Study the inhibition activity of the different concentrations of Alum on some pathogenic bacteria isolation from different sites of human body

Sura, H.N. AL- ajeely , Ryiam, F.S.AL-haditheyi
College of Sciences, Biology department, Tikrit University.

Abstract
This study included the test of antimicrobial activity for Alum on seven bacterial isolates: Proteus sp., Pseudomonas sp₁, Pseudomonas sp₂, Streptococcus sp., E. coli and Staphylococcus aureus. The results showed alum in concentrations (5 and 10)gm/100 ml sterilized by a Millipore filter and gauze was affected against most bacterial isolates while alum in concentration 2.5 gm/100 ml not showed any inhibited affect against all selective bacterial isolates. The best mean of Minimal Inhibition Concentration for the alum sterilized by the a Millipore filter was in (100 & 200) mg/cm² the MIC was (19,15 and 11) respectively while low (MIC) was in concentrations 25 mg/cm² the MIC was (3), the high MIC for alum sterilized by gauze was in concentration (100 and 200) mg/ cm² the MIC was (15,12 and 9) respectively, while low (MIC) of alum sterilized by gauze was in concentration (50 and 25) mg/cm² the MIC was (4,2 and 1) respectively. The high mean of inhibited activity of alum against bacterial isolates when it sterilized by a Millipore filter while low mean of inhibited activity when it sterilized by gauze. Alum which sterilized by a Millipore filter have high inhibited activity towards most selective bacterial isolates and showed high activity against Streptococcus sp. compared with antibiotics (Ampicillin, Tobramicin and Ceftriaxone) while alum which sterilized by gauze have high activity against Staphylococcus aureus comparison with Antibiotics (Ampicillin, Tobramicin and Ceftriaxone).

Key Words: Alum, Antimicrobial activity, Bacteria isolated from different sites of human body

Introduction
Despite the great progress in human medicine, infectious disease caused by bacteria, viruses and fungi are still a serious problem in public health. It is estimated that over 90% of staphylococci, pneumococci and enterococci are resistant to antibiotics. There has been reported the increasing prevalence of methicillin-resistant Staphylococcus aureus (MRSA), β-lactam- and macrolide resistant pneumococci, and glycopeptide-resistant enterococci.

The worldwide increase in multidrug resistance of pathogenic bacteria has led to an urgent need for identifying an alternative strategies to counter bacterial infection. The latest research have been focused on identifying the potential antimicrobial agent from the natural resources.

Natural products have been used for centuries in treating human diseases and they contain components of therapeutic value. are environmentally safer, easily available, alum has become a favorite antimicrobial and can therefore be used as a natural inhibiting the growth of the bacteria.

The addition of alum to water results in the production of chemical precipitates which remove pollutants by two primary mechanisms. Removal of suspended solids, algae phosphorus, heavy metals and bacteria occurs primarily by enmeshment and adsorption onto aluminum hydroxide precipitate according to the following net reaction.

$$\text{Al} + 3 + 6\text{H}_2\text{O} \rightarrow \text{Al(OH)}_3(s) + 3\text{H}_3\text{O} + \text{Al}$$
Alum (Aluminum potassium sulfate) KAl(SO4)2.12H2O is colorless crystalline solids that turn white in air which is a favorite amongst mineral. Potassium alum is antimicrobial and can therefore be used as a natural deodorant by inhibiting the growth of the bacteria (8)(9)(10)(11).

Materials and Methods

1- Collection of Samples:

The samples under study have collected of different parts of human body from Teaching Tikrit hospital/ Tikrit. It has been selected to medical importance and causing various diseases to humans. Different media were used to growing it after diagnostic pathogenic case and growing on cultural media (Blood agar, MacConkey agar and Nutrient agar then incubated at 37°C for 24 hours).

2- Identification of isolates under study

A- Morphological Identification:

Isolated bacterial colonies were identified according to morphology, color and consistency on Nutrient and MaCconkey's agar medium and type of hemolytic on blood agar medium (7).

