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Effects of Potash Alum and Lime on *Pseudomonas aeruginosa* and *Serratia marcescens* Isolated from Wound Infection

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Abstract*: The study was aimed at examining the effects of potash alum and lime on *Pseudomonas aeruginosa* and *Serratia marcescens* isolated from wound infection. With the aid of swab sticks, 250 wound samples were collected from surgical wounds patients admitted to different hospitals in Onitsha, Anambra State and cultured using spread plating technique. Isolates were characterized using standard microbiological technique. The Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration of the potash alum, lime and the combination of potash alum and lime were determined by preparing different concentration of these substances. Some of the experimental animal's immune response were suppressed with cyclophosphamide which was injected intraperitoneally without anesthesia, before they were inoculated with the test isolates and allowed to develop infection. Both the healthy and immunocompromised albino mice that developed infection were treated with potash alum, lime and the mixture of potash alum and lime. Phytochemicals screening of the lime juice showed the presence of alkaloids, flavonoids, tannins, saponins and reducing sugars. The MIC of potash alum was effective on *Serratia marcescens* at 6.25% and on *Pseudomonas aeruginosa* at 12.5%. The MIC of lime was effective on *Serratia marcescens* at 12.5% and on *Pseudomonas aeruginosa* at 25%. The MIC of the combination of potash alum and lime was effective on *Serratia marcescens* at 6.25% and on *Pseudomonas aeruginosa* at 12.5%. The combination of potash alum and lime was most effective in the wound healing, indicating the possibility of synergy between the two.

Keywords*: wound, lime, potash alum, *Pseudomonas aeruginosa.

1. Introduction

Nosocomial infections also known as hospital acquired infections (HAIs) are infections that occur in patients under medical care [1]. Hospital acquired infections are major challenges to patient's safety. It was estimated that in 2002, a total of 1.7 million hospital acquired infections occurred (4.5 per 100 admissions) and almost 99,000 deaths were associated with a hospital acquired infection [2]. This makes HAI the sixth leading cause of death in the United States [3]. Similar data have been reported from Europe [3].

Serratia marcescens is a species of rod-shaped Gram-negative bacterium in the family Enterobacteriaceae [4]. A human pathogen, *S. marcescens* is involved in hospital acquired infections (HAIs), particularly Catheter- associated bacteremia, urinary tract infections [5]. Most *S. marcescens* strains are resistant to several antibiotics because of the presence of R-factors which are a type of plasmid that carry one or more genes that encodes resistance, all are considered intrinsically resistant to ampicillin, macrolides and first generation Cephalosporins [6].

The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen that normally inhabits the soil and surfaces in aqueous environments. Its adaptability and high intrinsic antibiotic resistance enable it to survive in a wide range of other natural and artificial settings including medical facilities. Serious *P. aeruginosa* infections are often nosocomial and nearly all are associated with compromised host defences such as in neutropenia, severe burns or cystic fibrosis [7]. Therapeutic options are increasingly limited due to the continued emergency and spread of antimicrobial resistant strains, as a result, *P. aeruginosa* infections demonstrate high morbidity and mortality. Given the severity of *P. aeruginosa* infections and limited antimicrobial arsenal with which to treat them, finding alternative prevention and treatment strategies is an urgent priority. The success of P. *aeruginosa* as an opportunistic pathogen is due substantially to the versatility and adaptability encoded in its genome. As of September 2012, 26 strains of *P. aeruginosa* from both clinical and environmental sources had been fully or partially sequenced according to the NCBI (National Center for Biotechnology Information) Entreze database. Compared with the most other bacteria that caused disease, *Pseudomonas* has a relatively high genome ranging from 6.22 to 6.91Mb [8].

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value [9]. In the traditional indigenous medicinal system, sweet lime juice is valued for curing fever, malaria and jaundice [10]. To prevent scurvy during the 19th century, British sailors were issued a daily allowance of citrus, such as lemon, and later switched to lime.

Alum is both a specific chemical compound and a class of

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chemical compounds. The specific compound is the hydrated potassium aluminum sulfate (potassium alum) with the formula $KAL(SO4)_2.12H_2O$ [11]. Alums are useful for a range of industrial processes. They are soluble in water; have a sweetish taste; react acid to litmus; and crystallize in regular octahedral. When heated they liquefy; and if the heating is continued, the water of crystallization is driven off, the salt froths and swells, and at last an amorphous powder remains. They are astringent and acidic [12].

Antimicrobial resistance is recognized as a growing global threat. This is as a result of misuse and overuse of the drugs used in treating the infections caused by microorganisms. Compounding the problem of antimicrobial resistance is the immediate threat of a reduction in the discovering and development of new antibiotics. There is urgent need to discover novel acting compounds that are relatively cheap to overcome this problem. Natural substances like potash alum and lime juice could serve as better alternative to our conventional antibiotics if their antimicrobial activity is established, hence this study.

2. Materials and Methods

Specimen Collection:

With the aid of swab sticks, wound samples were collected from two hundred and fifty patients with surgical wounds admitted to different hospitals in Onitsha, Anambra State.

Media Preparation and Inoculation:

The media used (blood and nutrient agar plates) were carefully weighed and prepared according to manufacturer's instructions. Through spread plate inoculation technique, samples were inoculated on the prepared plates and at 37^{o}C for 24 hours.

Identification of Isolates:

Isolates were identified through standard identification protocol making use of their colonal features, cellular features and biochemical features.

Qualitative Phytochemical Analysis of lime:

The lime sample used was screened for the presence of the following compounds; alkaloids, flavonoids, glycosides, phenols, saponins, tannins and reducing sugars using standard laboratory techniques [13], [14]. Procedure for the tests:

Test for Alkaloids:

Few drops of the lime sample were mixed in 5 ml of 1.5 % v/v hydrochloric acid. These solutions were then used for alkaloid detection.

