

## Detection of Inhibitory Effect of Aluminium Potassium Sulfate Against Some Bacterial and Fungal Strains.

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### Abstract:

The percentage of microbial resistance to antibiotics has been remarkably increasing in the last decades. For this reason, there has been an increasing demand for using natural products molecules for medicinal purposes. The current study revealed the antimicrobial activity of aluminium potassium sulfate (alum) against the selected pathogens which includes gram positive and negative bacteria and fungus. Alum inhibits the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* at all concentrations (50,40,30,20, 10 and 5 %).

The percentage of microbial resistance to antibiotics has been remarkably increasing in the last decades. For this reason, there has been an increasing demand for using natural products molecules for medicinal purposes. The current study revealed the antimicrobial activity of aluminium potassium sulfate (alum) against the selected pathogens which includes gram positive and negative bacteria and fungus. Alum inhibits the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* at all concentrations (50,40,30,20, 10 and 5 %). However, *Vibrio cholera* growth was inhibited at the concentrations of (50, 40, 30, 20 and 10 %). The antimicrobial activity of alum against the selected pathogenic fungi (*Cryptococcus neoformance* , *Cryptococcus gattii*, *Rhizopus micrococcus* and *Mucor circinelloides*) was measured. The results showed that alum has excellent antimicrobial activity against all the selected pathogenic fungi in all concentrations. With no inhibitory effect against both *Rhizopus micrococcus* and *Mucor circinelloides* at the lowest concentration of alum (5%). The growth of fungi is significantly reduced by 4-fold in the presence of alum in comparison with those grown in YPD media alone. Which means that half of antimicrobial activity of alum is due to inhibitory mechanism differ from the acidification. The above results confirmed the antimicrobial activity of alum against the selected pathogens, and proved that the inhibitory activity of alum is not only due to the low pH produced by alum but also to the presence of other inhibitory mechanism of alum. Regarding these results further study is needed to understand the inhibitory mechanisms of alum with neglecting the acidity effect of alum.

**Keywords:** Aluminium potassium sulfate, antibacterial activity, antifungal activity, bacterial resistance, natural products.

دراسة فعالية مادة الشب كمضاد فطري ومضاد بكتيري

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**الخلاصة:**

ازدادت نسبة مقاومة الميكروبات للمضادات الحيوية بشكل ملحوظ في العقود الماضية. لهذا السبب، كان هناك طلب متزايد على استخدام جزيئات المنتجات الطبيعية للأغراض الطبية. كشفت الدراسة الحالية عن النشاط المضاد للميكروبات للشب ضد مسببات الأمراض المختارة. يمنع الشب نمو *Pseudomonas aeruginosa* و *Staphylococcus aureus* بجميع التركيزات (50، 40، 30، 20، 10 و 5٪). بينما، تم منع نمو ضمة الكوليرا بتركيزات (50، 40، 30، 20 و 10٪). تم قياس النشاط المضاد للميكروبات للشب ضد الفطريات الممرضة المختارة (*Cryptococcus neoformance*، *Cryptococcus gattii*، *Rhizopus micrococcus* و *Mucor circinelloides*). أظهرت النتائج أن الشب له نشاط مضاد للميكروبات ممتاز ضد جميع الفطريات الممرضة المختارة وبجميع التراكيز. مع عدم وجود تأثير مثبط ضد كل من *Rhizopus micrococcus* و *Mucor circinelloides* عند أدنى تركيز للشب (5٪)، ينخفض نمو الفطريات بشكل ملحوظ بمقدار 4 أضعاف في وجود الشب مقارنة بتلك التي تنمو في وسط YPD وحده. مما يعني أن نصف نشاط مضادات الميكروبات للشب يرجع إلى آلية تثبيط تختلف عن التأثير الناتج من ارتفاع ال pH. أثبتت هذه النتيجة أن النشاط التثبيطي للشب لا يرجع فقط إلى انخفاض الأس الهيدروجيني الناتج عن الشب ولكن أيضًا إلى وجود آلية مثبطة أخرى. فيما يتعلق بهذه النتائج، هناك حاجة إلى مزيد من الدراسة لفهم الآليات المثبطة للشب بغض النظر لتأثير الحموضة للشب.

**الكلمات المفتاحية:** الشب، الفعالية المضادة للبكتيريا، الفعالية المضادة للفطريات، المقاومة البكتيرية، المنتجات الطبيعية.