B- Microscopic examination:

Microscopic examination was used to classify bacteria to cocci, bacilli and to Gram negative or Gram positive bacteria, on the basis of their staining with gram stain, size, shape and arrangement of cells (7).

C- Biochemical test:

The biochemical test were done according to (7)(8)(9)(10)(11).

- Indole test:

Peptone water was inoculated with bacterial isolate and incubated at 37°C for 24 hours, 0.5ml of Kovacs reagent was added to incubated tube and shacked gently. The appearance of red color ring on upper part of broth is regarded as positive result color such as E.coli.

- Methyl red test

MR-VP broth was inoculated with the bacterial isolates and incubated at 37°C for 24 hours, 5 drops of Methyl red reagent was added. The appearance of red color reagent was added. The appearance of red color reagent was added. The appearance of red color regarded as positive result such as E. coli.

- Voges-Proskauer test:

MR-VP broth was inoculated with bacterial isolates and incubated at 37°C for 24 hours, 6 drops of α-naphthol and 2 drops of KOH were added. The appearance of change in color to red in 15 minutes is regarded as positive result.

- Urease test:

The urea base agar was sterilized by autoclave. It was left to cool to 50°C. Then urea was added to it and was poured in sterile tubes in slant position. The prepared medium was inoculated with pure bacterial colony by stabbing and streaking and incubated at 37°C for 24-48 hours. Change the color of media from yellow to pink color for 24-48 hours indicated a positive result while the result was regarded as negative after 4 days of incubation.

- Citrate utilization test:

This test was done by inoculating Simmon citrate slant media with bacteria and incubated at 37°C for 24 hours. Change the color of media from green to blue regarded as positive result.

- Growth on Kligler Iron Agar (KIA) test:

Bacterial isolates were inoculated on KIA by stabbing and streaking then incubated at 37°C for 24 hours. This test was used to detect the ability of bacteria to ferment lactose and glucose. Change of color from red to yellow in lower part of medium indicated glucose fermentation. When color of media doesn’t change indicates no fermentation of glucose or lactose. If gas bubble appears indicate production of CO₂ as a result of glucose fermentation. While change of color from red to yellow in upper part of medium indicated lactose fermentation and the black color indicated production of H₂S.

- Motility test:

Motility agar was inoculated with bacterial isolates by stabbing and incubated at 37°C for
24 hours. Appearance of turbid area around stabbing indicated a positive result.

- **Eosine Methylene Blue (EMB):**
  Bacterial isolates were inoculated on (EMB) and incubated at 37°C for 24 hours, appearance of growing colony of green metallic sheen indicated that is *E. coli*.

- **Oxidase test:**
  Few drop of Oxidase reagent was saturated on filter paper then part of colony from 24 hours cultures was transported by wooden stick and put on saturated filter paper, if the colony gave purple color during 10 second, this indicated a positive result.

- **Catalase test:**
  A drop of (3% H₂O₂) was put on a clean dry slide then a colony from 24 hours culture of bacteria was transported and mixed with this reagent. Appearance of gas bubbles means positive result. Such as *Staphylococcus* and *Micrococcus* because this bacteria produce the catalase enzyme, catalase enzyme breaks down hydrogen peroxide (H₂O₂) into water and oxygen and release bubble.

- **Coagulase test:**
  This test was used to determine the ability of microorganisms to clot plasma. This test prepared by taking one loopful of bacterial isolates that growth from 18- 24 hours and added to 0.5 ml of human plasma, the tube was incubated at 37 °C for 30 minutes. If no clot appeared, the tube was incubated for further 24 hours, Appositive coagulase was represented by appeared of clotting.

- **Mannitol fermentation test:**
  Mannitol salt agar was inoculated with pure colony of isolated bacteria and incubated at 37°C for 24 hours.

- **Antibiotics sensitivity test:**
  The antibiotic disc which are shown in table (1) were placed on the surface of Muller – Hinton agar media with a sterile forceps after spreading out the bacterial inoculums by streaking method. The cultivated plates were incubated for 24 hours at 37 °C (14).

