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Formulation and evaluation of Yemeni potash alum as hydrophilic topical preparations against bacterial skin infections

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ABSTRACT

Skin and soft tissue infections are common. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Pseudomonas aeruginosa* cause most bacterial skin infections. Yemen's alum is a natural mineral with potent antibacterial and antifungal activity. The current study aimed to verify Yemen alum's antibacterial activity against chosen bacterial strains to formulate a valuable topical preparation. We formulated twenty-three formulations involving four non-adjusted aqueous solutions, eight adjusted pH aqueous solutions, three Oil/Water cream formulations, and eight glycerin solutions, all with different alum con-

centrations. After that, we evaluated the antibacterial efficacy against the selected bacterial strains. Additionally, we performed stability testing (almost six weeks) to determine the chosen preparations' estimated shelf life (t90). Alum showed antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Pseudomonas aeruginosa*. Finally, it was concluded that Oil/Water cream (10% alum) is viable preparation for large-scale production.

Introduction

Skin and soft tissue infections (SSTIs) are common. It ranges from uncomplicated superficial infections to severe necrotizing infections of the skin and the underlying subcutaneous tissues and muscles. Its incidence exceeds approximately 7% of patients admitted to hospitals, two folds of urinary tract infections, and tenfold of pneumonia, counting around 6.3 million medical consultations annually [1, 2]. Despite SSTIs being often superficial and mild, it may be just a matter of days to turn into a systemic infection, extremely invasive or potentially lethal, particularly in immunosuppressed patients [1, 3]. Given the variety and multiplicity of pathogenic strains [4] in SSTIs, clinicians utilize broad-spectrum antimicrobials to ensure the most effective eradication. However, incorrect diagnosis, which accounts for 35.2% of cases reported by specialists compared to 30.2% of patients confirmed to have dermatoses such as skin infections, can dramatically exacerbate antibiotic resistance [5]. In addition, overusing antimicrobials for unconfirmed cases may produce multidrug-resistant bacteria [6, 7].

The infectious incidence of several bacterial species has increased, and some species are resistant to antibacterial drugs. The risk of acute infections is associated with substantial morbidity and death, especially in diabetes individuals[8]. *Staphylococcus aureus* and *Streptococcus pyogenes* are the most common gram-positive bacteria to infect the skin. They cause impetigo, erysipelas, and cellulitis [9]. *Klebsiella pneumonia* is a gram-negative bacterium that apart from skin infections also causes eye, brain, lung, liver, and genitourinary infections [10]. *Pseudomonas aeruginosa* is also gram-negative bacteria. It is associated with ear, lung, urinary tract, and skin infections [11].

Alum is a naturally occurring sulfate mineral rock that generally forms from the oxidation of potassium- and sulfide-containing minerals [12–14]. Using alum at a concentration of 4% can provide astringent effects [15, 16]. In addition, it is believed to shrink pores and minimize fluid discharges, thus used to relieve nosebleeds, haemorrhoids, and internal organ bleeding [15, 17, [18]. As FDA awarded alum category I ingredient in mouthwashes [20, 21], it has been used as an antiseptic mouthwash [17–19] to treat oral and gingival ulcers, gingivitis, and mucositis.

Alum can be found abundantly in the form of white sedimentary rocks containing aluminium in numerous mountain caves across Yemen governorates, including Amran. Yemeni natives have utilized alum as a deodorant, an astringent, and an aftershave. Furthermore, it has been used to purify water in rural areas due to its antibacterial properties, which help rid the water of bacterial contamination and make it suitable for drinking and bathing. In our previous study [22], we formulated topical skin preparations utilizing Aluminium Potassium Sulphate (Yemen's Alum), which were evaluated against various topical fungal infections. In this study, we will evaluate the effectiveness of these preparations against various topical bacterial infections.

Materials and Methods

Materials

Yemen's alum was gathered from its source. It occurs naturally as a rock-form precipitate in some mountains' caves in "Amran Governorate – Maswar District" and various governorates and districts in Yemen. Bacterial specimens of *Staphylococcus aureus* (SH1000), *Streptococcus pyogenes* (M1T1), *Klebsiella pneumonia* (ATCC 700603), and *Pseudomonas aeruginosa* (PAO1) have been brought from the central research Laboratory at Sana'a University. Other materials were: Mueller Hinton agar, sodium hydroxide

pellets, sodium sulfide, tartaric acid, dithizone, sodium lauryl sulfate and sodium acetate (Himedia, India), blood agar base (Conda, Spain), hydrochloric acid, sodium carbonate, paraffin wax (Uni– Chem, Serbia), barium chloride, ammonium hydroxide, zinc sulfate (ngec chemicals), EDTA disodium salt (acme-chemicals, India), ammonium acetate (E. Merck, India), glacial acetic acid (Al-Arifi medical, Yemen), ethanol (YSCO, Yemen), boric acid (El.nasr pharmaceutical chemicals co, Egypt), glycerin (Qualikems, India), paraffin oil (YSCO, Yemen), white petrolatum jelly (Optika, Yemen), ciprofloxacin cream 0.5 % (Ciplox®, Cipla, India, b.n.:g588), ciprofloxacin infusion 2mg/mL (ciplox®, Cipla, India, b.n.:zc2051).