**Introduction**

The percentage of microbial resistance to antibiotics has been remarkably increasing in the last decades and became a global public health concern and could threaten millions of lives in the world [1-3]. For this reason, there has been an increasing demand for using natural products molecules for medicinal purposes. These molecules were highly used for treating many diseases caused by microorganisms [4, 5]. One of these molecules which is found naturally is alum / potash, also called Aluminum Potassium Sulfate  $AlK(SO_4)_2$ . This salt is used as a buffer in the food and drugs production; it has been approved to be safe compound by the U.S Food and Drug Administration (FDA) [6]. Literatures showed that alum has the ability for the treatment of patients suffering bleeding, leucorrhoea, vomiting, diarrhea, antiseptic and other uses [7]. The need for studying the effect of such this product is highly increased in the last decades because of increasing the rate of resistance to antibiotics. The antibacterial activity of alum can be due to its ability to inhibit the growth of bacteria by different mechanisms and causing damages for cell wall / membrane [8].

**Materials and Methods**

The microbial activity of alum against selected pathogenic bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Vibrio cholera*) as well as fungi

(*Cryptococcus neoformance*, *Cryptococcus gattii*, *Rhizopus micrococcus* and *Mucor circinelloides*) was measured using agar well diffusion method using Muller Hinton/ YPD agar, respectively.

The different types of pathogenic fungi were cultured in Yeast Extract-Peptone- Dextrose (YPD) Medium from aliquots frozen in 10% glycerol, which then incubated for four days at 25 °C. Whereas the pathogenic bacteria were cultured in Luria Broth Agar from aliquots were frozen in 20% (v/v) glycerol in Luria Broth Medium (LB) in sterile cryovial, Luria Broth Medium (LB) contained 1% (w/v) tryptone, 0.5% (w/v) yeast extract, 0.5% NaCl in deionized water. The antibacterial activity of alum against selected pathogenic bacteria as well as fungi was measured using agar well diffusion method [9]. This method was performed to produce a loan of bacteria/fungi on Muller Hinton/ YPD agar, by spreading a particular amount of the bacterial inoculum all over the agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer or a tip, and a volume (100 µL) of the alum solution at (50, 40, 30, 20, 10 and 5 %) concentration was introduced into the well. Tetracycline (50 µg/ ml) and Amphotericin B (1.25µg/ml) were used as reference slandered. The petri dishes were then incubated under suitable conditions for bacterial and fungi strains.

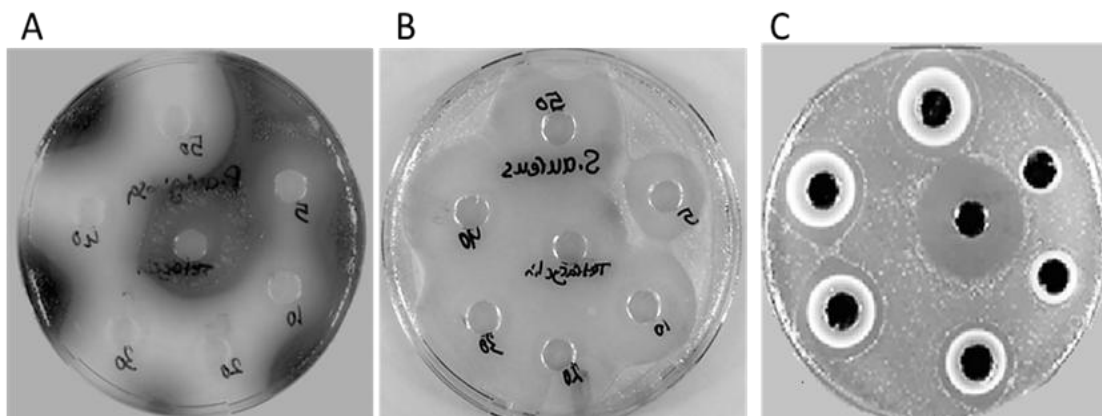
The effect of alum against *Cryptococcus neoformance* and *Cryptococcus gattii* was also determined using broth dilution method, containing different concentrations of alum (50, 40, 30, 20, 10 and 5 %). The *Cryptococcus* strains were grown in different YPD medium in the presence or absence of different concentrations of alum and in YPD with low pH (by adding HCl) which is the same pH produced by alum, in order to investigate whether the inhibitory effect of alum was due to acidification or as a result of other mechanisms.

