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"Evaluating Antimicrobial efficacy of 'potash Alum' on oral ulcers"

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Abstract

Recurrent Aphthous Stomatitis (RAS), or canker sores, impact the oral mucosa and arise from factors like trauma, nutritional deficiencies, infections, and systemic conditions. This research explores the healing potential of alum, a traditional Unani medicine, for mouth ulcers. Alum's antimicrobial efficacy was tested via agar well diffusion, showing significant inhibition against microorganisms including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Candida albicans*, *Penicillium digitatum*, and *Aspergillus niger*, with the highest inhibition zones observed at 20% concentration for *Pseudomonas aeruginosa* (26mm) and *Candida albicans* (30mm). Antibiotic susceptibility tests indicated that Gentamicin and Ciprofloxacin were most effective, whereas Ampicillin showed no inhibition. Combining alum with these antibiotics enhanced antibacterial effects, presenting a promising treatment for RAS. The study underscores alum's therapeutic potential in facilitating oral ulcer healing.

Key Words: Recurrent Aphthous Stomatitis (RAS), Oral Ulcer, Potash Alum, Antimicrobial

Introduction

Mouth ulcers, also known as Recurrent Aphthous Stomatitis (RAS), are a type of ulcerative ailment that affects the oral mucosa and can be acute, chronic, or recurrent. (Mekseepralard et al., 2010)They can be classified into major, minor, and herpetiform lesions and can be caused by various factors such as inadvertent cheek biting, food sensitivities, aggressive tooth brushing, hormonal fluctuations, vitamin deficiencies, bacterial infections, and illnesses. Oral diseases manifest with varying degrees of mucosal alterations, presenting as pigmentation, white or

hyperkeratotic patches, ulceration, erythema, or atrophy. These manifestations, often chronic, can be linked to underlying systemic diseases.(Meiller et al., 1991).

This study demonstrates that healing mouth ulcers can be facilitated by using iron oxide nanoparticles modified with vitamins, specifically vitamin B2 (VB2), which possess both anti-inflammatory and antibacterial properties(Gu et al., 2020). A comparative analysis of microorganisms presents in the oral cavities of individuals with peptic ulcers and a control group of healthy individuals revealed the isolation of several bacterial species, including *Staphylococcus aureus, Klebsiella, Pseudomonas, and Escherichia coli*. Anaerobic bacteria such as *Bacillus* and *Candida albicans* were also identified.(Deepa et al., 2014).

a) b).

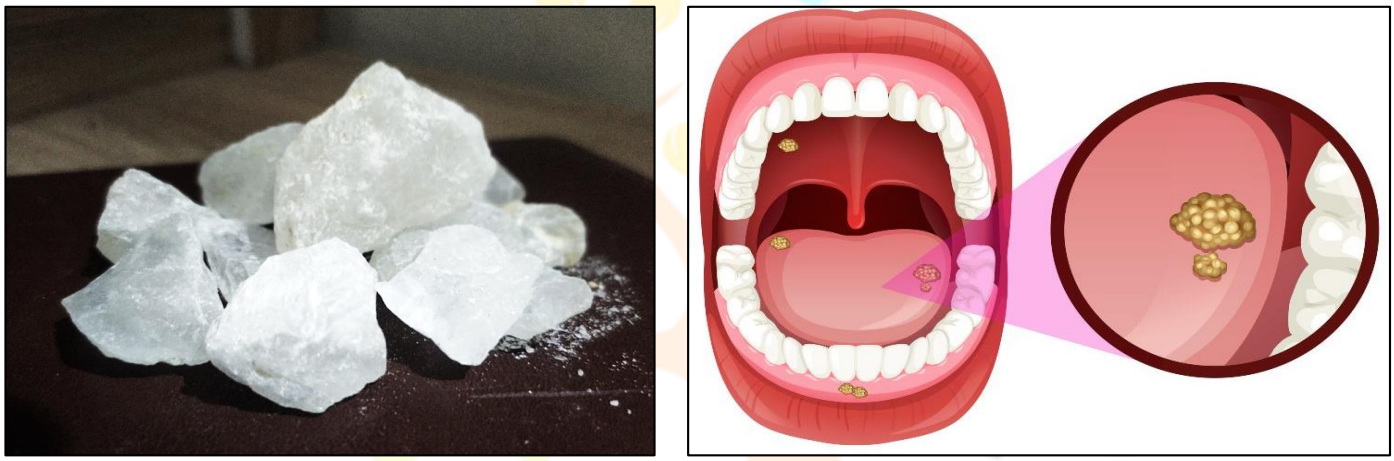


Fig. 1. a) Potash alum and b).Recurrent Aphthous Stomatitis (RAS)