Methods

A flowchart in **Figure 1** makes it easier to understand this research better. The figure is recreated based on a comparable one in the previous research, considering any necessary modifications. There were four stages of experimentation, each of which was broken into multiple substages. As numerous experiments were discussed in our previous study, and as they were fully detailed in our previous article [22], we will not detail them

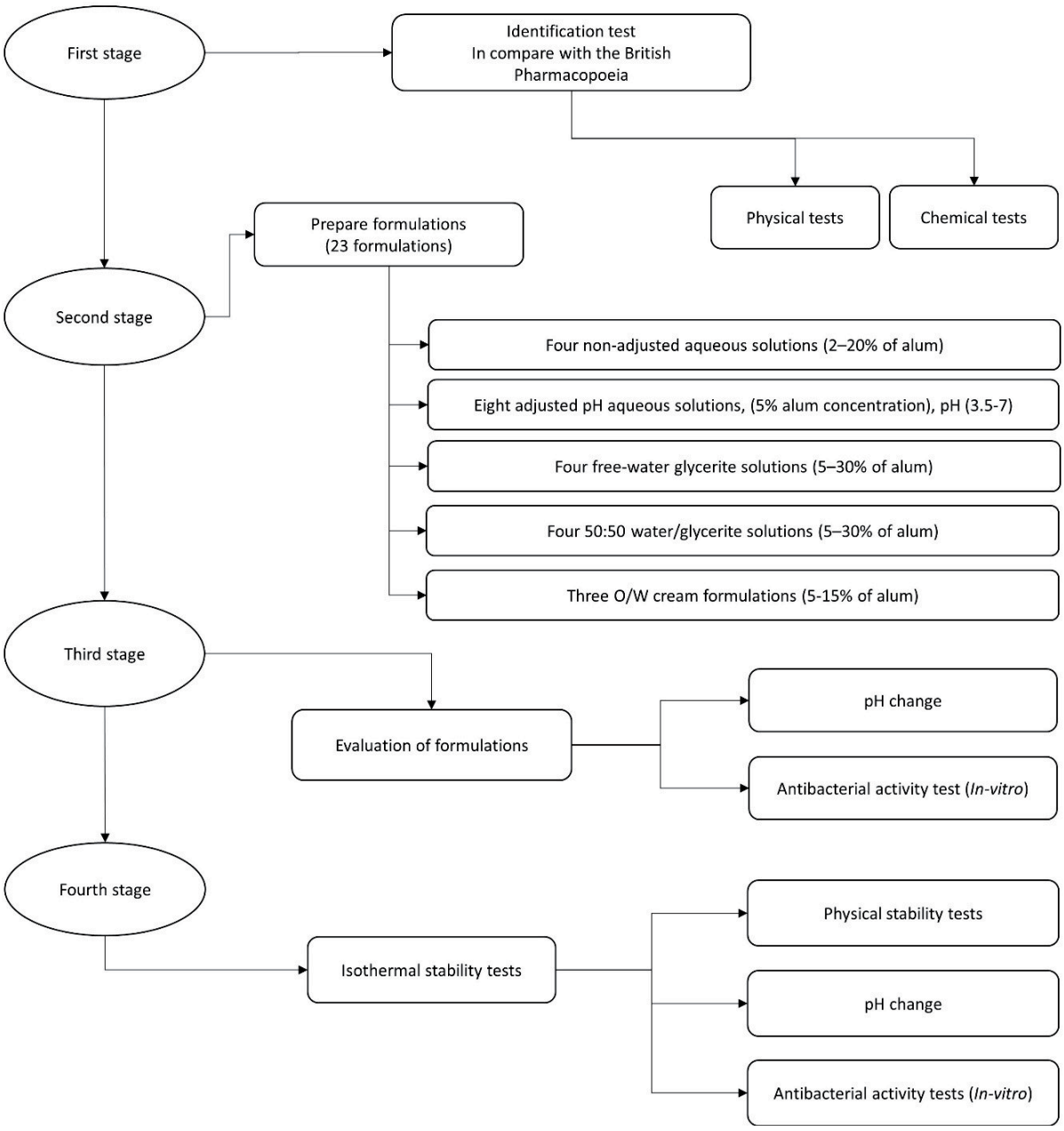

Figure 1. Experimental work flowchart

again here. We have chosen only to discuss the new experiments. Please refer to our previous article for further details.

Formulation of Alum preparations

Non-adjusted pH aqueous solutions

Alum powder with a particle size ranging between 180–250µm was dissolved in water with continuous stirring and filtering (the simple solution method) to prepare four aqueous solutions with various alum concentrations, including A1 (2%), A2 (5%), A3 (10), and A4 (20%), as shown in **Table 1**.