Test for Saponins:

Foam Test: A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, a froth is obtained, saponins are present [14].

Test for Tannins:

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagent:

Ferric Chloride Test:

A 5 % solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. If dark green or deep blue color is obtained, tannins are present [14].

Test for Flavonoids:

Shinoda Test: A small quantity of test residue was dissolved in 5 ml of ethanol (95 % v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta color is developed within a minute or two, if flavonoids are present [14].

Test for Sugars:

Molisch's Test: The Molisch's reagent was prepared by dissolving 10 g of α- naphthol in 100 ml of 95% alcohol. A few mg of the test extract was placed in a test tube containing 0.5 ml of water, and it was mixed with two drops of Molisch's reagent. To this solution, was added 1 ml of concentrated sulphuric acid from the side of the inclined test tube, so that the acid formed a layer beneath the aqueous solution without mixing with it. If a red brown ring appears at the common surface of the liquids, sugars are present [14].

Test for Glycosides:

Borntrager's Test: Borntrager's test is employed for presence of anthraquinones. The drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides [14].

In vitro Antibacterial Assay with Conventional Antibiotic Disc:

Conventional antibiotic susceptibility of the isolates was performed on Mueller Hinton Agar by Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines (Cheesbrough, 2000, CLSI, 2007). Antibiotics used were Amoxicillin (30*u*g), Streptomycin (30*u*g), Gentamycin (l0*u*g), Ciprofloxacin (10*u*g), Septrin (l0*u*g).

Inoculums standardization of culture was done according to the methods described by [15] and [16]. Universal bottles containing nutrient broth were suspended with 24hrs culture of the test bacteria. Normal saline was gradually added to it so as to compare the turbidity with 0.5 MacFarland standards which is approximately 10^8cells/ml. The inoculum was mixed properly and fresh sterile cotton tipped swab was dipped into the inoculum. The swab was pressed against the side of the tube to remove excess liquid. The swab was then spread evenly on the surface of the solidified Mueller Hinton agar contained in petri dishes and left for 5mins. The antibiotic sensitivity disc were asceptically placed on the already seeded agar plate. They were incubated at 37^{o}C for 24hrs. The antibacterial susceptibility was determined by measuring the zone of growth inhibition. The mean of the triplicate result was taken.

Processing of Potash Alum:

Impurities were physically removed from the potash alum. 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% (g/v) of the potash alum were placed in the shaker at 60rpm at 40°C. The samples dissolved in water within one hour.

Processing of lime:

Impurities were removed from the lime fruit by thoroughly

washing with sterile water. The lime juice was squeezed out from the lime fruit. 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% (v/v) of the lime juice were placed in a shaker at 60rpm at 40°C. The samples mixed in sterile water within 2 minutes.

Determination of pH Values of Different Concentrations of Potash alum and lime juice:

pH values of the different concentrations of potash alum was determined by the aid of a pH meter. The pH value of lime juice at different concentrations were also determined by the aid of a pH meter.

In vitro antibacterial activity of potash alum and lime juice:

The antibacterial activity of potash alum and lime juice were determined using the agar well diffusion method. 1ml of 24hrs culture of the test bacteria previously adjusted to 0.5 MacFarland's standard corresponding to approximately 1.0 x 10^8cfu/ml was pipette into a sterile plate and mixed with molten agar (Mueller Hinton) at 45°C added and the plate shaken gently so as to mix the contents. The molten agar and the test organism were allowed to solidify on a flat table for 40mins. A sterile cork borer of 6mm diameter was used to bore wells 4mm deep. Four wells were bored on each plate. The wells were filled with 0.5ml each of different potash alum concentrations (10%-80%g/v) and lime juice (10%-80%v/v). Standard antibiotic solution (Gentamycin 10*u*g) and sterile water was used as positive and negative control respectively. The plates were incubated at 37°C for 24hrs. Antibacterial activity was determined by measuring the zone of growth inhibition of the different concentrations and mean of the triplicate results taken.

Determination of the Minimum Inhibitory Concentration (MIC) of potash alum sample:

The Minimum Inhibitory Concentration of potash alum was determined using doubling broth dilution method of Sahm and Washington (1990). The powdered sample was reconstituted by dissolving 1g of the powder in 1ml of nutrient broth for potash alum and the concentration was transferred to another 1ml of sterile broth in another test tube and this continued until a 7th test tube was reached. The 8th test tube containing sterile nutrient broth served as control. 1ml of an 24hrs culture with a cell density 1.0 x 10^8cfu/ml was inoculated into each test tube and the contents thoroughly mixed on a votex mixer. The test tubes were inoculated at 37°C for 24hrs. The test tube with the lowest dilution with no detectable growth in form of turbidity was considered the minimum inhibitory concentration.

Determination of the Minimum Inhibitory Concentration (MIC) of Lime:

The MIC of lime was determined using doubling broth dilution method of Sahm and Washington (1990). 1ml of crude extract of lime was dissolved in 1ml of nutrient broth and the concentration was transferred to another 1ml of sterile broth in another test tube and this continued until a 7th test tube was reached. The eight-test tube containing sterile nutrient broth served as control. 1ml of an 24hrs culture with a cell density 1.0 x 10^8cfu/ml was inoculated into each test tube and the contents thoroughly mixed on a votex mixer. The test tubes were inoculated at 37°C for 24hrs. The test tube with the lowest dilution with no detectable growth in form of turbidity was considered the minimum inhibitory concentration [17].

Determination of the Minimum Bactericidal Concentration (MBC) of the Potash Alum, lime juice and their combination:

The MBC, 100*ul* of the broth culture was taken from the MIC test tube that showed no growth by visual inspections in both potash alum, lime and the combination of potash alum and lime and inoculated on the surface of a solidified Mueller Hinton agar contained in a petri dish. The MBC is the lowest concentration that results in killing 99.9% of the test bacteria [18].