Alum, a mineral-based medication used in Unani medicine, exhibits various therapeutic properties such as astringent, analgesic, hemostatic, desiccative, expulsive, antipyretic, corrosive, expectorant, emetic, detergent, and irritating. It plays a role in bacterial vaccines, eliciting a robust humoral response in vaccinations against tetanus, hepatitis B, and diphtheria. Alum is recommended for pharyngitis and stomatitis as a mouthwash or gargle and can be applied as a solid or solution for hemostatic treatment on cuts and abrasions. It finds application in the treatment of gingivitis, mucositis, and oral ulcers, particularly demonstrating significant improvements in the healing process of recurring aphthous ulcerations when applied at concentrations of 1000, 2000, and 4000 PPM.(Idan et al., n.d.)

Material and methodology

IN-SILICO ANALYSIS OF POTASH ALUM AGAINST RAS

To investigate the effectiveness of alum against RAS, an in-silico analysis was conducted. This involved obtaining the canonical smile for the structure of alum from the PubChem database, and then identifying the target genes using tools such as Swiss Target Prediction, Binding database, and Superpred. Target genes associated with RAS disease were obtained from disease databases such as GeneCard,DisGeNETand OMIM. Following data retrieval, the target genes for both Alum and RAS were subjected to Venn analysis, resulting in the identification of the genes specifically targeted by alum (Chen et al., 2020).

COLLECTION /ISOLATION/ IDENTIFICATION

In this study twenty-five oral samples from individuals experiencing recurrent aphthous stomatitis (RAS) were collected, a painful mucosal lesion commonly found on the tongue, gums, and cheeks. Saliva samples were taken from patients with ulcers using a practical method. The isolates were obtained using the streak plate method and then inoculated on nutrient agar and potato dextrose agar. Bacteria were incubated for 18-24 hours, while fungi were incubated for 3-5 days. After incubation, standard microbiological and biochemical techniques were used to obtain and characterize bacterial colonies. Following identification, pure bacterial isolates were cultured in broth after incubation on selective media for 18-24 hours (Ai-Huwaizi & Al-Alousi, 2013).

ANTIMICROBIAL ACTIVITY

The growth of microorganisms in the presence of antibiotics and various concentrations of alum was assessed using antibiotic susceptibility testing. The well-diffusion method was employed to evaluate the antimicrobial activity of antibiotics, alum, and their combination, by measuring inhibition zones. After nutrient broth was poured as the inoculum, the plates were incubated at 37°C for 18 to 24 hours (Khurshid et al., n.d.). The zones of inhibition, formed by alum, antibiotic discs, and their combination, were measured using a meter rule. Alum's antibacterial activity was tested at concentrations of 4%, 8%, 16%, and 20%. Four antibiotics—AMPICILLIN, TETRACYCLINE, GENTAMICIN, and CIPROFLOXACIN—were used in the tests. This additional investigation aimed to evaluate the antibacterial efficacy of the antibiotics.(Khurshid et al., n.d.) When tested on the same bacterial isolates responsible for mouth ulcers, alum demonstrated high inhibitory activity, while the antibiotics showed the highest growth suppression. A combined investigation was conducted to determine the effectiveness of alum and antibiotics in treating recurrent aphthous stomatitis.(Hasan et al., 2024)