Table 1. Ingredients and their quantities to prepare 100 ml of non-adjusted pH aqueous alum solutions

Ingredient	A_1	A_2	A_3	A_4
Alum (g)	2	5	10	20
Water up to (mL)	100	100	100	100

Adjusted pH aqueous solutions

A similar concentration of alum (5%) was used to make eight aqueous alum solutions, as was previously stated. As stated in **Table 2**, borate buffer was used to adjust the pH between 3.5 to 7. The primary goal of adjusting the pH of the solutions was to find the pH of maximal alum activity at different pH levels.

Glycerin solutions

Eight alum's glycerin solutions were prepared using a shaker water bath at 70°C with different (water: glycerin) co-solvent ratios. The preparations included four water-free glycerin solutions: G1(5%), G2(10%), G3(20%) and G4(30%), as well as four 50:50 (water: glycerin) solutions: Gw1(5%), Gw2(10%), Gw3(20%), Gw4(30%). **Table 3** indicates all formulations' concentrations.

O/W creams

Three O/W cream formulations with various alum concentrations were prepared by the fusion method; the formulations were C1 (5%), C2 (10%), and C3 (15%). **Table 4** refers to the amount of each formulation in detail.

Table 4. Ingredients and their quantities (g) to prepare 100 g of semisolid O/W cream formulation of alum

Ingredient	C_1	C_2	C_3
Alum	5	10	15
Glycerin	12.3	11.7	11.05
liquid paraffin	0.95	0.90	0.85
Sodium lauryl sulfate	0.95	0.90	0.85
Paraffin wax	9.5	9	8.5
White petrolatum jelly	23.75	22.50	21.25
Water	47.55	45	42.5

Evaluation of the antibacterial activity of the alum preparations

Culture medium preparation

Mueller Hinton agar

Molar Hinton agar powder (38g) was added to 1000mL of water, which was heated until boiling over a flame and then cooled at room temperature (around 5 minutes). The heating/cooling cycle has been done thrice to obtain total solubility and proper sterilization. Finally, the mixture was cooled to 40°C, and then 25mL of it was

Table 2. Ingredients and their quantities to prepare 100 ml of adjusted pH aqueous alum solutions

Formulation	A_{A1}	A_{A2}	A_{A3}	A_{A4}	A_{A5}	A_{A6}	A_{A7}	A_{A8}
pH	3.5	4	4.5	5	5.5	6	6.5	7
Ingredient								
Alum (g)	5	5	5	5	5	5	5	5
Boric acid buffer pH 10.4 (mL)	-	7.5	17.5	22.5	26.5	30	33.5	35
Water Up to (mL)	100	100	100	100	100	100	100	100

Table 3. Ingredients and their quantities to prepare 100 mL of glycerin-alum solutions

Ingredient	G_1	G_2	G_3	G_4	G_{w1}	G_{w2}	G_{w3}	G_{w4}
Alum (g)	5	10	20	30	5	10	20	30
Water	-	-	-	-	50	50	50	50
Glycerin up to (mL)	100	100	100	100	100	100	100	100

poured into separate sterile Petri dishes, carefully capped, and left to solidify.

Blood agar

Blood agar powder (40g) was added to 1000mL of water, boiled using a flame, and then cooled at room temperature (about 5 minutes). The heating/cooling cycle has been done thrice to obtain complete solubility and proper sterilization. Eventually, the mixture was cooled to 40°C, and then 25mL of it was poured into separate sterile Petri dishes, carefully capped, and left to solidify.

Specimen collection and culturing

Four pathogenic bacterial specimen types were collected, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were cultured in the Mueller Hinton agar, But *Streptococcus pyogenes* were cultured in Blood agar. For culturing on plate culture, a sterile loop was used to spread bacterial specimens as parallel lines on a plate culture, with the plate being rotated to facilitate spreading.

Testing for the antibacterial activity

The antibacterial activity of the twenty-four formulations prepared previously was investigated on four different bacteria, including *staphylococcus aureus, Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa*. The standard cup plate method [23] was utilized to investigate the antibacterial effect of alum in the formulation. We also tested positive control formulations with 150 µL ciprofloxacin 5% solution and 0.2 g ciprofloxacin 0.5% O/W cream throughout the inspection process and compared them to the aqueous and glycerin alum solutions or alum O/W cream formulations, respectively. The MIC of the antibacterial-containing preparation was determined utilizing the broth dilution method; then, MIC was estimated depending on the presence or absence of bacterial growth. Please check our prior research [22] for more details on this part..

Isothermal accelerated stability study

Alike the previously mentioned study, the successful three preparations A2 (5% alum aqueous solution), G2 (10% alum water-free glycerin solutions), and C2 (10% O/W cream) underwent an isothermal stress stability test in an oven at 37°C, 50°C, and 75°C [25] for six weeks. Then, samples were taken from the stored preparations and assessed at 0, 1, 3, 4, and 6 weeks of storage. The assessment only assessed physical appearance, pH, and antibacterial activity against Streptococcus pyogenes. The degradation kinetics has been done previously in the former study. Please refer to our previously mentioned study [22] for more details.