In vivo Antibacterial Assay:

The *in vivo* antibacterial assay of potash alum solutions, lime juice, and their combinations on the isolates was carried out using albino mice.

Sample (albino mice) Collection:

Six weeks old healthy albino mice (40) weighing between 100g-120g were obtained from a private animal house in Awka. The mice were housed in a standard metal cage, fed a stock diet and given water to drink. Twenty of the albino mice served as the healthy mice while twenty served as immunocompromised mice. The mice were kept at room temperature before and during the experiment. This was done as described by [19].

Immunosuppression:

The experimental animals' immune response were suppressed by injecting them (using 1ml insulin syringe) with 0.1ml cyclophosphamide (100mg/kg body weight). Intraperitoneally without anesthesia as described by [19]. After 3days, the immunosuppressed mice were inoculated with the test isolates.

3. Excision Wound Model Induction

Inoculation of albino mice (infection):

The experimental animals were infected with the selected bacterial isolates cutaneously as described by [20]. The dorsal area, just above the tail region was gently disinfected with 70%(v/v) ethanol and shaved carefully using sterile surgical blades. The size of the injured area was (1.5 x 1.5cm^2). Bacterial inoculums were prepared by adding the bacterial isolates in Nutrient broth at 37°C overnight followed by repeated centrifugation and washing with a final re-suspension in normal saline. To determine the LD_{100} (Lethal dose causing 100% mortality) value of the bacterial culture, doses ranging from 10^2 to 10^{10} colony forming unit/ml (CFU/ml) were evenly applied topically using a sterile swab stick on the injured site. The animals were carefully observed till they develop signs of infections.

Effects of topical application of potash alum, lime juice and their combination in treating the isolate in healthy and immunocompromised albino mice:

Five groups of healthy mice A, B, C, D and E (4 mice in each group) were taken. A full cutaneous injury was induced at the dorsal region of all groups and infected with LD_{100} of the selected bacterial culture directly at the site of injury. All the mice were monitored for any signs of infection for three days before commencement of treatment. In groups A, B, and C, mice were treated with a daily topical application of 0.5ml of potash alum, lime juice and their combination respectively. In group D, all the mice infected with bacterial inoculum acted as

negative control while in group E all the mice were treated with a daily application of 100% Gentamicin solution which served as positive control.

Post Inoculation Analysis:

Koch's postulate analysis:

This analysis was done to establish Koch's postulate in the disease process. Wound swabs were carefully and asceptically taken from the infected areas after three days of post inoculation using sterile swab stick. It was inoculated onto Nutrient agar and incubated at 37°C for 24 hrs.

The colonies were further identified as described earlier.

Post Inoculation Treatment:

The animals were treated and monitored for two weeks till full recovery. Groups A, B, C of both healthy and immunocompromised mice were treated with potash alum, lime juice and a combination of both respectively. Groups D of healthy and immune compromised (negative control) mice were left untreated while groups E of both healthy and immunocompromised mice were treated with Gentamicin (positive control).

Wound Contraction Measurement:

A transparent tracing paper was used to measure the wound size on the 1st, 4th, 8th, 11th and 14th day of post treatment. The transparent paper was placed on a 1mm^2 graph sheet and the wound area was measured. The percentage wound contraction was calculated using the formula of [21].

Percentage of wound contraction =

$$\frac{\text{Wound size on day 0} - \text{Specific day wound size}}{\text{Wound size on day 0}} \times 100$$

4. Results

The results of the morphological and biochemical characteristics of the bacterial isolates are presented in table 1. Table 2 shows the result of sugar fermentation test.

The result of the phytochemical analysis of lime juice used is shown in Table 2, and it contained moderate amount of reducing sugar and glycosides. There were also presence of alkaloids, phenols, flavonoids, tannins and saponins. The intensity of colour change is a semi quantitative measure of the amount of each chemicals present in the sample and is represented in the table by the number of plus (+) signs.

Table 2
Phytochemical analysis of lime juice concentrate

Parameters	value (inference)
Alkaloids	+
Phenols	+
Flavonoids	+
Tannins	+
Reducing sugar	+ +
Saponins	+
Glycosides	++

+ slightly detected; ++ moderate amount; +++ high amount

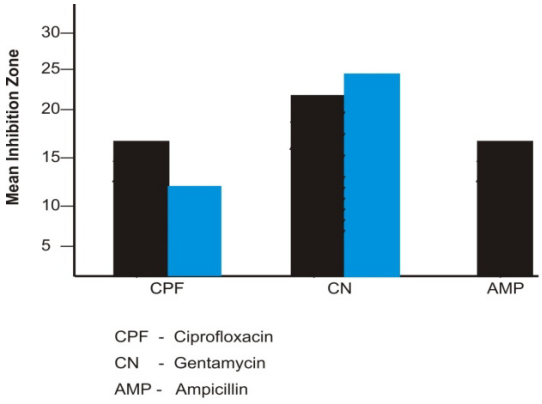


Figure 4.0: Mean inhibition zone of conventional antibiotic sensitivity rise on both *Pseudomonas aeruginosa* and *Serratia marcescenes* Isolates

The pH values of the different concentration of potash alum, lime and the combination of potash alum and lime were shown

Table 1
Cellular and biochemical features of the bacterial isolate

Bacteria	G.	Mot	Cat	Lac	Ind.	MR	VP	Oxi	Ur	Lac	Glu	Sor	Mann	Suc
Serratia marcescens	-	+	+	-	-	+	+	-	-	-	+	+	+	+
Pseudomonas aeruginosa	-	+	-	+	-	+	+	+	-	+	+	-	+	+