Results

Determination of potential targets for alum and RAS

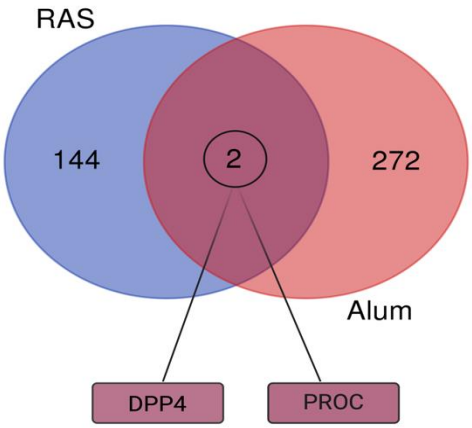
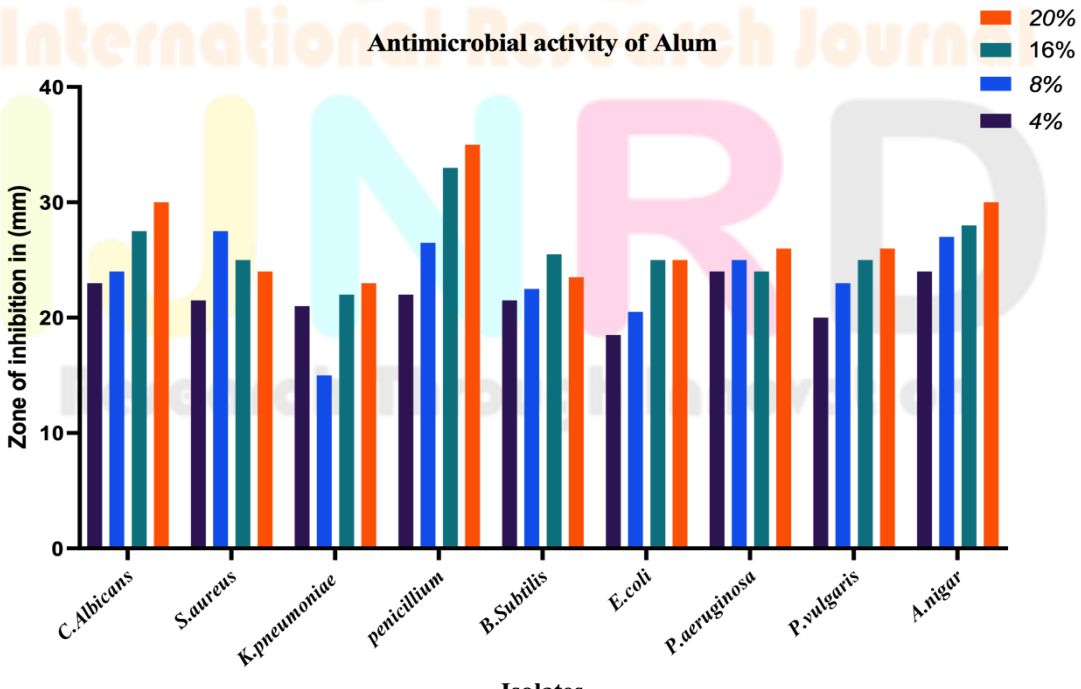


Fig.2. Venn Diagram that shows genes that are targeted by Potash Alum in the RAS.

A preliminary selection identified 274 targets for alum activity using Swiss Target Prediction, Binding Database, and SuperPred. Concurrently, 152 targets linked to recurrent aphthous stomatitis (RAS) were identified from GeneCard, OMIM, and DisGeNET databases. After integrating these datasets and eliminating duplicates, two intersecting targets were identified. These targets potentially align with alum's carcinogenicity in RAS (Fig. 2).

ANTIMICROBIAL ACTIVITY OF ALUM AGAINST ORAL ISOLATES



Graph.1. Antimicrobial assay of alum (In mm)

Alum's antimicrobial activity was assessed against various microorganisms using an in vitro bioassay with the agar well diffusion method. The zones of inhibition for *Escherichia coli* were largest at 16% and 20% concentrations (25mm each), followed by 8% (20.5mm) and 4% (18.5mm). For *Staphylococcus aureus*, the highest inhibition zone was at 16% (25mm), followed by 20% (24mm), 8% (27.5mm), and 4% (21.5mm). *Klebsiella pneumonia* showed maximum inhibition at 20% (23mm) and 16% (22mm), with lesser zones at 8% (21mm) and 4% (15mm). *Pseudomonas aeruginosa* exhibited the greatest inhibition at 20% (26mm), followed by 8% (25mm), 4% (24mm), and 16% (24mm).For *Bacillus subtilis*, the maximum zone was at 16% (25.5mm), followed by 20% (23.5mm), 8% (22.5mm), and 4% (21.5mm). *Protease vulgaris* showed the highest inhibition at 20% (26mm), followed by 16% (25mm), 8% (23mm), and 4% (20mm). *Candida albicans* had the largest inhibition zone at 20% (30mm), followed by 16% (27.5mm), 8% (24mm), and 4% (23mm). *Penicillium digitatum* was most inhibited at 20% (35mm), followed by 16% (33mm), 8% (26.5mm), and 4% (22mm). *Aspergillus niger* showed maximum inhibition at 20% (30mm), followed by 16% (28mm), 8% (27mm), and 4% (24mm).Overall, alum's inhibitory activity decreased at 4% and 8%, but was significantly higher at 16% and 20%, with 16% being the most effective concentration against the tested microorganisms.