Data analysis

All data were analyzed, and graphs were generated using GraphPad Prism 8 software. The data were presented with appropriate replicates of each experiment, and one-way analysis of variance (ANOVA) with LSD posthoc test was used to compare statistical differences between the groups. Results are shown as (mean ± S.D., n = 3) compared with the control group. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, ns > 0.05.

Results and Discussion

With an increased need to find more powerful, and safe antibacterial drugs, many studies have been performed to reveal alum's activity against some types of bacterial infections [15, 17, 20, 21, 26–28], fungal infections [29, 30], and viral infections. Moreover, as a vaccine adjuvant [31, 32], the leishmania vaccine [33–36], and hepatitis vaccine [37, 38]. In their study, Kelber et al. [39] suggest various potential modes of action regarding alum antifungal and antibacterial activity. However, the alum's mechanism of action behind its fungicidal and bactericidal properties is still unclear [28].

In their study, K. Alzomor et al. aimed to formulate and evaluate different preparations of alum, including deodorant lotion and after-shaving astringent as cream and gel [21]. As shown in **Figure 1**, throughout the four stages of the study, we aimed to formulate and evaluate effective topical preparations of Yemen's potash alum against bacterial skin infections. We considered all the time that bacterial species in this study could cause invasive systemic bacterial infections. However, the relationship between these species is that they could begin as topical/mild infections and then transmit via the bloodstream into multiple tissues, including the brain, liver, lungs, kid-

ney, soft tissues, and others. Finally, this might cause life-threatening illness or patient death, with an overall case mortality rate overtaking 27% [40].

First of all – alum's verification – all findings met the British Pharmacopeia specifications, as depicted in **Table 5.** Kindly refer to our previously mentioned research [22] for more details.

The antibacterial activity of the twenty-four formulations prepared previously was investigated on four different bacteria, including *staphylococcus aureus, Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa*. Alzomor et al. [21] reported that the MIC varied between 0.9 and 2 based on the bacterial type. However, as shown in **Figure 2**, for non-pH adjusted aqueous solutions, the 5% alum concentration (A2 formulation) had the lowest value of (MIC) with inhibition zones of ≥ 20 mm diameter in comparison to the ciprofloxacin (the positive control,). For adjusted pH aqueous alum solutions **Figure 3**, the antibacterial activity had dramatically declined at pH > 3.5 and almost vanished over pH 4. That was noticed against all the tested bacteria, where there was little action, demonstrating a clear relationship between the medium's pH and the alum's antibacterial effectiveness.

Table 5. Potash alum identification tests

Test	Result
Physical aspects	Granular powder or translucent, colourless, crystalline bulk.
Solubility	Freely soluble in water, highly soluble in boiling water, soluble in glycerol, and almost insoluble in ethanol (96%).
Potassium detection	White precipitation crystals developed.
Sulfates detection	A white-coloured precipitate was formed.
Aluminium detection	A gelatinous, white precipitate that was insoluble in the excess reagent was produced.
Melting point (°C) range	93–95°C
pH Average (± S.D.; C.V.%)	3.2 (± 0.103; 3.22)

In the second stage, we prepared twenty-three formulations involving: four alum non-adjusted pH aqueous solutions (concentration between 2–20%); eight adjusted pH aqueous solutions (5% alum concentration and pH between 3.5–7); eight glycerin solutions (four water-free solutions, and four 50:50 water\glycerin solutions, alum concentration 5 to 30%); and three alum O/W cream preparations (concentration between 5–15%). Please refer to **Tables 1–4** in our earlier research for extra information on the second-stage results.

The evaluation of formulations was the topic of interest in the third research stage. The antibacterial activity of all the formulations was tested; accordingly, because of their antibacterial efficacy, only three preparations were involved in this stage. Concerning preparations pH, as the alum concentrations increased, a decrement of pH was observed in non-adjusted pH aqueous preparations and the glycerin solutions in water-free and water-contain glycerin, attributed to the acidic nature of the materials. As alum content rose from 5 to 10, the rate of pH decrement in water-free glycerin solutions varied, particularly compared to other preparations. In contrast, the pH of the O/W cream preparations was almost identical regardless of the alum concentration differences. For further details on the results of the third stage, kindly refer to **Tables 7–8** in our earlier research for more details.

Likewise, among water-free glycerin solution in **Figure 4**, G2 (10% alum concentration) showed the MIC with inhibition zones of ≥ 20 mm diameter against all the tested bacteria. However, because of the instability of water-containing glycerin solutions, as alum crystals were remarkably observed shortly after the following storage at room temperature, all water-containing glycerin preparation was neglected despite MIC values. Conversely, the optimum MIC of the O/W cream formulations is shown with C2 (10 % alum concentration). As in **Figure 5**, the inhibition zone is ≥ 20 mm in diameter against all bacteria.

