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ORIGINAL ARTICLE

Potassium Aluminium Sulphate (Alum) Inhibits Growth of Human Axillary Malodor-Producing Skin Flora in Vitro

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ABSTRACT

Introduction: Axillary malodor is caused by microbial biotransformation of non-smelling molecules present in apocrine secretions, into volatile odorous molecules. This study aimed to determine the antimicrobial activities of potassium aluminium salts (alum) against four malodor-producing axillary bacterial flora, as an alternative natural product for reducing axillary malodor. **Methods:** The antimicrobial activity of alum against axillary bacterial flora [*Micrococcus luteus* (ATCC 49732) (*M. luteus*), *Staphylococcus epidermidis* (ATCC 14990) (*S. epidermidis*), *Corynebacterium xerosis* (ATCC BAA-1293) *C. xerosis* and *Bacilus subtilis* (ATCC 19659) (*B. subtilis*)], was tested in vitro using broth dilution method for different concentrations (0.937 – 20mg/mL) on Luria-Bertani broth. Subculture was done to determine colony-forming units (CFUs) and the minimum inhibitory concentrations (MICs). **Results:** Alum showed excellent inhibitory effects on all tested bacteria. The lowest MIC of alum was against *C. xerosis*, at 1.88 mg/mL. *M. luteus*, *B. subtilis* and *S.epidermidis* showed a higher MIC of 3.75, 5.00 and 7.50 mg/mL, respectively. All of the tested bacteria were completely inhibited at a concentration of 7.50 mg/mL. **Conclusions:** This study revealed that alum has excellent antimicrobial effects against axillary malodor -producing bacteria and is recommended to be used either directly by topical application or as an active ingredient in deodorants and antiperspirants.

Keywords: Antimicrobial, Potassium aluminium, Alum, Malodorous, Axilla

INTRODUCTION

Body odors especially in axilla and foot are unique scents of adults. The eventual source of axillary odor is apocrine sweat which is naturally odorless and sterile [1]. The human scent is genetically controlled and systemically influenced by dietary and medicinal intake [2]. Generation of malodor on axilla of the human body is caused by the microbial biotransformation of odorless volatile fatty acids secreted by apocrine glands into volatile odorous molecules. This results in unpleasant smell which might cause social embarrassment and reduce self-confidence [3]. Excessive sweating and moisture at axilla makes the environment optimal for sustained growth and proliferation of bacteria and this contributes to unpleasant odor [1]. Axillary flora was found to be a stable mixture of gram-positive bacteria like *Micrococcus* spp., coagulase negative staphylococci, *Corynebacterium* spp., and *Bacillus spp*. [4]. Different bacteria produce different odors according to their sugar digestion. *Bacillus subtilis* and *S. epidermidis* contribute to foot odor, while *S. epidermidis* and *Corynebacterium* spp. contribute to underarm odor [5,6]. Although higher bacterial densities were associated with higher malodor intensities in axilla, there was no association between odor intensities and any particular microorganism [7]. Alum (molecular formula: $KAl(SO_4)_2.12H_2O$) is a colourless, odorless crystalline solid that turns white in air [8]. The medical uses of alum in mouth rinses, vaccines development, haemostasis and inhibition of *V. cholerae* growth in water have been described [9-12]. However, to date there is no study on the effect of alum salt against bacterial skin flora. Therefore, the aim of this study was to evaluate the inhibitory effects of alum solution against certain bacterial skin flora namely *Micrococcus luteus*, *Staphylococcus epidermidis*, *Corynebacterium xerosis* and *Bacilus subtilis* that cause axillary malodor.

METHODS

Bacterial strains

Four standard bacterial strains which cause axillary malodor were used in this study. All bacteria were obtained from Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM). Bacterial strains used in this study were *Micrococcus luteus* (ATCC 49732) (*M. luteus*), *Staphylococcus epidermidis* (ATCC 14990) (*S. epidermidis*), *Corynebacterium xerosis* (ATCC BAA-1293) *C. xerosis* and *Bacilus subtilis* (ATCC 19659) (*B. subtilis*). All bacterial strains were inoculated in blood agar and incubated for 24 hours at 37°C. The bacterial suspension was prepared by inoculating two bacterial colonies in Luria-Bertani broth (LB - broth) for 3 hours at 37°C and the turbidity was adjusted in phosphate buffered saline to 0.5 McFarland's scale.

Preparation of alum solutions

Pure aluminum potassium sulfate (Sigma-aldrich, KL, Malaysia) was used in this study. Alum crystals were completely dissolved in hot sterile distilled water at 100 °C, to obtain a final concentration of 1 g/mL, at pH 3.6. Broth dilution method was used to assess the antimicrobial activity of alum against previously mentioned strains. Different concentrations ranging from (20, 15, 10, 7.5, 5, 3.75, 2.5, 1.875, 1.25, 0.937 mg/mL) of alum broth media were prepared aseptically. Positive control LB - broth without alum crystal was used to confirm the growth of the tested bacteria. For negative control a non – inoculated LB - broth was used to exclude contamination.