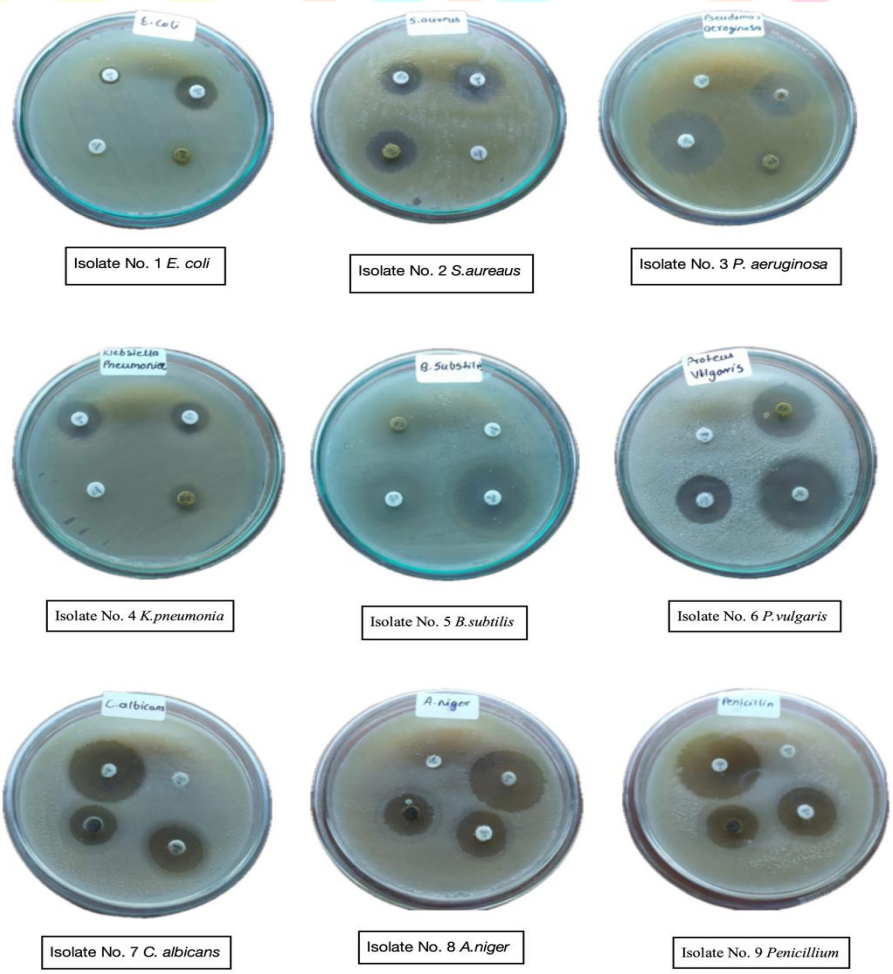
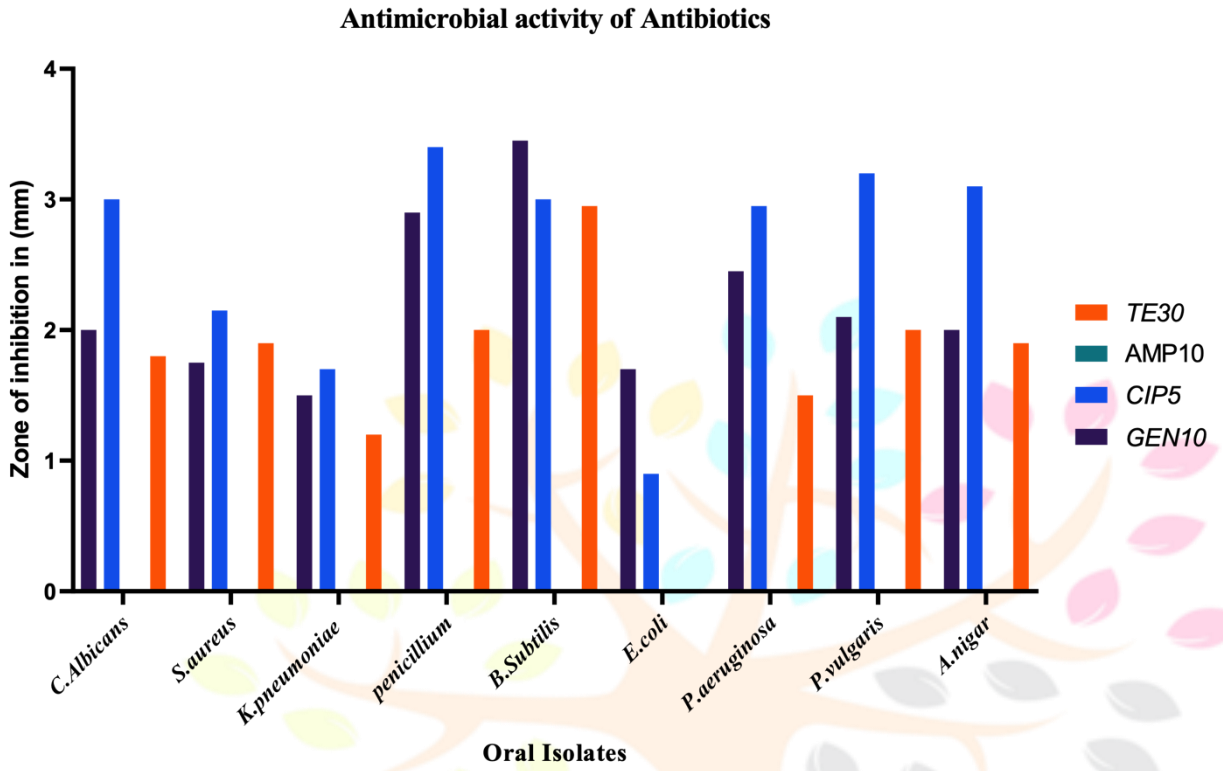