## Results and Discussion

### The inhibitory effect of Alum against pathogenic bacteria:

In the current study the experiments of antimicrobial activity of alum against the selected pathogens revealed that alum has an inhibitory effect on these pathogens.

The results showed that alum has antimicrobial activity against the selected pathogenic bacteria which includes gram positive and negative bacteria. Alum inhibits the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* at all concentrations. However, *Vibrio cholera* growth was inhibited at the concentrations of (50,40,30,20 and 10 %) (Table 1, Figure.1).



**Figure 1: The evaluation of antimicrobial activity of alum using agar-well diffusion methods against pathogenic bacteria. The inhibitory effects of Alum against (A) *S. aureus*, (B) *P. aeruginosa* and (C) *V. cholera* were determined by the zone of bacterial inhibition around the wells containing different concentrations of alum.**

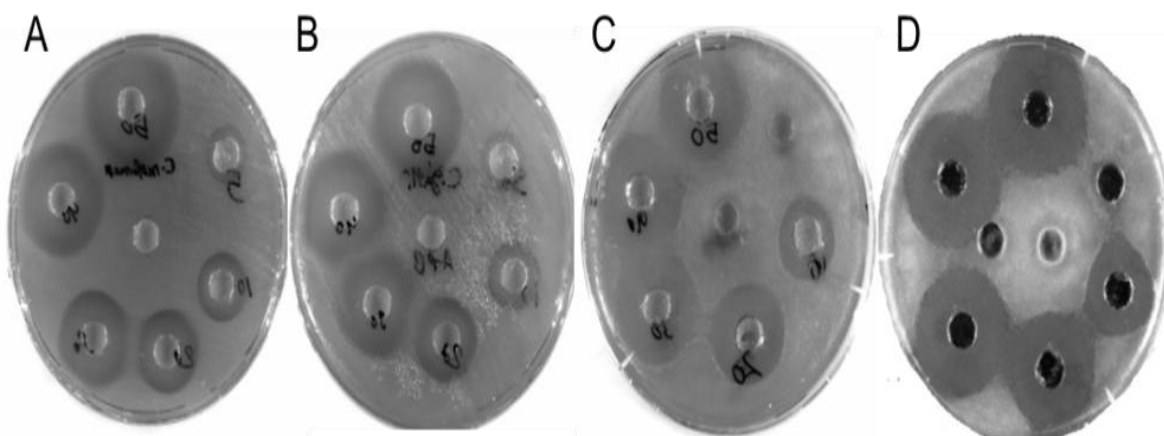
**Table 1. The inhibitory effect of alum against pathogenic bacteria**

Pathogenic bacteria	Concentration of alum (%)						Antibiotic
	Inhibition Zone (mm) ± SD						
	50%	40%	30%	20%	10%	5%	Tetracyclin (50 µg/ml)
<i>Pseudomonas aeruginosa</i>	33.7±1.154	32.3±2.516	29.3±1.527	24.7±2.081	22.7±1.154	18.7±1.55	18.7±1.55
<i>Vibrio Cholera</i>	23.7±0.57	20.7±0.577	19.6±0.577	17.6±1.527	9.66±2.081	—	26±1.52
<i>Staphylococcus aureus</i>	35.66±1.52	33.66±1.15	31.66±2.08	27.66±0.57	23±1.73	20.33±0.57	33.7±1.15

**The inhibitory effect of Alum against fungi:**

The antimicrobial activity of alum against the selected pathogenic fungi was measured using agar-well diffusion method. The results showed that alum has excellent antimicrobial activity against all the selected pathogenic

fungi in all concentrations (Table 2, Fig. 2), with no inhibitory effect against both *Rhizopus micrococcus* and *Mucor circinelloides* at the lowest concentration of alum (5%).