Evaluation of antimicrobial activity of the alum solution

The 0.5 McFarland standard (1×10^8 CFU/mL) of overnight pure culture bacterial suspensions were made in LB - broth. Ten μL of each suspension (1×10^6 CFU/mL) was inoculated in five mL of different concentrations of alum broth media and incubated at overnight 37ºC [13]. Subsequently, 100 μL of each inoculated alum broth media was streaked evenly onto blood agar plate to obtain uniformly distributed growth. The streaked plates were incubated aerobically at 37ºC and inspected after 24 hours. The broth cultures showing heavy growth were serially diluted with sterile broth before being incubated onto blood agar as mentioned. After incubation, the number of bacterial colonies was counted and expressed as colony forming unit per mililiter (CFU/mL).

RESULTS

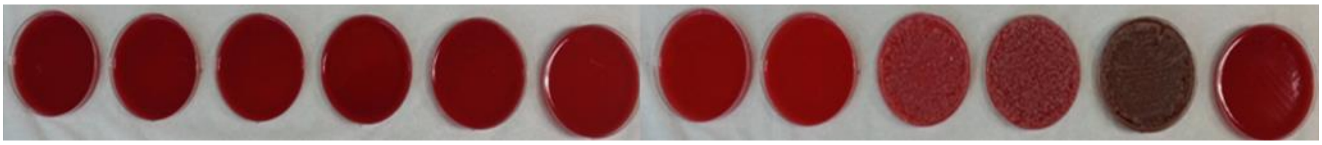
The antimicrobial effects of alum against different malodour -producing bacteria are summarized in Table 1. Alum solution showed excellent inhibitory effects on all bacterial strains at various concentrations (7.5–1.875 mg/mL). All tested bacteria showed no growth with alum at concentrations of 7.5, 10, 15 and 20 mg/mL. The lowest MIC of alum was against *C. xerosis* 1.875 mg/ mL (Figure 1). While those for *S. epidermidis, B. subtilis* and *M. luteus* were 7.5, 5 and 3.75 mg/mL, respectively. Positive control samples showed growth of all tested bacteria in all groups, while, negative control samples showed no bacterial growth in all tested samples.

DISCUSSION

Antibiotic resistance is a major clinical and public health problem which forces researchers to look for alternatives choices. Natural chemical compounds are among these alternatives. In this study, alum salt was tested against axillary normal bacterial flora which produces unpleasant smell. To the best of our knowledge, this is the first study on antimicrobial effects of alum against malodor - producing bacteria in Malaysia. The preliminary results from this study could lead to future effort to investigate how alum salt inhibits growth of the bacteria and potential use of alum salt. The results showed that alum had potent inhibitory effects against *M. luteus*, *S. epidermidis*, *C. xerosis* and *B. subtilis* at different concentrations.

Based on the broth dilution assays, the MIC of 7.5 mg/mL appeared as optimal concentration of alum against four major bacteria responsible for axillary malodor.Previous studies have revealed that alum is effective against a wide variety of microbial pathogens [16, 17] including *Staphylococcus aureus*, *Escherichia coli and Klebsiella pneumoniae* [14, 15].. In 2014, Bnyan et al. also observed a significant bactericidal effect of alum against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Klebsiella pneumoniae* [15]. However, the mechanism of bactericidal effect of alum is not well known [18]. Some assumptions attribute the antibacterial effect of alum to reduction in acidity or deleterious effects on bacterial cell wall. Furthermore, histological studies confirm the safety of alum salt for mammalian consumption [19].It cannot be directly absorbed due to its negatively charged molecule, which are unable to pass through the cell membranes and therefore alum remain a harmless substance [8]. However higher concentration of alum might cause nephrotoxicity and intestinal bleeding [15]. Alum salt is used in cosmetics as antiperspirant to reduce axillary odor by blocking sweat ducts and preventing sweat secretion [20]. Alum crystals are highly soluble in water and when used under arm, they are dissolved by the body's sweat leaving a dry thin layer on the skin's surface which prevents sweat to come in contact with odor-causing bacteria [21]. Further studies are required to investigate the safety, allergy and efficacy of alum on human skin when used as antiperspirant. According to this study a concentration of 7.5 mg/mL could be considered appropriate for formulation of deodorant lotion, cream and gel.

Since, no previous studies have been done to investigate the effects of alum salt on axillary flora, the results from this study, although preliminary, could lead to future efforts to investigate how alum salt inhibits growth of the bacteria and its potential uses.



20 mg/mL 15 mg/mL 10 mg/mL 7.5 mg/mL 5 mg/mL 3.75 mg/mL 2.5 mg/mL 1.875 mg/mL 1.25 mg/mL 0.937 mg/mL Positive Negative control

Figure 1 Antimicrobial effects of alum crystals on *Corynebacterium xerosis*.

Table 1 Antimicrobial effects of alum crystals against malodor-producing bacteria.

Alum concentrations (mg/mL)	Tested organisms (CFU/mL)			
	B. subtilis	*M. luteus*	*S.epidermidis*	*C. xerosis*
20	0	0	0	0
15	0	0	0	0
10	0	0	0	0
7.5	0	0	0	0
5	0	0	66	0
3.75	210	0	102	0
2.5	full	9360	540	0
1.875	full	1.05×10^4	2328	0
1.25	full	1.31×10^4	1.51×10^4	1.25×10^4
0.937	full	1.91×10^4	full	1.58×10^4
Positive control	full	full	full	full
Negative control	0	0	0	0

CONCLUSION

From this study it can be concluded that alum has excellent antimicrobial inhibitory effects on malodor-producing skin bacteria. It can therefore be used as either natural deodorants or as an alternative to other existing chemicals, currently used as active ingredients in deodorants.

Conflicts of interest

Authors declare none.

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