Fig.3. Antimicrobial Assay to check efficacy of Potash Alum against Oral Isolates

Evaluating antibiotic effectiveness on oral isolates



Graph.2. Antimicrobial assay of antibiotic (In mm)

The antimicrobial susceptibility of various isolates was tested using four antibiotics: Ampicillin, Tetracycline, Gentamicin, and Ciprofloxacin. The results indicated that Ampicillin showed no inhibitory effect on any of the isolates. Tetracycline demonstrated minimal inhibitory activity. In contrast, Gentamicin and Ciprofloxacin exhibited the highest levels of inhibition. *Escherichia coli* showed maximum inhibition zones with Gentamicin (17 mm) and Ciprofloxacin (9 mm), but none with Ampicillin and Tetracycline. *Staphylococcus aureus* had the highest inhibition with Ciprofloxacin (21.5 mm), followed by Gentamicin (17.5 mm) and Tetracycline (19 mm), and no inhibition with Ampicillin. *Klebsiella pneumoniae* showed maximum inhibition with Ciprofloxacin (17 mm) and Gentamicin (15 mm), and Tetracycline (12 mm), with no effect from Ampicillin. *Pseudomonas aeruginosa* exhibited the greatest inhibition with Ciprofloxacin (29.5 mm), followed by Gentamicin (24.5 mm) and Tetracycline (11.5 mm), with no inhibition from Ampicillin. For *Bacillus subtilis*, Gentamicin had the highest inhibition (34.5 mm), followed by Ciprofloxacin (30 mm) and Tetracycline (29.5 mm), with no inhibition from Ampicillin. *Proteus vulgaris* showed inhibition zones with Ciprofloxacin (32 mm), Gentamicin (21 mm), and Tetracycline (20 mm), but none with Ampicillin. *Candida albicans* exhibited inhibition with Ciprofloxacin (30 mm), Gentamicin (20 mm), and Tetracycline (18 mm), and no inhibition with Ampicillin. *Penicillium digitatum* showed the highest inhibition with Ciprofloxacin (34 mm), followed by Gentamicin (24 mm) and Tetracycline

(20 mm), and no effect from Ampicillin. *Aspergillus niger* had maximum inhibition with Ciprofloxacin (31.5 mm), Gentamicin (20 mm), and Tetracycline (19 mm), with no inhibition from Ampicillin.