G = Gram, Mot. = Motility, Cat =Catalase, Lac. = Lactose, Ind = Indole, MR = Methyl Red, VP = Vogues Proskaeur, Oxi = Oxidase, Ur =Urease, Lac = Lactose, Glu=Glucose, Sor = Sorbitol, Mann = Mannitol, Suc = Sucrose

Table 3
pH values of the different concentrations of potash alum, lime and combination of potash alum and lime

	pH		
Concentration (%)	Potash Alum	lime	Potash alum and lime
10	2.84±0.00	3.69±0.00	2.82±0.00
20	2.78±0.00	3.54±0.00	2.73±0.00
30	2.60±0.00	3.43±0.00	2.65±0.00
40	2.69±0.00	3.36±0.00	2.60±0.00
50	2.53±0.00	3.10±0.00	2.51±0.00
60	2.45±0.00	2.92±0.00	2.42±0.00
70	2.31±0.00	2.84±0.00	2.30±0.00
80	2.37±0.00	2.71±0.00	2.33±0.00

Result presented as Mean ± SEM

in Table 3, here the pH values of all the samples reduced, becoming more acidic as their concentration increased.

The antimicrobial activities of potash alum, lime and its combination on *Serratia marcesens* and *Pseudomoans aeruginosa* using well-in-agar diffusion method are shown in tables 4, 5 and 6. *Serratia marcesecens* and *Pseudomonas aeruginosa* were sensitive to the combination of potash alum and lime. There was slight increase in the zone of inhibition diameter of the formulation of potash and alum compared to the individual samples.

Table 4
Antimicrobial effect of potash alum and lime at different concentrations on Serratia marcescens (zone of inhibition diameter)

Concentrations (%)	Potash alum (mm)	Lime (mm)
10	20.10±0.10	-
20	24.40±0.20	-
30	26.75±0.15	3.80±0.10
40	29.55±0.15	6.50±0.10
50	31.80±0.10	10.45±0.15
60	34.10±0.10	13.65±0.15
70	37.40±0.10	17.01±0.1
80	40.20±0.10	22.23±0.01

KEY: Positive control - Gentamycin 35.00 mm; Negative control - Distilled water 0.00mm
MM – Millimetre; Result presented as Mean ± SEM

Table 5
Antimicrobial effect of potash alum and lime at different concentrations on *Pseudomonas aeruginosa* (zone of inhibition diameter)

Concentrations (%)	Potash alum (mm)	Lime (mm)
10	10.40±2.05	-
20	16.00±1.50	-
30	20.20±1.50	4.10±1.40
40	23.50±1.00	5.50±2.14
50	27.30±3.06	7.30±0.50
60	30.00±2.15	11.70±1.10
70	32.30±1.00	16.20±1.75
80	35.80±1.60	20.00±0.01

KEY: Positive control (Gentamycin) - 32.00mm, Negative control (distilled water) - 0.00mm; mm-Millimetre. Result presented as Mean ± SEM

Table 6
Antimicrobial effects of a combination of potash alum and lime at different concentrations on the isolates

Concentration (%)	Formulation of Potash alum and Lime (mm)	
	Serratia marcescens	*Pseudomonas aeruginosa*
30	27.00	21.00
40	31.60	22.90
50	33.10	25.10
60	35.00	28.80
70	35.50	31.10
80	41.20	33.10

Positive control - Gentamycin 38.00mm and 35.00mm for Serratia marcescens and Pseudomonas aeruginosa respectively. Negative control – Distilled water 0.00mm; mm – millimeter

The MIC of potash alum was effective on *Serratia marcescens* at 6.25% and on *Pseudomonas aeruginosa* at 12.5%. The MIC of lime was effective on *Serratia marcescens* at 12.5% and on *Pseudomonas aeruginosa* at 25%. The MIC of the combination of potash alum and lime was effective on *Serratia marcescens* at 6.25% and on *Pseudomonas aeruginosa* at 12.5%, although a very slight synergy was noticed when in their combination against the bacterial isolates (tab. 7).

The MBC of potash alum was effective on *Serratia marcescens* at 12.5% and on *Pseudomonas* aeruginosa at 25%. The MBC of lime was effective on *Serratia marcescens* at 25% and on *Pseudomonas aeruginosa* at 50%. The MBC of the combination of potash alum and lime was effective on *Serratia marcescens* at 12.5 and on *Pseudomonas aeruginosa* at 25% (Tab. 7).

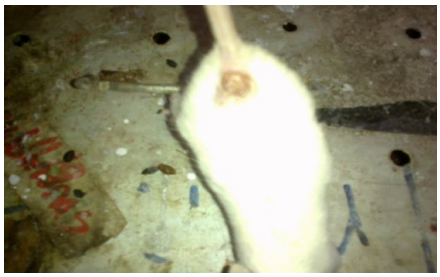
Table 7
Minimum inhibitory concentration and minimum bactericidal concentration of the bacterial isolates on potash alum, lime and the combination of potash alum and lime

Antimicrobial agents	*Serratia marcescens*		*Pseudomonas aeruginosa*	
	MIC (%)	MBC (%)	MIC (%)	MBC (%)
Potash alum	6.25±0.00	12.50±0.00	12.50±0.00	25.00±0.00
Lime	12.50±0.00	25.00±0.00	25.00±0.00	50.00±0.00
Potash alum and Alum	6.25±0.00	12.50±0.00	12.50±0.00	25.00±0.00

Result presented as Mean ± SEM

5. Wound Healing Studies on Healthy Albino Mice

The use of potash alum and lime in the treatment of wound infection in healthy albino mice caused by *Pseudomonas aeruginosa* and *Serratia marcescens* are shown (plates 1- 6). The photographs of the infection site from the first day of treatment with potash alum and lime and a combination of both to the gradual closure of the wound interface and restoration of an epithelial cover in the healthy albino mice are shown.