Similar to our former antifungal study, as referred to in the related **Tables 10–12** and **Figures 7–8**, **Supp. Fig. 2**, three preparations (A2, G2, and C2) were selected for the last stage to undergo isotheral stability testing. The physical stability and general appearance are incon-

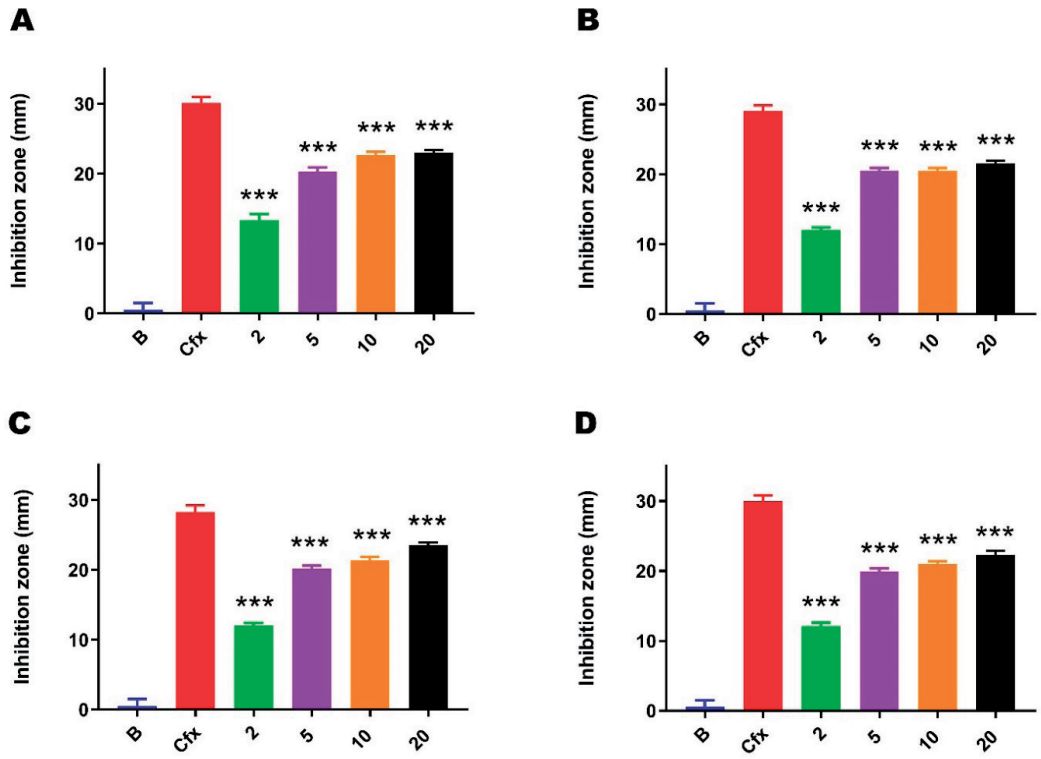

Figure 2. Antibacterial activity of non-adjusted pH aqueous alum solutions on (A) staphylococcus aureus, (B) Streptococcus pyogenes, (C) Escherichia coli, (D) Pseudomonas aeruginosa. Preparations included blank formulation, ciprofloxacin positive control, and alum formulations with different concentrations ranging (from 2%–20%). Results are shown as (mean ± S.D., n=3) compared with the control group. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, ns 0.05