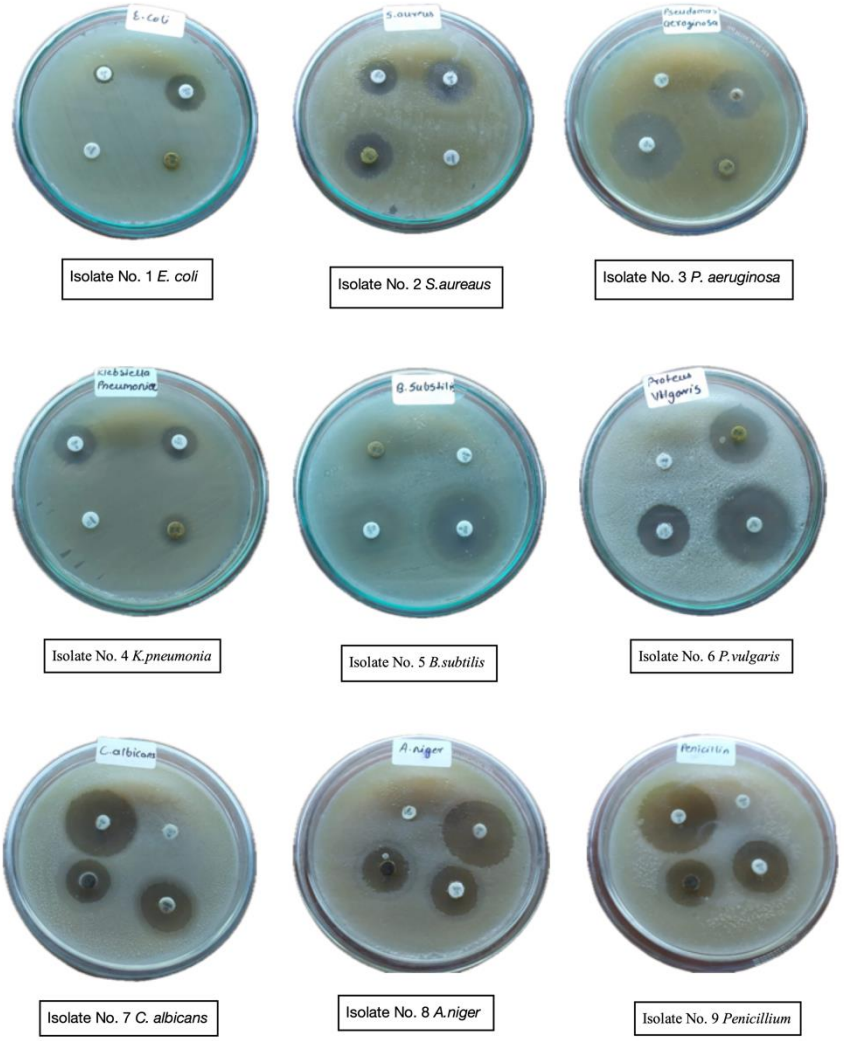
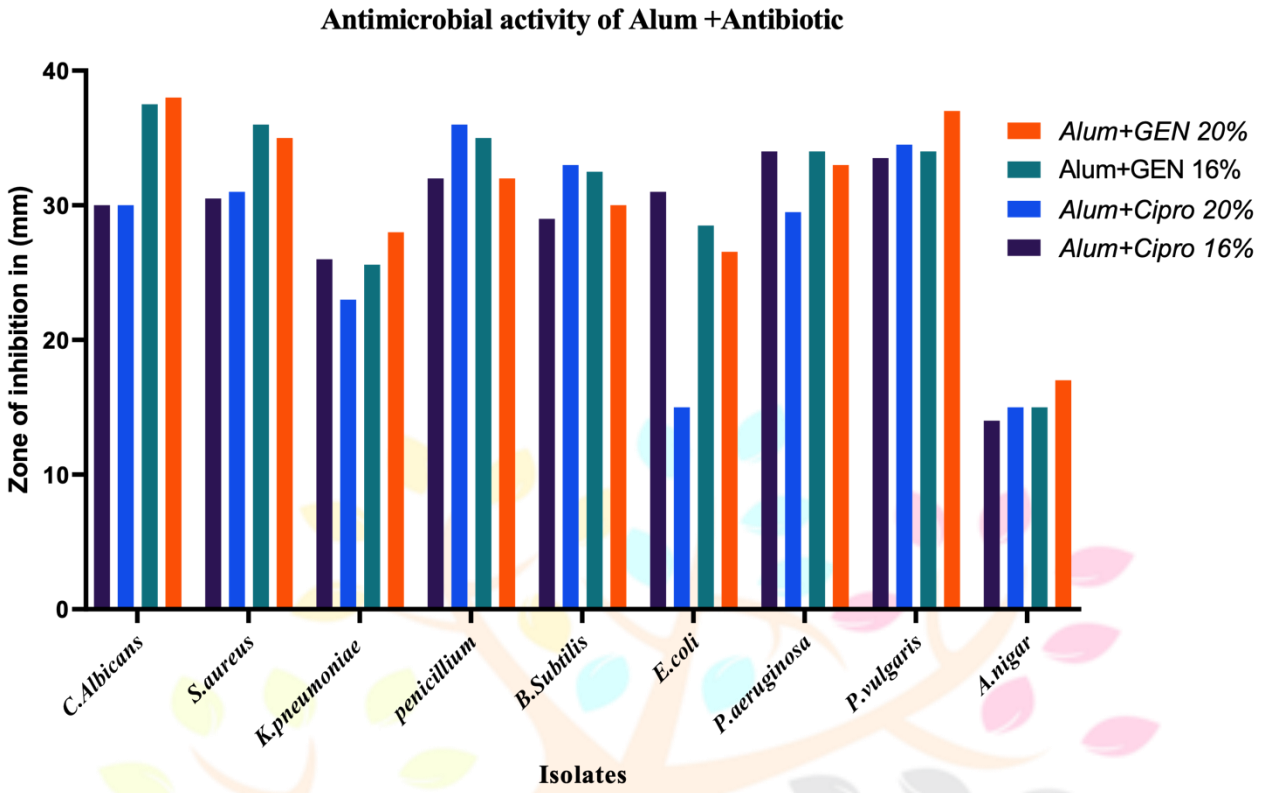


Fig.4. Antimicrobial plate Assay to check efficacy of Antibiotics against Oral Isolates.