**G-Evaluation of antibacterial activity of the alum solution**

Agar well diffusion method was used to evaluate, in vitro, the antibacterial effect of the alum against the common bacterial isolates from different parts of human body, by means of of agar-well diffusion assay, Mueller Hinton agar (45 °C) were poured into sterile petri dishes.50 μl from each bacteria isolate (Cell suspensions containing were taken separately and evenly spread onto the surface of the agar plates of Mueller Hinton agar using a micropipette. Wells (6mm diameter, and 4mm height) were bored using a sterile cork borer. Different concentrations of the test solution were placed into the wells and the plates were incubated aerobically at 37°C for 24 h. After 24 hours of incubation, the plates were removed from the incubator and are examined for the inhibition zone around each well (if present), by using the ruler minimum calibration: 1mm.

**3- Alum solution preparation:**

A stock solution of Alum was prepared by mixing (2.5, 5 and 10)g of Alum with 100 ml of sterile distilled water which made 200% solution. 4 serial concentrations were prepared including the stock solution were used in the study prepared from three stocks The Alum stocks solutions was sterilized by filtration through a Millipore filter and gauze the of a milli pore diameter was 0.22mm. All concentrations were prepared from stock solution in a sterile conditions (16)(17).

**4- Statistical Analysis**

The data in this study were analysis according to Duncan's Multiple Range Test (DMRT) the statically test results were considered highly significant at \( p \leq 0.05\) and not significant at \( p \leq 0.05\) (18) and by using Minitab program in computer.

**Results**
The results showed that effect of different concentration of alum against bacterial isolates under study by using well diffusion method. Table (3) showed that effect of Minimum inhibition concentration (MIC) of Alum on bacterial isolates. The study showed significant variation found between Minimal inhibitions concentrations (MIC) against different bacterial isolates. The concentrations (200 and 100) mg/cm$^3$ were highest rate inhibited bacterial growth, the (MIC) were 19,15 and 12 in concentration 200 mg/cm$^3$, the (MIC) of concentration 100 mg/cm$^3$ was 15,11 and 9 while lowest concentration that inhibited bacterial growth was in concentrations (50 and 25) mg/cm$^3$ the (MIC) was 9 and 6 in concentration 50 mg/cm$^3$ and 3 in concentration 25 mg/cm$^3$. In concentration 2.5 of alum stocks which sterilized by a Millipore filter or by gauze was not affect in all concentrations against bacterial isolates.

In results explained in table (4) showed the mean of alum sterilized by Millipore filter was more effect against bacterial isolates comparison with alum sterilized by gauze which showed less effect from alum sterilized by a Millipore filter. Table (5) showed the inhibition effect of (alum in 2.5, 5 and 10) gm/100 ml with sterilized by a Millipore filter and gauze compared with antibiotic sensitivity. From the results alum that sterilized by a Millipore filter in stock's concentration (2.5)gm/100 ml that sterilized by (a Millipore filter and gauze) do not showed any inhibition efficiency toward all bacterial isolates (5 and 10)gm/100 ml have high inhibition efficiency against all bacterial isolates under study compared with Ampicillin.

The inhibition efficiency of alum in concentration 10 gm/100 ml sterilized by a Millipore filter was high compared with (Ceftriaxone) on bacteria (E. coli, Pseudo sp.$^1$, Pseudo sp.$^2$, Proteus sp., Streptococcus sp. Staph. aureus.$^1$ and Staph. aureus $^2$) the inhibition zone was (15, 19, 17, 24 and 16) . From the results the alum have equal inhibition activity on (Staphylococcus aureus.$^1$) compared with Tobramycin antibiotic and inhibition zone was (15) while alum was high inhibition efficiency against (Staph aureus.$^2$) compared with Tobramycin.

Alum in concentration 5 gm/100 ml showed high inhibition effect against bacteria (Pseudo sp.$^1$, Proteus sp., Streptococcus sp. and Staph aureus $^2$) compared with Ceftriaxone antibiotic also alum showed high effect against (E. coli, Pseudo sp.$^1$, Streptococcus sp. and Staph aureus $^2$) compared with Tobramycin. Alum in concentration (10 and 5) gm/100 ml sterilized by gauze showed high inhibition effect against all bacterial isolates under study compared with the antibiotics (Ampicillin, Tobramycin and Ceftriaxone).