Plates 1a: Day 0 before treatment with lime in *Pseudomonas aeruginosa* wound infection in a healthy mice, presence of inflammation and exudates

Plate 1b: Day 6 after treatment with lime in *Pseudomonas aeruginosa* wound infection in a healthy mice. Here the wound has reduced in size showing that it is gradually healing

The ability to heal wound in the albino mice were compared between the positive control groups (mice treated with gentamycin), mice treated with potash alum, lime and its combination as experimental groups and mice treated with distilled water as negative control groups during the wound healing process. In all groups, wounds were covered by a

dehydrated wound crust at day 3 after infection with the bacterial isolates. The scab disappeared before day 12. By 18th day after infection with the bacterial isolates, the wounds were fully healed (In all potash alum, lime and potash alum/lime treated mice). Wound healing was based on the macroscopic closure of the wound interface and restoration of an epithelial cover.

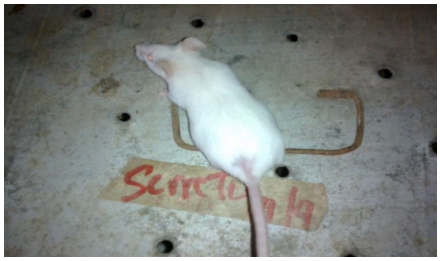


Plate 1c: Day 12 after treatment with lime in *Psedomonas aeruginosa* wound infection in healthy mice. Here the wound had completely healed and closed up

Plate 1d: Day 18 after treatment with lime in *Pseudomonas aeruginosa* wound infection in healthy mice. Here the skin has returned to normal

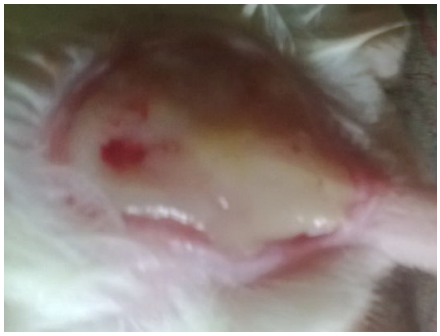


Plate 2a: Day 0 before treatment with Potash alum in *Pseudomonas aeruginosa* wound infection in healthy mice

Plate 2b: Day 6 after treatment with potash alum in *Pseudomonas aeruginosa* wound infection in a healthy mice. There is a clear reduction in the size of the wound

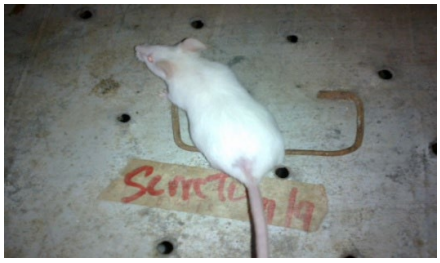


Plate 2c: Day 12 after treatment with Potash alum in *Psedomonas aeruginosa* wound infection in a healthy mice. The wound has closed up completely

Plate 2d: Day 18 after treatment with Potash alum in *Pseudomonas aeruginosa* wound infection in a healthy mice

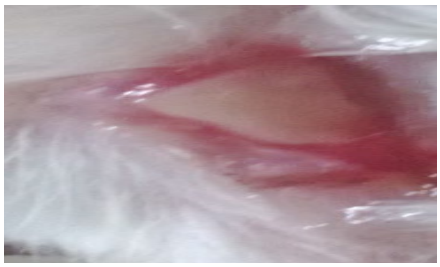


Plate 3a: Day 0 before treatment with combination of potash alum and lime in *Pseudomonas aeruginosa* wound infection in a healthy mice

Plate 3b: Day 6 after treatment with combination of potash alum and lime in *Pseudomonas aeruginosa* wound infection in a healthy mice

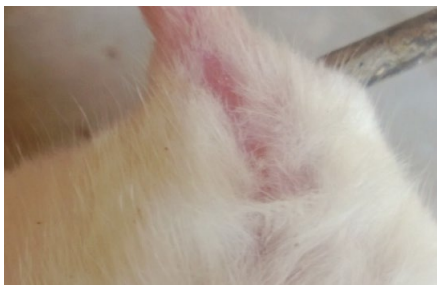


Plate 3c: Day 12 after treatment with combination of Potash alum and lime in *Pseudomonas aeruginosa* wound infection in a healthy mice

Plate 3d: Day 18 after treatment with combination of Potash alum and lime in *Pseudomonas aeruginosa* wound infection in a healthy mice

Plate 4a: day 0 before treatment with lime in *Serratia marcescens* wound infection in healthy albino mice

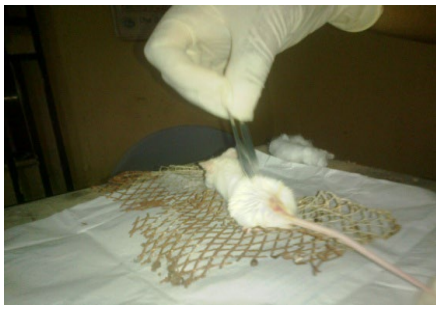


Plate 4b: Day 6 after treatment with lime in *Serratia marcescens* wound infection in healthy albino mice. There is a significant reduction in the size of the wound

Plate 4c: Day 12 after treatment with lime in *Serratia marcescens* wound infection in healthy albino mice

Plate 5d: Day 18 after treatment with Potash alum in *Serratia marcescens* wound infection in a healthy albino mice

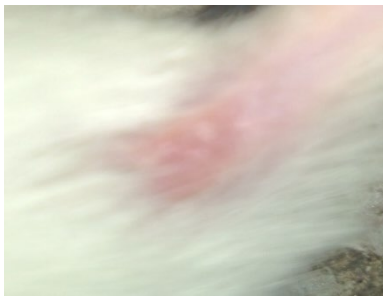


Plate 6a: Day 0 before treatment with combination of Potash alum and lime in *Serratia marcescens* infection in healthy albino mice