Evaluation of the antimicrobial efficacy of alum in combination with antibiotics against oral Isolates



Graph.3. Antimicrobial assay of Alum with combination of Antibiotics.

This study is performed to evaluates the efficacy of combining alum with antibiotics for the treatment of Recurrent Aphthous Stomatitis (RAS). Alum has been discovered to possess potent inhibitory activity against a range of microbial isolates that cause mouth ulcers, such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis, Protease vulgaris, Candida albicans, Penicillium digitatum,* and *Aspergillus niger*. The effective concentration range for each isolate was determined, with the greatest concentration observed for *E.coli* at 16% and the highest concentration observed for *S. aureus* at 16%. The optimal concentration range for *Bacillus subtilis* was determined to be 32.5mm, with the same concentration range of 32.5mm seen for B. subtilis at a 20% concentration. The concentration zone of *Protease vulgaris* was highest at 37mm, followed by 34.5mm, 34mm, and 33.5mm for *P. vulgaris* at a concentration of 20%. The highest concentration zone observed was 38mm for *Candida albicans*, followed by 37.5mm, 36mm, and 30mm for *C. albicans* at a concentration of 20%. *Penicillium digitatum* exhibited the most significant concentration zone measuring 36mm, followed by 35mm, 32mm, and 32mm for *P. digitatum* at a concentration of 20%. *Aspergillus niger* exhibited the largest zone of concentration measuring 17mm, followed by 15mm, 15mm, and 14mm for *A.niger* at a concentration of 20%.

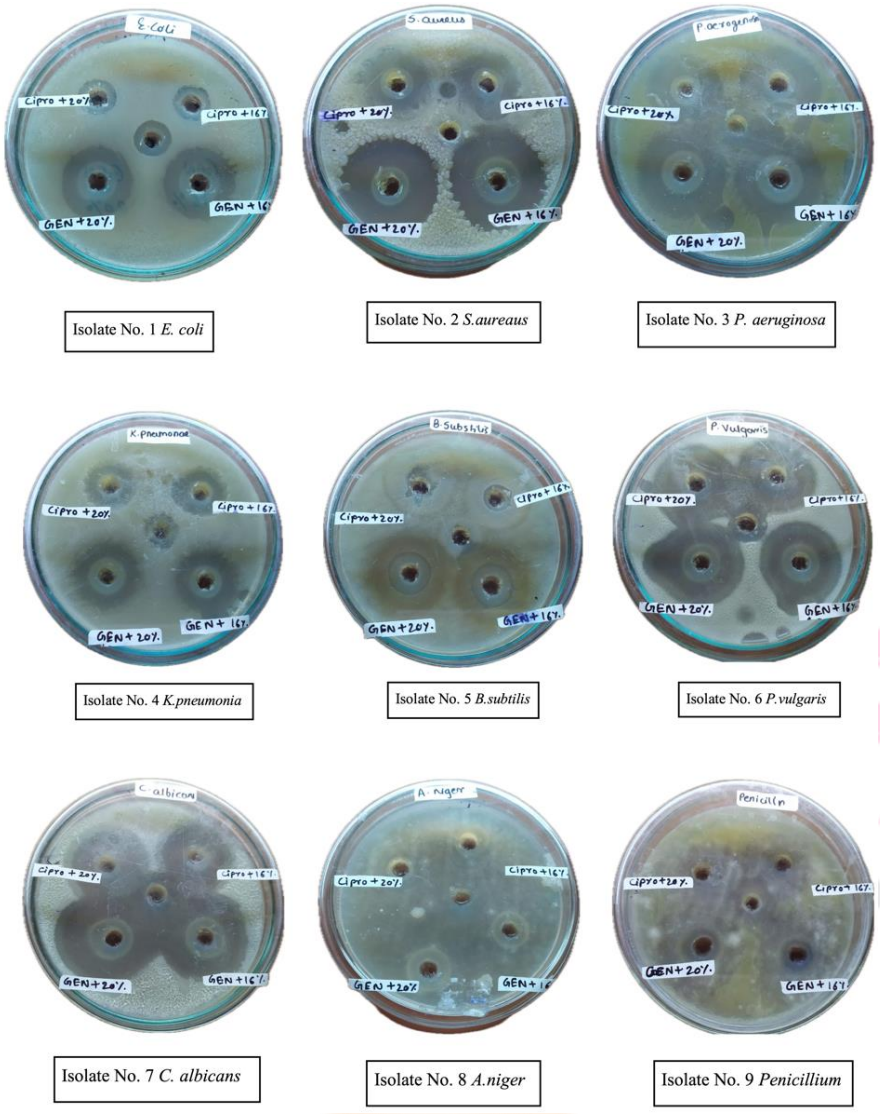


Fig.5. Antimicrobial plate Assay to check efficacy of Alum with combination of Antibiotics.