**Discussion**

A wide variety of natural products has been under scrutiny for their clinical potential, both in terms of disease prevention and treatment. In this study Alum (Aluminum potassium sulfate) a naturally occurring was tested as inhibitor bacterial growth. In this study is was found that-Alum inhibits growth of bacterial isolates (E. coli, Pseudomonas sp.$^1$, Pseudomonas sp.$^2$ Proteus sp. Staphylococcus aureus$^1$ and Staphylococcus aureus $^2$) The study showed significantly was found between Minimal inhibition concentrations(MIC) against different bacterial isolates (Table 1) and we showed significantly affect the growth rate of the bacteria. The concentration of (200 and 100) mg/cm$^3$ was highest rate inhibited bacterial growth. This material hydrolyzes in water to form sulfuric acid, which is responsible for rising the acidity in the environment therefore precipitate the protein ($^1$ 19) so the flagella formation and growth rate of bacteria will inhibition ($^2$ 20). From the results the mean of alum sterilized by a Millipore filter was more effect against bacterial isolates was more effect comparison with sterilize by gauze, this effect may return to the foreign material in solution after sterilized by gauze inhibit the efficiency of alum by change the PH of media or present some compounds in solution after filtration of alum solution that encourage of bacterial growth. Alum effectively reduces bacteria.
because is an acidic producing compound that inhibits ammonia production by lowering pH, Hydrogen ions produced from the dissolution of alum react with ammonia to form non volatile ammonium(NH4+) (21) alum reduces ammonia emissions by biological (inhibition of ureolytic microorganisms) and chemical means (conversion of NH3 to NH4–N). this explain why alum eletively reduces ammonia emissions. In a low-pH, environment where bacteria are inhibited this study is agree with study of (15) (17)(22) were founds alum solution able demonstrate antibacterial against bacteria (E.coli, Staphylococcus sp. and Pseudomonas aerogenosa, Proteus mirailis and Streptococcus mutans) but this study disagree with (23) who found that alum did not show any antimicrobial effect.

As a result alum in concentration 200mg/cm3 that sterilized by a Millipore filter have high inhibition affect against Streptococcus sp. in dose 10 gm/ml sterilized by a Millipore filter the inhibition zone was 24 and lowest inhibition affect was in E.coli the inhibition zone was 12 in dose 5 gm/ml while alum sterilized by gauze in concentration 10 gm/ml have highest affect against bacteria (Staphylococcus sp.1 ) the inhibition zone was 18 and lowest affect was against (E.coli, Staphylococcus sp.1 and Staphylococcus sp.2 ) in concentration 5 gm/ml comparision with antibiotics (Ampicillin, Tobramycin and Ceftriaxone) .Alum has a low toxicity inexperience animals, and because the body doesn't absorb aluminum, it's harmless when swallowed. but ingestion of 30 grams (one ounce) has killed adult humans. Concentrated solutions have caused breakdown of gum tissues, kidney damage, and fatal intestinal bleeding (24). Oneada and others proved that administration of Aluminum potassium sulphate (alum) 1.0, 2.5, 5.0 and 10.0% (w/w)does not exert tumorigenic or any other toxic actions in B6C3F1 mice (25). The emergence of bacterial strains that exhibit resistance to various antibiotics possess a major threat to medicine and public health. As a consequence, there is renewed interest in antibacterial targets which, by attenuating virulence, disrupt the capacity of pathogenic bacteria to cause infection. Cheong and others proved that the combinations of aluminum with chlorhexidine or erythromycin, are potentially useful as antibacterial agent. It is therefore, more beneficial to develop antibacterial agent using aluminum salts. However, more studies on the effects of these salts on the physical properties as well as toxicity are necessary (26).