Plate 6b: Day 6 after treatment with combination of potash alum and lime in *Serratia marcescens* wound infection in healthy albino mice

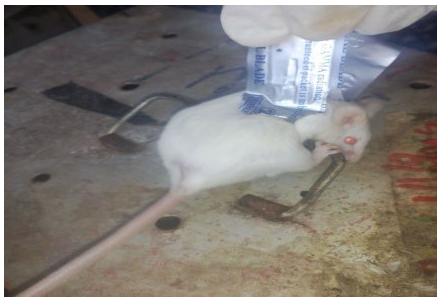


Plate 6c: Day 12 after treatment with combination of potash alum and lime in *Serratia marcescens* wound infection in healthy albino mice

Table 8
The wound size diameter in the experimental groups treated with potash alum, lime and the combination of both in *Pseudomonas aeruginosa* wound infection in healthy albino mice

Day	Distilled Water Negative Control (mm)	Potash alum (mm)	Lime (mm)	Potash alum/Lime (mm)	Gentamycin positive Control (mm)
1	14.50±2.60	14.66±2.82	14.42±2.55	15.21±2.94	14.80±2.58
3	13.25±1.80	11.25±2.63	11.56±2.67	11.00±2.51	12.00±2.74
6	10.81±1.52	7.17±1.10	7.27±1.14	6.41±1.07	8.82±1.39
9	7.67±1.23	3.13±0.70	4.32±0.96	2.50±0.50	2.90±0.69
12	5.04±1.08	0.00	0.00	0.00	0.00
15	2.11±0.56	0.00	0.00	0.00	0.00

Results presented as mean ± SEM

Table 9
The wound size diameter in the experimental groups treated with potash alum, lime and the combination of both in *Serratia marcescens* wound infection in healthy albino mice

Day	Distilled Water Negative Control (mm)	Potash alum (mm)	Lime (mm)	Potash alum and Lime (mm)	Gentamycin positive Control (mm)
1	16.20±2.50	16.51±2.71	15.48±2.42	16.20±2.50	16.70±2.78
3	15.51±2.45	12.12±2.22	13.00±2.32	11.42±2.17	13.81±2.39
6	12.74±2.16	8.81±1.33	9.42±1.66	9.57±1.69	10.25±1.80
9	9.23±1.73	4.30±0.81	5.11±0.89	3.51±0.52	4.91±0.94
12	6.11±1.01	0.00	0.00	0.00	1.90±0.08
15	3.94±0.83	0.00	0.00	0.00	0.00

Table 10
Percentage contraction of *Pseudomonas aeruginosa* induced wound infections treated with potash alum, lime and a combination of potash alum and lime in healthy albino mice

Day(s)	Negative Control (%)	Potash alum (%)	Lime (%)	Potash alum and Lime (%)	Gentamycin positive Control (%)
1	3.57	4.71	3.00	8.64	5.71
3	5.35	19.64	17.43	21.42	14.28
6	22.78	48.78	48.07	54.21	37.00
9	45.21	77.64	69.14	82.14	79.29
12	64.00	0.00	0.00	0.00	0.00
15	84.92	0.00	0.00	0.00	0.00

Table 11
Percentage contraction of *Serratia marcescens* induced wound infection treated with potash alum, lime and a combination of potash alum and lime and controls in healthy mice

Day(s)	Distilled Water (Negative Control) (%)	Potash alum (%)	Lime (%)	Potash alum and Lime (%)	Gentamycin (positive Control) (%)
1	1.25	3.19	3.25	1.25	4.38
3	3.06	24.25	18.75	28.63	13.69
6	20.38	44.94	41.13	40.19	35.94
9	42.31	73.13	68.06	78.06	69.93
12	61.81	0.00	0.00	0.00	88.13
15	75.36	0.00	0.00	0.00	0.00

Plate 6d: Day 18 after treatment with combination of lime and potash alum in *Serratia marcescens* wound infection in healthy albino mice

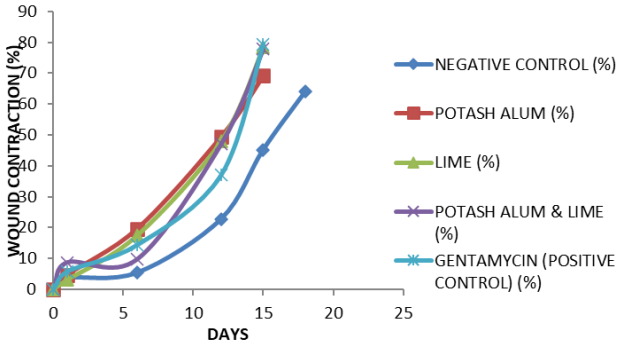


Fig. 1. Percentage contraction of *Pseudomonas aeruginosa* induced wound infection treated with potash alum, lime and combination of potash alum and lime and controls in healthy albino mice

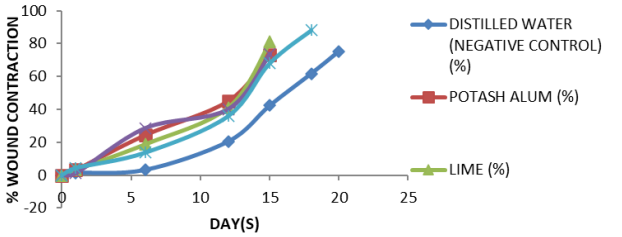


Fig. 2. Percentage contraction of *Serratia marcescens* induced wound infection treated with potash alum, lime and combination of potash alum and lime and controls in healthy albino mice

Tables 8 and 9 show the wound diameters in the experimental group, which was treated with the potash alum, lime and the combination of both. This was done by marking the wound diameter on a transparent paper and measuring the mark with 1mm graph note. There was a significant reduction in the wound diameters as the number of days for the treatment increased. The positive control group were healed but at a slower rate than the experimental group.

