceptible strains. *P. emblica* extracts also showed strong inhibitory effects against *S. aureus*, *S. epidermidis*, and *M. luteus* with only 5 mg/mL concentration. Nevertheless, *P. emblica* extracts were ineffective in inhibiting the growth of *P. aeruginosa* and *C. albicans* (Table 1, 2).

The lowest MIC of potassium aluminum sulfate solution, found on *S. epidermidis*, was 0.0195 mg/mL, while the highest MIC of potassium aluminum sulfate solution, which was 0.156 mg/mL, was found in *S. aureus* and *P. aeruginosa*. The lowest MIC of *P. emblica* extract, observed in antibiotic-susceptible *C. acnes*, was 0.097 mg/mL, whereas 0.78 mg/mL was the highest MIC *P. emblica*, detected in *S. aureus* (Table 3).

Synergy test

Synergistic interaction between potassium aluminum sulfate and *P. emblica* extract was evaluated by checkerboard analysis. FIC index was the sum of FIC_{Alum} and $FIC_{P. emblica}$ on the same organism. FIC index determined the interaction between the two tested substances, whether they were synergistic, antagonistic, or non-interactive.

The results showed that every FIC index of tested bacteria was below 0.5, thus there was synergistic interaction between potassium aluminum sulfate solution and *P. emblica* extracts on antibiotic-susceptible *C. acnes* no. 50 and 74, *S. aureus*, *S. epidermidis*, and *M. luteus* (Table 4).

Discussion

The present study found that potassium

Table 4. FIC index of potassium aluminium sulfate solutions and *P. emblica* extracts by checkerboard assay

Tested organisms	FIC_{Alum} (% w/v)	$FIC_{P.\ emblica}$ (mg/mL)	FIC index
Antibiotic-susceptible strains			
C. acnes (no. 50)	0.031	0.123	0.154
C. acnes (no. 74)	0.007	0.123	0.130
S. aureus	0.015	0.031	0.046
S. epidermidis	0.123	0.062	0.185
M. luteus	0.338	0.083	0.421

FIC=fractional inhibitory concentration; FIC_{Alum}=FIC of alum solution; $FIC_{P.\ emblica}$=FIC of *P. emblica*; w/v=weight per volume

aluminum sulfate showed stronger inhibitory effect on antibiotic-susceptible *C. acnes* than antibiotic-resistant *C. acnes*. However, the exact mechanism of potassium aluminum sulfate resistance is not fully understood. Since no previous study has been conducted to evaluate the antimicrobial effect of alum on *C. acnes*, more studies should be carried out to compare the results among them. Potassium aluminum sulfate also demonstrated a very strong inhibitory effect on both Gram-positive and Gram-negative bacteria. The results concurred with a previous study[19] that demonstrated that potassium aluminum sulfate exhibited broad-spectrum antibacterial potency, particularly against Gram-negative bacteria.

The results of the present study showed that alum has antibacterial effects that increase with higher alum concentrations and concurred with the study of Ali[15] who described that the inhibition zones of six bacterial isolates (*S. aureus*, *K. pneumoniae*, *Proteus vulgaris*, *P. aeruginosa*, *Pseudomonas fluorescens*, *E. coli*) and one fungus (*C. albicans*), increased with the incremental concentration of alum solutions. However, none of the tested potassium aluminum sulfate concentrations exhibited antifungal activity against *C. albicans*.

In contrast to potassium aluminum sulfate, *P. emblica* extracts in the present study demonstrated strong inhibitory effects on both antibiotic-susceptible and antibiotic-resistant *C. acnes*. This agreed with the previous study of Asavaphark et al.[20] that reported that the mean inhibition zones of *P. emblica* extracts on antibiotic-susceptible and antibiotic-resistant *C. acnes* were 17.48 and 13.76 mm, respectively. Furthermore, the results indicated that *P. emblica* extracts exhibited a stronger inhibitory effect against Gram-positive bacteria than against Gram-negative bacteria and fungi, which concurred with an earlier study of Gandhi[21]. Inhibition zones were observed at a concentration of 25 mg/mL for both *S. aureus* and E. coli with mean diameters of 19 mm and 9 mm, respectively. However, the inhibition zones for *C. albicans* began to appear clearly at a concentration of 75 mg/mL with a mean diameter of 7±0.49 mm. It was concluded that *P. emblica* leaf extract was more effective against Gram-positive bacteria than Gram-negative bacteria and showed very limited efficacy against fungi.