**Figure 2: The evaluation of antimicrobial activity of alum using agar-well diffusion method against pathogenic fungi. The inhibitory effect of Alum against (A) *Cryptococcus neoformans*, (B) *Cryptococcus gattii*, (C) *Rhizopus micrococcus* and (D) *Mucor circinelloides* was determined by the zone of inhibition around the wells containing different concentration of alum.**

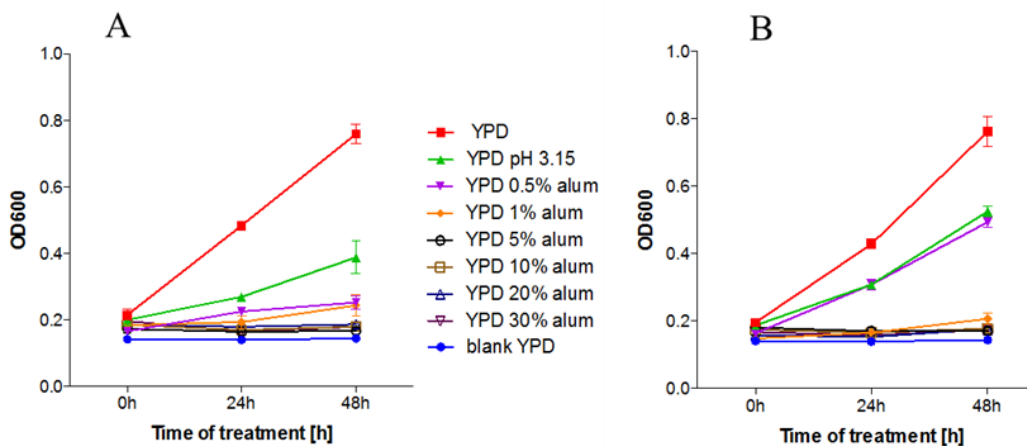


**Table (2): The inhibitory effect of Alum against fungi.**

Fungi	Concentration (%)						Antibiotic Amphotericin B(1.25µg/ml)
	Inhibition Zone(mm)±SD						
	50%	40%	30%	20%	10%	5%	
<i>Cryptococcus gatti</i>	26.67±3.05	26.33±1.52 7	24.67±2.0 8	21.67±2.8 8	17.66±2.0 8	10.7±0.577	R
<i>Cryptococcus neoformance</i>	29.7±0.57	27.3±2.516	24.6±2.51	22±2.645	14.3±4.04	12±1.73	R
<i>Rhizopus micrococcus</i>	29.66±0.57	28±1.73	27.3±0.57 7	24.67±0.5 7	21.3±0.57	–	R
<i>Mucor circinelloles</i>	29.67±0.57	28±1.73	27.33±0.5 7	24.67±0.5 7	21.3±0.57	–	R

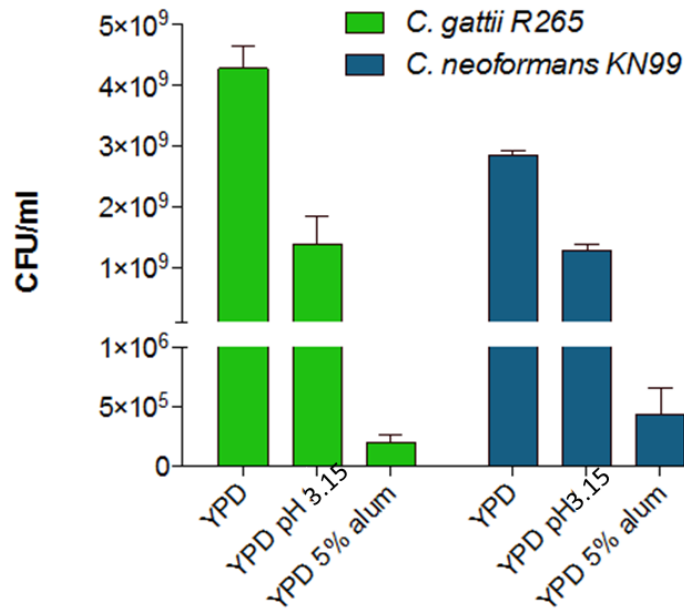
Impaired growth of *Cryptococcus* under different concentrations of alum and acid stress condition: The result revealed that the growth of *Cryptococcus* was reduced by 2 folds under low pH condition (the same pH of alum) from those grown in YPD media only, however, the growth of fungi is significantly reduced by 4 folds in the presence of alum in

comparison with those grown in YPD media. Which means that half of antimicrobial activity of alum was due to inhibitory mechanism differ from the acidification. This result proved that the inhibitory activity of alum is not only due to the low pH produced by alum but might be also to the presence of other inhibitory mechanisms.