A graph showing the percentage contraction of wounds infection treated with potash alum, lime, combination of potash alum and lime and controls for both Pseudomonas aeruginosa and Serratia marcescens infected wounds from day(s) 0 to 22 in healthy albino mice albino mice were presented in figure 1 and figure 2.

Wound Healing Studies in Immunocompromised Albino Mice

Topical treatment of wound infection caused by *Pseudomonas aeruginosa* and *Serratia marcescens* in immunocompromised mice using potash alum, lime and

combination of potash alum and lime. The ability to heal wound in immunocompromised albino mice were compared based on pathological changes. Pathological changes in laboratory animals: Animals of all experimental groups showed dehydrated wound crust covering hypremia, swelling with abscess formation, inflammation after 3 days of wound infection with *Pseudomonas aeruginosa* and *Serratia marcescens*. By 20 days, the wounds were fully healed in all alum, lime and combination of potash alum and lime treated immune compromised mice. Wound healing was determined based on macroscopic closure of the wound interface and restoration of the epithelial cover.

Plates 4.7 to 4.11 show the photograph of the infection site from the first day of treatment with potash alum, lime and combination of potash alum and lime to gradual closure of the wound interface and restoration of an epithelial cover in the immune compromised mice.

Plates 1a: Day 0 before treatment with lime in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice, presence of inflammation and exudates

Plate 1c: Day 12 after treatment with lime in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice. Here the wound had completely healed and closed up

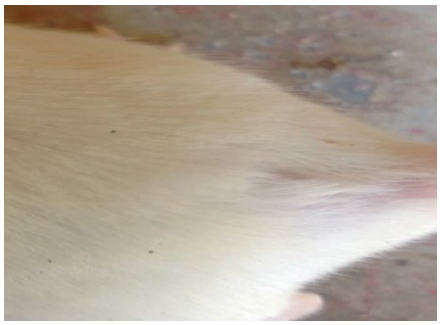


Plate 1d: Day 18 after treatment with lime in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice. Here the skin has returned to normal

Plate 2a: Day 0 before treatment with Potash alum in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice

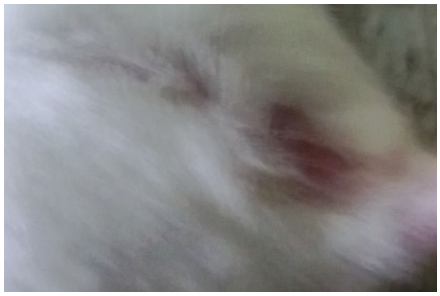


Plate 2c: Day 12 after treatment with Potash alum in *Psedomonas aeruginosa* wound infection in immunosuppressed mice. The wound has closed up completely

Plate 2d: Day 18 after treatment with Potash alum in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice

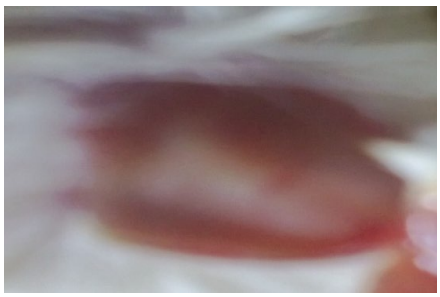


Plate 3a: Day 0 before treatment with combination of potash alum and lime in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice

Plate 3c: Day 12 after treatment with combination of Potash alum and lime

in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice

Plate 3d: Day 18 after treatment with combination of Potash alum and lime in *Pseudomonas aeruginosa* wound infection in immunosuppressed mice

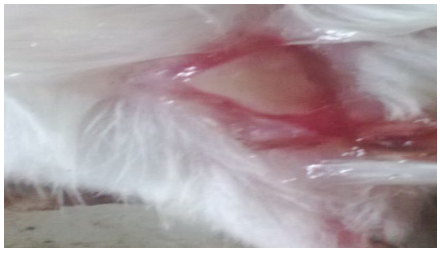


Plate 4a: day 0 before treatment with lime in *Serratia marcescens* wound infection in immunosuppressed albino mice

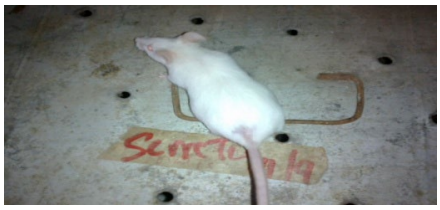


Plate 4c: Day 12 after treatment with lime in *Serratia marcescens* wound infection in immunosuppressed albino mice

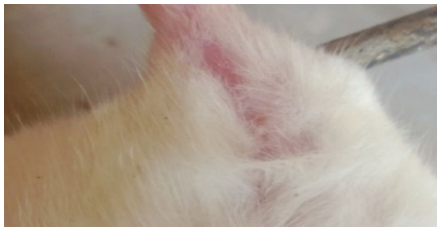


Plate 5d: Day 18 after treatment with Potash alum in *Serratia marcescens* wound infection in immunosuppressed albino mice

Plate 6a: Day 0 before treatment with combination of Potash alum and lime in *Serratia marcescens* wound infection immunosuppressed albino mice

Plate 6c: Day 12 after treatment with combination of potash alum and lime in *Serratia marcescens* wound infection in immunosuppressed albino mice

Plate 6d: Day 18 after treatment with combination of lime and potash alum in *Serratia marcescens* wound infection in immunosuppressed albino mice

A graph showing the percentage contraction of wounds infection treated with potash alum, lime, combination of potash alum and lime and controls for both *Pseudomonas aeruginosa* and *Serratiamarcescens* infected wounds from day(s) 0 to 21 both in healthy albino mice and immune compromised albino mice are presented in figures 3 and 4.