Conclusions
The present study conclude that:
— The alum solution was more affect against pathogenic bacteria under study at concentrations (100 and 200) mg/ cm3. While it was lowest affect at concentration 25 mg/ cm3.
— The mean of alum sterilized by Millipore filter was more effect against bacterial isolates comparison with alum sterilized by gauze which showed less effect from alum sterilized by a Millipore filter.
— The alum stocks concentrations at (5% and 10%) that sterilized by A millipore filter and gauze was very effect against pathogenic bacteria compared with antibiotics (Ampicillin, Tobramycin and Cefitriaxone).

Recommendations
— Using the alum solutions or powder in sterilized instead of disinfectants in many scopes.
— Using the alum solution or powder in limited rate because it has high poison.
— The alum may using as alternate of antibiotics.
— The alum may using in industries of drugs.
— Doing more studies on alum and its effectives.
References


12- NCCLS. Performance Standards for Antimicrobial Disc Susceptibility Tests. Approved Standard NCCLS Publication 1993; M2- A5, Villanova, PA, USA.


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Appendix

Table (1) the Antibiotics used in the study

<table>
<thead>
<tr>
<th>No.</th>
<th>Antibiotics</th>
<th>Symbol</th>
<th>Conc. Of Antibiotic µg/disk</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ampicillin</td>
<td>AM</td>
<td>10</td>
<td>Bioanalyse (Turkey)</td>
</tr>
<tr>
<td>2.</td>
<td>Tobramycin</td>
<td>TOB</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Ceftriaxone</td>
<td>CRO</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Table (2) Test concentration of Alum

<table>
<thead>
<tr>
<th>Alum</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock solution</td>
<td>200</td>
</tr>
<tr>
<td>2 ml of Stock + 1ml of H2O</td>
<td>100</td>
</tr>
<tr>
<td>2 ml of conc. 2 + 1 ml of H2O</td>
<td>50</td>
</tr>
<tr>
<td>2 ml of conc. 3 + 1 ml of H2O</td>
<td>25</td>
</tr>
</tbody>
</table>


Table (3) showed the Minimal Inhibitory Concentration (MIC) to bacteria isolated from different sites of human body.

<table>
<thead>
<tr>
<th>Sterilized type</th>
<th>Stock solutions Concentrations (%)</th>
<th>Concentrations (mg/cm³)</th>
<th>Minimal Inhibitory concentrations (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millipore filter</td>
<td>10%</td>
<td>200 19 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 15 A</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50 9 B</td>
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<tr>
<td></td>
<td></td>
<td>25 3 C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>200 15 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 11 A</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50 6 B</td>
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<td>25 —</td>
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<td></td>
<td>2.5%</td>
<td>200 —</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>100 —</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50 —</td>
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<td></td>
<td></td>
<td>25 —</td>
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<tr>
<td>Gauze</td>
<td>10%</td>
<td>200 15 A</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>100 9 B</td>
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<td></td>
<td></td>
<td>50 4 C</td>
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<tr>
<td></td>
<td></td>
<td>25 1 C</td>
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<tr>
<td></td>
<td>5%</td>
<td>200 12 A</td>
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<td></td>
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<td>100 9 B</td>
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<td>50 2 C</td>
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<td>25 —</td>
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</tbody>
</table>

The different vertically letters means significant differentiation present at (P ≤ 0.05)

Table (4) showed the means of alum sterilized by a Millipore filter and gauze

<table>
<thead>
<tr>
<th>Sterilized types</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millipore filter</td>
<td>17</td>
</tr>
<tr>
<td>Gauze</td>
<td>13.25</td>
</tr>
</tbody>
</table>
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Table (5) showed the effect of alum in different concentrations on bacterial isolates comparison with antibiotics