Regarding synergistic effect of potassium aluminum sulfate solutions and *P. emblica* extracts, all of the FIC indexes were below 0.5 which indicated synergistic interaction between potassium aluminium sulfate solutions and *P. emblica* extracts on every studied organism. The highest FIC index (0.421) was reported on *M. luteus* and the lowest FIC index was seen on *S. aureus*. The antimicrobial mechanism of potassium aluminum sulfate solution was not fully understood. However, studies suggested that it damaged or reduced the acidity of bacterial cell walls. *P. emblica* extract has tannin as the main active ingredient for antibacterial action. Tannin or tannic acid is a natural polyphenol that can strongly bind to proteins or metal ions and consequently forms complexes[22]. Hence, it can inhibit bacteria's proteins transport, microbial adhesion, and extracellular microbial enzymes such as lipase, protease, or hyaluronidase[23]. This study found that there was a synergistic interaction between potassium aluminum sulfate solution and *P. emblica* extract. Thus, the mechanism of synergy might be explained as the bacteria's cell walls were weakened by potassium aluminum sulfate solution. Consequently, *P. emblica* extract, containing tannin, can react more easily to bacterial proteins.

Moreover, this study found that the combinations of potassium aluminum sulfate solutions and *P. emblica* extracts at high concentration exhibited unexpected incidents. In particular, the concentrations, which were higher than the MIC, of both potassium aluminum sulfate solution at 0.078%, and *P. emblica* extracts at 0.195 mg/mL, failed to inhibit *C. acnes* growth. On the contrary, the combinations of low concentration potassium aluminum sulfate and *P. emblica* extracts tended to inhibit bacteria's growth. This phenomenon could be explained as tannin-metal complex formation. Slabbert reported that aluminum can form colorless complexes with tannins[22]. Another study concluded that the formation of tannin-metal complexes was enhanced by increasing initial tannic acid concentration[24]. In summary, high concentration of potassium aluminum sulfate solutions and

P. emblica extracts reacted to each other and formed complexes more readily than at low concentrations. Therefore, they lost their antibacterial property more than at low concentrations.

According to Food Safety Commission of Japan (FSCJ), aluminum in potassium aluminum sulfate has no genotoxic or carcinogenic effects in humans. The association between aluminum and the effects on bone or neurological diseases, such as Alzheimer's disease, were still controversial[25]. Aluminum derivatives, such as aluminum oxide and aluminum hydroxide, have been approved by the U.S. Food and Drug Administration (FDA) and European regulations for use in various cosmetics and personal care products, including antiperspirants and toothpaste. Moreover, only a small amount of aluminum was absorbed through the skin. Therefore, it should not pose a risk to human health[26].

This study has limitations. Firstly, it included limited strains of microorganisms that might affect the generalizability of the results. Secondly, the quality and concentration of the *P. emblica* extracts could be inconsistent due to the maceration method, therefore, more studies are needed to compare the results from each extraction method. Lastly, since this research is an in-vitro study, the authors suggest that the clinical trials should be conducted to investigate the therapeutic effects of both potassium aluminum sulfate and *P. emblica* against cutaneous infections, caused by *C. acnes*, *S. aureus*, *S. epidermidis*, *M. luteus*, and *P. aeruginosa*.

Conclusion

In summary, the present study demonstrated that potassium aluminum sulfate solutions exhibit antibacterial effect against only antibiotic-susceptible *C. acnes*, with no inhibitory effect on antibiotic-resistant strains. While these solutions showed significant inhibitory effects on both Gram-positive and Gram-negative bacteria, they were ineffective against *C. albicans*. In contrast, *P. emblica* extracts displayed a strong inhibitory effect on Gram-positive bacteria, including antibiotic-resistant *C. acnes*, but had no effect on *P. aeruginosa* and *C. albicans*. A synergistic interaction between potassium aluminum sulfate solutions and *P. emblica* extracts was observed. However, at high concentrations, the formation of tannin-metal complexes caused both substances to lose their antimicrobial properties.

What is already known in this topic?

Potassium aluminum sulfate and *P. emblica* are known to have antimicrobial activity against various microorganisms. However, no studies have been conducted on the antibacterial properties of potassium aluminum sulfate on *C. acnes* or the synergistic interaction between potassium aluminum sulfate and *P. emblica*.

What does this study add?

Potassium aluminum sulfate could inhibit the growth of clinically-isolated *C. acnes*. Moreover, synergistic interactions were observed between potassium aluminum sulfate solutions and *P. emblica* extracts.

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Conflicts of interest

The authors declare no conflict of interest.

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