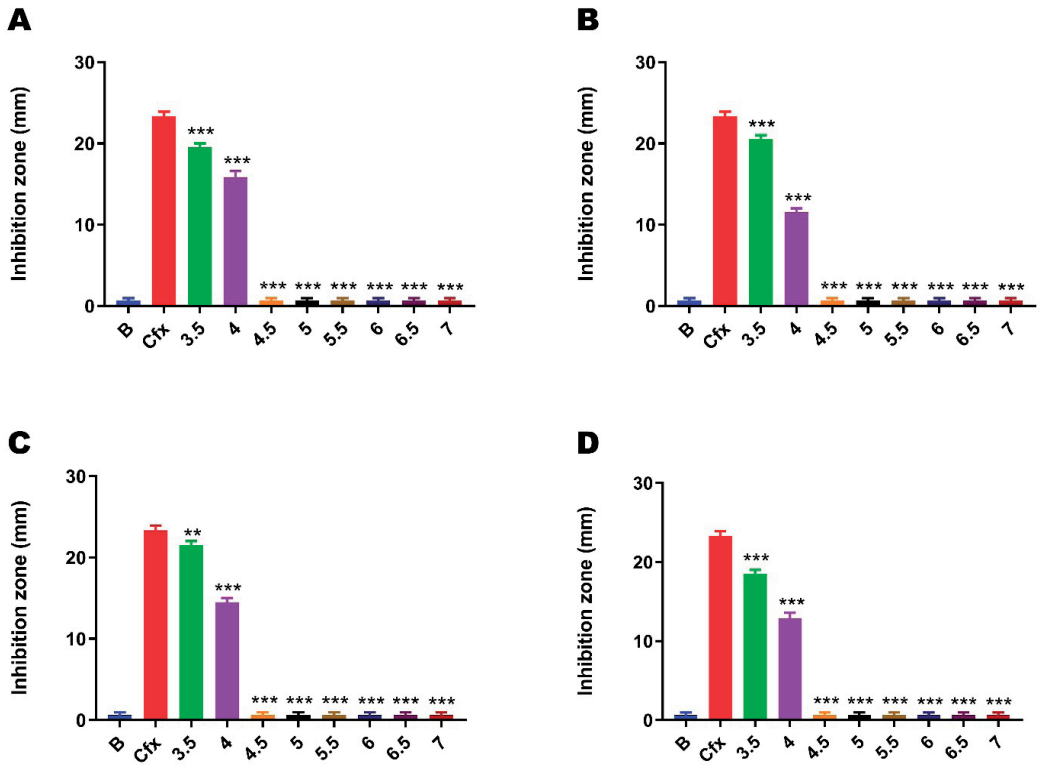

Figure 3. Antibacterial activity of adjusted pH (5%) aqueous solutions on (A) staphylococcus aureus, (B) Streptococcus pyogenes, (C) Escherichia coli, (D) Pseudomonas aeruginosa. Preparations included blank preparation, ciprofloxacin positive control, and alum formulation with eight different pH values ranging from 3.5–7. Results are shown as (mean ± S.D., n=3) compared with the control group. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, ns 0.05

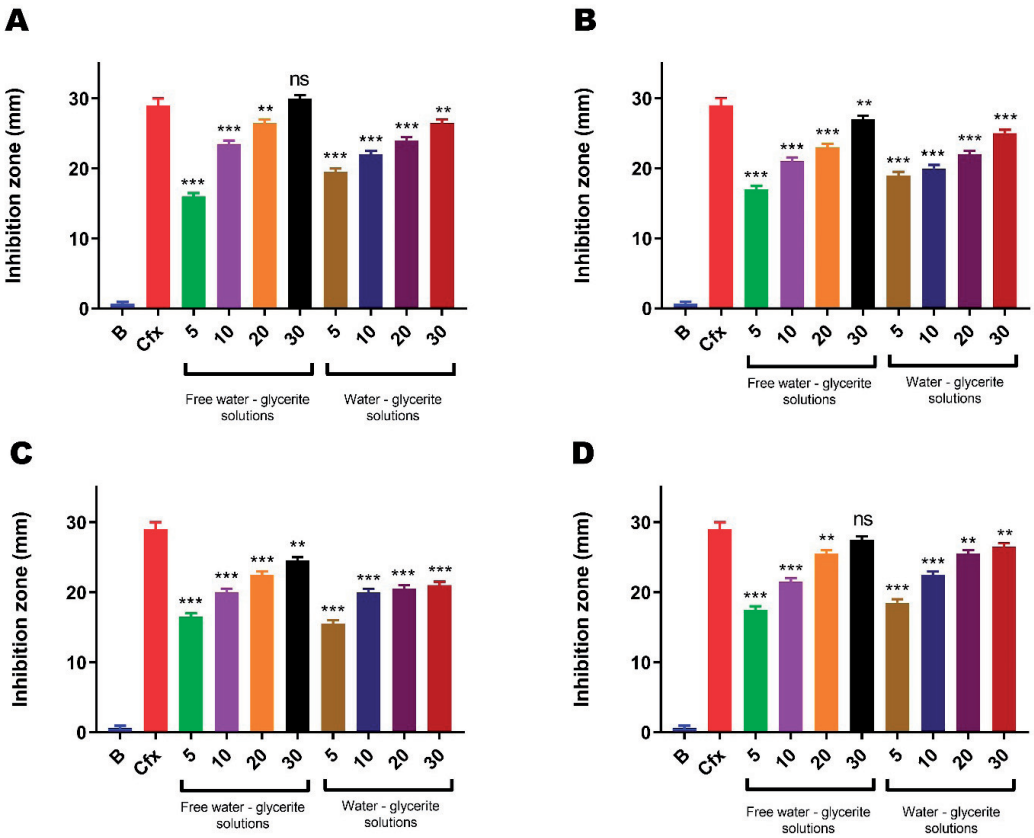

Figure 4. Antibacterial activity of glycerin preparations on (A) staphylococcus aureus, (B) Streptococcus pyogenes, (C) Escherichia coli, (D) Pseudomonas aeruginosa. Preparations included blank formulation, ciprofloxacin positive control, and alum formulations with different concentrations ranging (from 5%–30%). Results are shown as (mean ± S.D., n=3) compared with the control group. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, ns 0.05