Discussion:-

Oral samples from people with recurrent aphthous stomatitis or mouth ulcers demonstrated to the study that alum successfully inhibits microorganisms from causing oral ulcers. (Alhusayni et al., 2023) Nine individuals were surveyed using swabs from various areas of the mouth, and the samples were injected with nutrient broth, regardless of age, sex, or eating habits. Isolates were obtained using the streak plate method was used to identify the bacteria. At 37°C, samples were streaked on blood, nutrient, and MacConkey the isolated colonies were subcultured in agar slants and then incubated. Bergey's Manual of Determinative Bacteriology agar plates. On the nutrient agar slant, separate colonies were maintained as pure cultures (Mazumdar et al., 2023).

Identification of isolates were done by using Gram staining, morphological characteristics, biochemical test, carbohydrate fermentation test, and enzyme test. Upon identification pure culture of the isolates were cultured. The isolates were analyzed for biochemical characteristics, and sugar fermentation tests.(Maskare et al., n.d.) Hemolysis patterns were examined on Blood Agar with 5% sheep blood, and pigment-producing cells were

identified using King's B medium. The approach was based on the distinctive cultural morphology of bacteria that form biofilms on Congo red medium. The isolates were incubated for 48 hours at 37°C. A study was conducted to evaluate the antimicrobial efficacy of an inorganic alum extract against various microorganisms, using the agar well diffusion method to measure the zone of inhibition. Alum concentrations of 4%, 8%, 16%, and 20% were tested (Ai-Huwaizi & Al-Alousi, 2013).Results showed that lower concentrations (4% and 8%) had reduced inhibitory activity, while higher concentrations (16% and 20%) exhibited significant zones of inhibition, with 16% being the most effective against the tested microorganisms (Laheij et al., 2012). Antibiotics used in the study included Ampicillin, Tetracycline, Gentamicin, and Ciprofloxacin. Alum demonstrated strong inhibitory effects on microbial isolates from mouth ulcers, particularly at 16% and 20% concentrations. Gentamicin and Ciprofloxacin also showed high zones of inhibition against the same isolates. The findings are consistent with previous reports by on the antimicrobial properties of alum. The study highlighted that alum at 16% and 20% concentrations effectively inhibited antibiotic-resistant strains of *E. coli* and *C. albicans* (Alhusayni et al., 2023). Additionally, alum combined with Tetracycline or Cefotaxime exhibited greater inhibitory effects than either antibiotic alone, with zones of inhibition measured at 38 mm for alum alone, 46 mm with Cefotaxime, and 40 mm with Tetracycline.

Conclusion:

Alum, used in Unani medicine, possesses therapeutic properties such as astringent, analgesic, hemostatic, desiccative, expulsive, antipyretic, corrosive, expectorant, emetic, detergent, and irritant effects. Despite extensive research showing its antibacterial, hemostatic, healing, anti-obesity, and larvicidal properties, a complete understanding of its medicinal applications in Unani literature is still lacking. The active compounds in alum, aluminum potassium sulfate or aluminum ammonium sulfate, function as astringents by precipitating proteins on cell surfaces and in interstitial spaces. This study assessed the effectiveness of potash alum in combination with antibiotics to reduce mouth ulcers. Twenty-five samples from individuals with mouth ulcers were analyzed, identifying nine strains*: C. albicans, S. aureus, K. pneumoniae, Penicillium, B. subtilis, E. coli, P. aeruginosa, P. vulgaris,* and *A. niger.* Identification was performed using IMVIC, sugar fermentation, and enzyme tests. Antimicrobial tests with alum concentrations of 4%, 8%, 16%, and 20% revealed reduced inhibitory activity at 4% and 8%, while 16% and 20% concentrations showed high or nearly equivalent zones of inhibition. The 16% concentration was the most effective for treating oral ulcers. Combining alum with antibiotics Gentamicin (GEN10) and Ciprofloxacin (CIP5) enhanced efficacy against Recurrent Aphthous Stomatitis (RAS).

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