**Figure 3: Impaired the growth of *Cryptococcus* under different concentration of alum and low pH. A: *Cryptococcus neoformance* and B: *Cryptococcus gatti* were grown in YPD media with alum (30,20,10 and 5 %) and in low pH YPD media (pH3.15) or no addition. PYD media without fungal inoculation was used as negative control.**





**Figure 4: the additional reduction of Cryptococcus growth in the presence of 5% alum in comparison with their growth in YPD (pH 3.15). Both *Cryptococcus neoformans* and *Cryptococcus gattii* were grown overnight at 25 with no shaking in YPD media with 5 % alum, in low pH YPD media (pH 3.15) or no addition.**

Regarding the effect against a pathogenic gram-positive bacteria *S.aureus* the result is consistent with finding of Bnyan *et al.*, (2014) [10] which showed that alum has antimicrobial activity against different types of pathogenic bacteria including *S.aureus*, *S. epidermidis*, *Klebsiella pneumonia* and *E. coli*. The subsequent published study by Shahriari *et al.*, (2017) indicates that alum can inhibit the growth of *E. coli* O157:H7 [11]. Previous publications due the inhibitory effect of alum either to its ability to damage the cell membrane of bacteria or to the low pH causing acid stress condition [12].

*Mucor circinelloides* is one of the three types of fungus used in the current study can cause a lethal disease, mucormycosis in patients with immunocompromised diseases [13,14]. *Cryptococcus* spp. is also responsible for causing pulmonary diseases in those patients [15,16].

Vellanki *et al.* (2018) showed that *Mucor circinelloides* lose the ability to form and grow mycelia and they attribute this to the effect of low PH. *Risopus micrococcuse* is also appeared to be affected by alum [17].

Until now there is no publication regarding the effect of alum on this type of fungus, however Sparringa *et al.*, 2022 publication demonstrating the optimum conditions for *Rizopus oligosporus* and determined that the optimum pH for the growth is approximately 5.8, which is belong to the same genus of *Risopus micrococcuse*, and interestingly showed that the growth rate of this fungus is highly sensitive to the pH level [18].

Previous publication suggest that the growth of *Cryptococcus* spp. in zinc redox stress condition was maintained in high pH, when they detect a combination between *Zip2* and *ZrfC* genes, which are responsible for zinc acquisition at high pH value, in *C. gattii* and *A. fumigatus* respectively, in accordance to the theoretical results of the analysis of phylogenetic tree [19]. The same mechanism is used by immune cells of the human body particularly the phagocytic cells. The extreme low pH condition inside these cells in addition to the antimicrobial substances in the cellular components are highly affected the growth of the engulfed infectious agents [20]. These together highly support the results of this study, which showed that the

inhibitory effect of alum on the growth of *Cryptococcus spp.* was notably higher than those under low pH condition (pH 3), which is the same pH caused by the presence of alum, and reached to the suggestion that alum can suppressed some essential growth pathways in fungus which is initiated by creating low pH conditions <sup>[21]</sup>.

## Conclusion

the above results confirmed the antimicrobial activity of alum against the selected pathogens however There is no publication up to date have been conducted to evaluate the effect of alum on the fungal pathogen which is associated with a high-level mortality like *Cryptococcus neoformance*. The output of this study highly promising and encouraging testing its effect in-vivo using laboratory animals to evaluate its effect for the treatment of infections caused by the selected pathogens used in the current study accurately.

It is true that one of the alum antimicrobial activity mechanisms is by acidification the results of the current study also proved that, and it was consistent with previous researches, However, the results of this study revealed that the inhibitory effect of alum on the growth of *Cryptococcus spp.* was notably higher than that under low pH condition (pH 3), which is the same pH as caused by the presence of alum.

From the results of this study, it is appeared that the growth of fungi is significantly reduced by 4-fold in the presence of alum in comparison with those grown in YPD media alone. Which means that half of antimicrobial activity of alum is due to inhibitory mechanism differ from the acidification. This result proved that the inhibitory activity of alum is not only due to the low pH produced by alum but also to the presence of another inhibitory mechanism. Regarding these results further study is needed to understand the inhibitory mechanisms of alum with neglecting the acidity effect of alum.

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## Authors Declaration

We hereby confirm that all the Figures and Tables in the manuscript are ours.

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