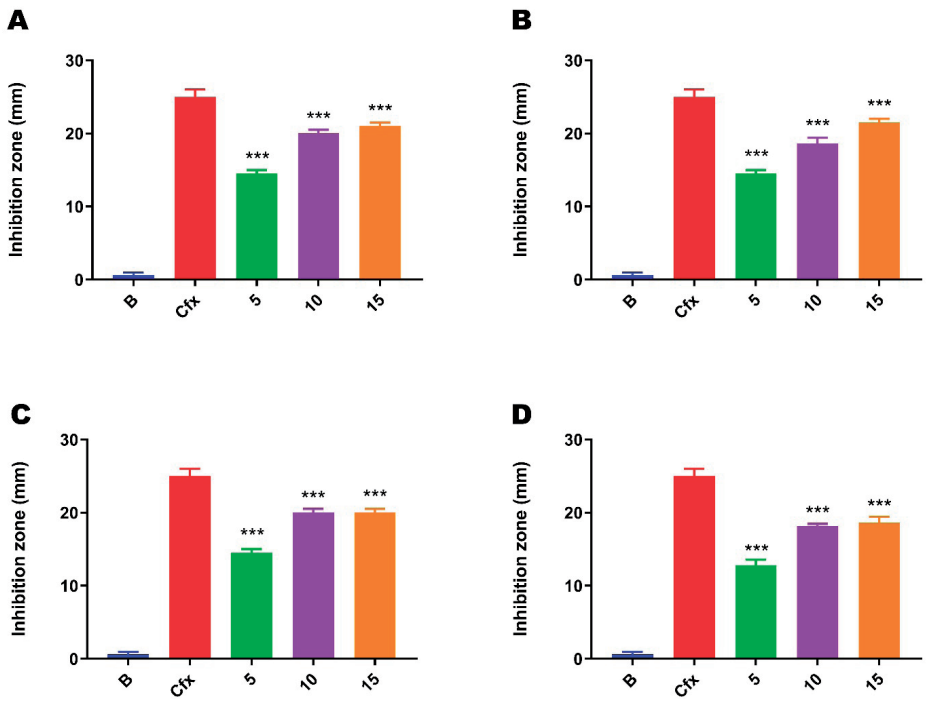

Figure 5. Antibacterial activity of cream preparations (A) staphylococcus aureus, (B) Streptococcus pyogenes, (C) Escherichia coli, (D) Pseudomonas aeruginosa. Preparations included blank formulation, ciprofloxacin positive control, and alum formulations with different concentrations ranging (from 5%–15%). Results are shown as (mean ± S.D., n=3) compared with the control group. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, ns 0.05