<table>
<thead>
<tr>
<th>Alum’s sterilized Methods</th>
<th>Alum stocks concentrations (%)</th>
<th>Inhibition zone (mm)</th>
<th>E.coli</th>
<th>Pseudo. sp¹</th>
<th>Pseudo. sp²</th>
<th>Proteus sp</th>
<th>Streptococcus sp</th>
<th>Staph. Aureus¹</th>
<th>Staph. aureus²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millipore filter</td>
<td>2.5%</td>
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<td></td>
<td>5%</td>
<td></td>
<td>12</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>17</td>
<td>15</td>
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<tr>
<td></td>
<td>10%</td>
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<td>15</td>
<td>19</td>
<td>16</td>
<td>17</td>
<td>24</td>
<td>16</td>
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<tr>
<td>Gauze</td>
<td>2.5%</td>
<td></td>
<td>___</td>
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<td></td>
<td>5%</td>
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<td>11</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>14</td>
<td>11</td>
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<td></td>
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<td>14</td>
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<td>Antibiotics</td>
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<td></td>
<td>8</td>
<td>___</td>
<td>___</td>
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<td>9</td>
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<td></td>
<td>Tobramycin</td>
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<td>___</td>
<td>13</td>
<td>15</td>
<td>11</td>
<td>11</td>
<td>15</td>
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<td></td>
<td>Ceftriaxone</td>
<td></td>
<td>___</td>
<td>6</td>
<td>10</td>
<td>14</td>
<td>___</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Staphylococcus aureus¹ means bacteria isolated from burns
Staphylococcus aureus² means bacteria isolated from Abscess
Pseudomonas sp.¹ means this bacteria isolated from UTI
Pseudomonas sp.² means this bacteria isolated from wounds
( ___ ) Means no inhibition zone
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Figure -1 : Zones of bacterial inhibition formed around different concentrations of alum (Sterilized by a Millipore filter) on *Streptococcus* sp.

Figure-2: Zones of bacterial inhibition formed around The different concentrations of alum (sterilized by gauze) on *Pseudomonas* sp.
دراسة الفعالية التثبيطية لتراكيز مختلفة من الشب على بعض البكتيريا الممرضة المعزولة من مناطق مختلفة من جسم الإنسان

م.م. سرا حميد نايف العجيلي
م.م. رياض فالس صالح الحذيثي
كلية العلوم / قسم علوم الحياة / جامعة تكريت

الخلاصة:
تضمنت الدراسة اختبار الفعالية التثبيطية لمادة الشب على (7) عزلات بكتيرية (Pseudomonas sp1, Pseudomonas sp2, Proteus sp, Staphylococcus aureus1, Staphylococcus aureus2, Escherichia coli, Streptococcus sp) وتم العزل مختلفة من جسم الإنسان، وقد أظهرت نتائج الدراسة أن الشب يتراكيزه المختلفة المعمق بطريقة المرشحات الغشائية الدقيقة والشاش بالتركيز (5 و 10) غم/لمل. قد اتضح تأثير فعالية تثبيطية ضد أغلب العزلات البكتيرية المختبرية ولكن لم يظهر أي تأثير ضد العزلات البكتيرية المنتهية عند التركيز (2.5 غم/لمل). أن أفضل متوسط تركيز مثبط أدنى للشب المعم باستخدام المرشحات الغشائية الدقيقة والشاش كان عند التركيزين (100 و 200) ملمغم/سمم. بينما أرتفع تركيز مثبط أدنى للشب المعم باستخدام المرشحات الغشائية الدقيقة كان عند التركيز (25) ملمغم/سمم. وأرتفع تركيز مثبط أدنى للشب المعم باستخدام الشاش كان عند التركيز (5 و 50) ملمغم/سمم.

ان الشب المعم بواسطة المرشحات الغشائية اتضح فعالية تثبيطية تجاه أغلب العزلات البكتيرية المختبرية، حيث أظهر Ampicillin, Tobramycin فعالية تثبيطية عالية ضد البكتيريا. فعالية تثبيطية عالية ضد البكتيريا Strepococcus sp1، لذي مقارته مع المضادات الحيويه Ceftriaxone، بينما اظهر الشب فعالية تثبيطية عالية ضد البكتيريا Staphylococcus aureus1, Ceftriaxone و مقارنة مع المضادات الحيوية Ampicillin, Tobramycin.