Formular for percentage wound contraction:

Percentage of wound contraction =

$$\frac{\text{Wound size on day 0} - \text{Specific day wound size}}{\text{Wound size on day 0}} \times 100$$

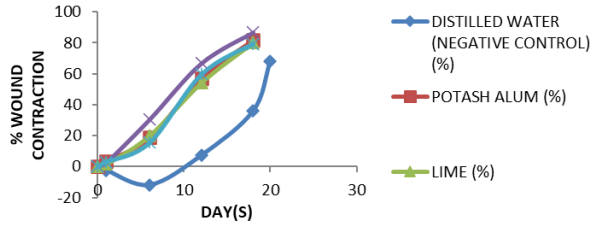


Fig. 3. Percentage contraction of *Pseudomonas aeruginosa* induced wound infection treated with potash alum, lime and combination of potash alum and lime and controls in immunocompromised albino mice

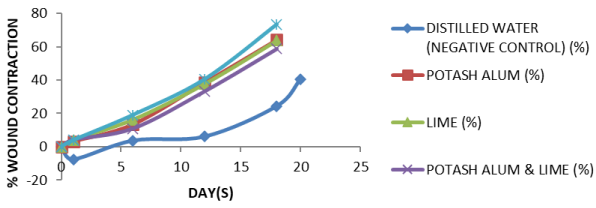


Fig. 4. Percentage contraction of *Serratia marcescens* induced wounds infection treated with potash alum, lime and combination of potash alum and lime and controls in immunocompromised albino mice

6. Discussion

The *in vitro* sensitivity pattern of the clinical isolates to conventional antibiotics in this research is in agreement with the work of [22], which also stated that Gentamycin and Ciprofloxacin have effect on *Pseudomonas aeruginosa. Serratia marcescens* was resistant to ampicillin and this is consistent with the 1997 study conducted by [23], which recorded that the problem in the treatment of infections due to *S. marcescens* is that the organism shows a resistance to a wide variety of antimicrobial agents including ampicillin and both second and third generation cephalosporins.

Phytochemicals confer certain health benefits such as anti-inflammatory, antimicrobial, antihypertensive and antidiabetic effects [24]; [25]. The result of the phytochemical analysis of lime in this work (Table 4.3) shows presence of alkaloids, flavonoids, glycosides, saponins and reducing sugar which is in concordance with the work of [26], which also showed the presence of saponins, alkaloids and reducing sugar.

Lime showed antibacterial activity against *Pseudomonas aeruginosa* and *Serratia marcescens*. This is in agreement with the work of [27], which reported that the phenolic components of lime juice have antibacterial action against Gram negative organisms. It is also supported by the statement of [28], that the saponins, tannins, steroids and glycosides of lime have antibacterial activities against Gram positive and Gram negative bacteria. Studies by [28], [30] and [31] revealed that alum is effective against a wide variety of microbial pathogens such as *Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia*. In [32] also observed a significant bactericidal effect of alum against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Klebsiella pneumonia.*

Potash alum and lime are excellent antimicrobials [33]. This was revealed by the results of the *in vitro* and *in vivo* sensitivity tests conducted (table 4.5/ figure 4.7). Potash alum gave the best result, followed by lime (table 4.5/ figure 4.7). All bacteria isolates (*Serratia marcescens* and *Pseudomonas aeruginosa*) showed similar sensitivity result to the potash alum and lime *in vitro* and likewise *in vivo* (figure 4.0).

The inhibition zone (mm) for alum increased with increase in the concentration of the solution (table 4.5). This agrees with the works of [30]. This also indicates that alum has an antibacterial action (table 1). This action increases with increased alum concentration which agrees with the findings of [34], who observed that the effect of 100ppm alum solution on microorganisms was less when compared to solutions containing 1000ppm alum or more.

This study shows that *Serratia marcescens* and *Pseudomonas aeruginosa* have high MIC and MBC values (table / figure) which translated that a higher concentration of potash alum are required to inhibit their growth and this work agrees with the reports of [35] in this study, the potash alum, lime and their combination exhibit favourable antimicrobial activities against the bacteria isolates. The concentration that gave the best result was 80% g/v. It is the best solvent for potash alum. The potency of the potash alum and lime was also indicated by the gradual reduction in the wound surface area during treatment, with combination of potash alum and lime showing the best reduction rate.

When applied topically at the wound site daily, there was a significantly higher percentage of wound contraction (healing) of 73.13% and 77.64% in *Serratia marcescens* and *Pseudomonas aeruginosa* induced wound infection respectively compared to 42.31% and 45.21% in the untreated group of the healthy mice on the 15th day (table 11 – 12). Also, when compared with the untreated control group in the immunocompromised mice there were high significant difference in the percentage contraction of the treated wound (figure 3 – 4).

Lime showed antibacterial activity against *Serratia marcescens* and *Pseudomonas aeruginosa in-vitro* as shown in tables 4.5 and 4.6 respectively, with its zone of inhibition diameter increasing as the concentration increases. Mice with wounds treated with a daily topical application of lime had a significantly high wound contraction rate of 100% and 69.14% as compared to 64.00% and 45.21% in untreated group of healthy mice on the 15th day of post treatment.

The efficacy of the topical application of potash alum in combination with lime was also evaluated for treating *Serratia marcescens* and *Pseudomonas aeruginosa* induced wound infection in healthy and immunocompromised mice.

The result shows that when potash alum was mixed with lime, there was a slight increase in the percentage of wound contraction compared to using them singly topically in both healthy and immunocompromised mice.

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