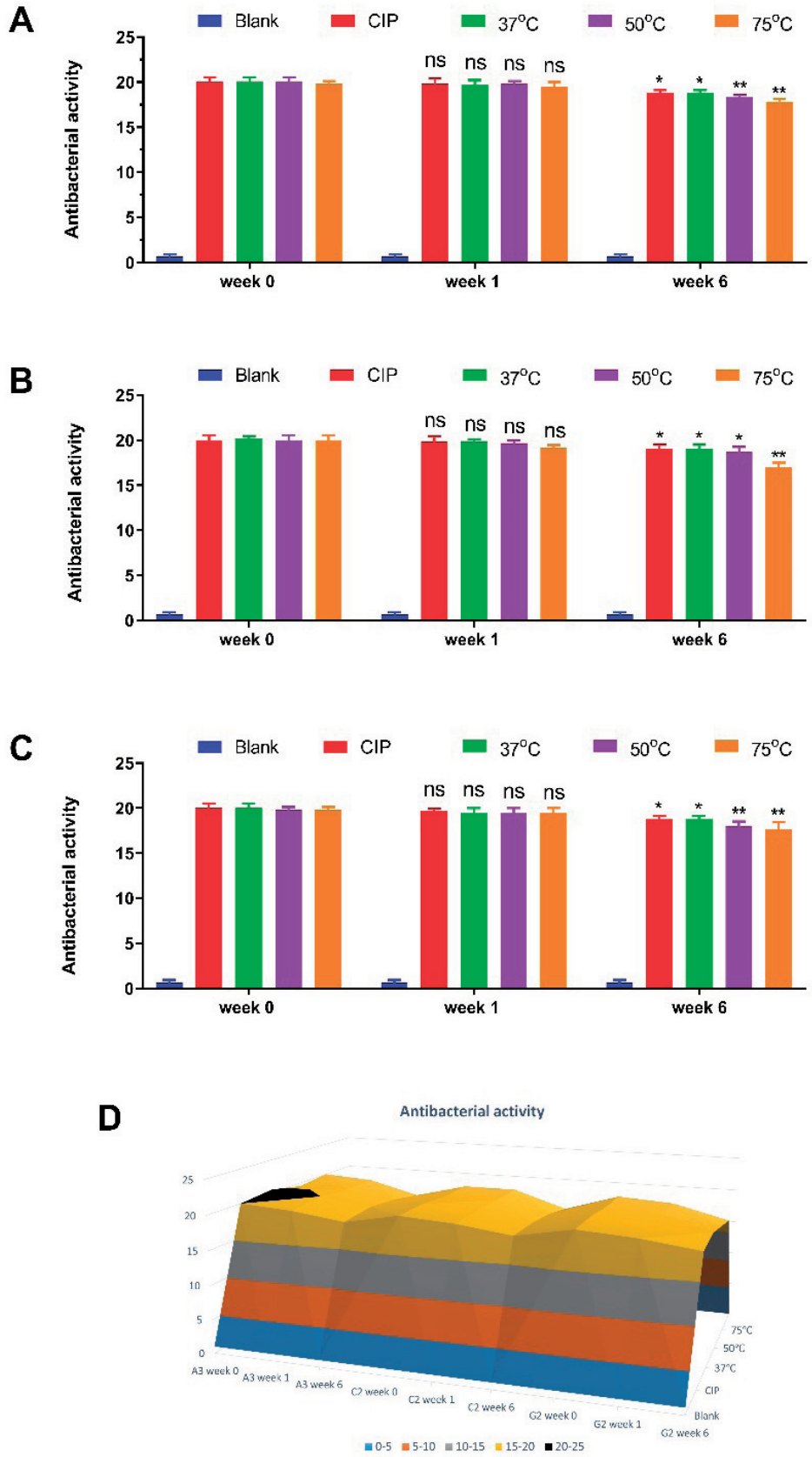

Figure 6. Stability study testing. Figures (A-C) show the antibacterial activity of aqueous formulations 5% (A2), O/W cream formulations 10% (C2), and glycerin formulations 10% (G2) on Streptococcus pyogenes during the stability study. Results are shown as (mean ± S.D., n=3) compared with the control group. ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05, ns 0.05

sistent with what was observed by K. Alzomor et al.[21], no valuable changes were observed in all formulations at 37°C, as reported in **Table 6**. However, at 50°C and 75°C, the glycerin formulation and cream formulation exhibited patterns of physical instability as colour and odour change, in addition to phase separation for the latest one.

For preparations' pH, neither the cream preparations nor the aqueous solution exhibited a remarkable variation — for more details, please refer to **Figure 7** in our earlier research. In contrast, a considerable decrement in pH was seen in the glycerin preparation at the three-storage temperature. Furthermore, as in **Figure 6,** the antibacterial activity of the preparations showed no significant changes as expressed by the variation in the inhibition zone.

Regarding the degradation kinetics of alum in the stored formulation, as we discussed in our earlier study, the content (%) of alum remained in the aqueous solution, and its kinetic parameter revealed that alum exhibited first-order degradation with higher R^2. Furthermore, the predicted shelf-life (t90) of alum in that formulation determined from the Arrhenius plot was approximately two years. Similar findings were observed with the cream formulation and the glycerin formulation. According to the Arrhenius plot, the cream formulation predicted shelf-life (t90) approximated 1.52 years. However, for the glycerin formulation, the t90 of alum was significantly shorter (0.16 years) – please refer to our earlier research for more details on this part.

Conclusion

To conclude, alum proved to have antibacterial activity. Therefore, the 10% alum O/W cream and 5% alum aqueous solution presented by this study are promising preparations for large-scale production as safe, stable hydrophilic topical preparations of Yemen's alum preparations owing to remarkable antibacterial activity. However, increasing the pH over 3.5 of the medium in aqueous alum solutions can significantly reduce the alum's antibacterial activity.

This study had some potential limitations; first, the bacterial species included in this study were limited to four species because the scope of the study was prone to formulate an effective antibacterial preparation regardless of bacterial species.. Bacterial species covered in this study have been chosen upon their prevalence and spreading among the local community. Second, no topical routes of administration were considered in this study except the dermal preparations because working on other dosage forms might consume much more time and need more funds.

In addition, inhibitory zones and minimum inhibitory concentration (MIC) values were used to evaluate the antibacterial activity of alum in preparations. However, the MIC was visually determined utilizing the broth dilution method. If we had utilized a statistical method including MIC range, MIC50, and MIC90 values, the evaluation might have been more accurate; however, this would have required the testing of more than 100 isolates, which was not possible considering the limited funds we had.

Depending on the stated limitations, we suggest covering more bacterial strains in addition to fungal strains as well. This study will significantly impact considering natural product preparations as an acceptable choice for treating dermal infections. Furthermore, we aim to broaden the scope of this study to include more administration routes, including eyes and nose washes or douches, the same for the mouth, and a gargle and rinse. Burn and injury dermal washes also could be considered for further studies.

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CRediT authorship contribution statement
Moath Refat: Methodology, Conceptualization, Investigation, Formal analysis, Visualization, Software, Data curation, Writing – original draft, Writing – review & editing. **Anes A.M. Thabit:** Supervision, Methodology, Conceptualization, Formal analysis, Writing – review & editing. **Hesham Siddick:** Investigation, Formal analysis, Data curation, Writing – original draft. **Abdul-Rahman Maqboli:** Investigation, Formal analysis, Data curation, Writing – original draft. **Mohammed Sharah:** Investigation, Formal analysis, Data curation, Writing – original draft. **Abdul-baqi A. Thabet:** Conceptualization, Methodology, Supervision. **Manar Refat:** Investigation. **Ahmed Al-Sabati:** Supervision, Methodology, Conceptualization, Resources, Funding acquisition.

Conflict of interest statement
The authors declare no conflict of interest.

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