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Antibacterial activity of aluminum potassium sulfate and *Lepidium sativum* seeds against *Vibrio cholera* compared to doxycycline and tetracycline

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ABSTRACT

Background: Cholera is a severe lethal secretory diarrheal disease caused by the *Vibrio cholera* with a serious threat to public health globally particularly in developing countries. Aluminum potassium sulfate (Alum) is crystallized double sulfate, generally odorless, colorless and crystalline solid, which is used as an antiseptic. *Lepidium sativum* is an erect, branched, glabrous herb 60 cm height. The aim of the study was to evaluate activity of Alum and *Lepidium sativum* seeds extract against clinically isolated *Vibrio cholera*. **Material and methods:** *V. cholera* O1 serotype ogawa were isolated from fifteen Iraqi patients. Isolates were obtained from watery diarrheal stool then cultured in peptone water for 8 hrs followed by overnight culture on blood agar, MacConkey agar and TCBS agar. Then they were diagnosed following the standard diagnostic methods. Isolates were confirmed by VITIK-2. Alum concentration 2% was prepared by dissolving 2grams of white alum in 100 ml distilled water then putting the solution in autoclave at 120 °C for 20 min. The antibiotic susceptibility testing was done by the standard well diffusion method on Mueller-Hinton agar. **Results:** The antibacterial activity of 2% alum was 100 % active against *V. cholera*, while the activity of doxycycline and tetracycline was 86% and 80%, respectively. *Lepidium sativum* seeds showed no activity against isolates. **Conclusions:** Alum is a promising agent for *V. cholera* treatment; it is superior to both doxycycline and tetracycline. *Lepidium sativum* seeds revealed no activity against *V. cholera*.

Introduction

Cholera is a severe lethal secretory diarrheal disease caused by *Vibrio cholera*, Gram-negative, motile, curved-rod bacterium. There are more than 200 serogroups of *V. cholera*. Bacterial action is made possible through its attachment and colonization on the small intestine [1]. The spreading of cholera mainly occurs from ingestion of contaminated water and food from environmental

or household sources. Direct transmission can also result from exposure to contaminated inanimate objects or household surfaces within a household cluster and thus may lead to epidemics [2].

Infectious dose of *V. cholerae* shed in human stool with hyper-infectious state show lower infectious dose (around one-fifth) than *V. cholera* grown in vitro [3]. Although vomiting possesses the

potential for cholera transmission, little is known about survivability of *V. cholerae* on vomiting [4].

Alum occurs naturally in rocks that are located in areas where sulfide materials and potassium-bearing minerals. It is used as an astringent and antiseptic in various food preparation processes such as pickling and fermentation and as a flocculant for water purification [5,6]. Food and Drug Administration over the counter advisory panel has recommended alum as category I active ingredient in mouthwashes. Alum is used medicinally in many subunit vaccines as an adjuvant to enhance the body's response to immunogens. Alum is also widely used in some rural areas for the treatment of pediatric cough [7]. Alum is used during the rainy season as most of the potable water sources become turbid and contaminated [8].

Lepidium sativum is an erect, branched, glabrous herb 60 cm height. The basal leaves have long petioles and are lyrate pinnatipartite; the couliner leaves are lanceolate. The inflorescence is in dense racemes. The flowers have white or slightly pink petals, measuring 2 mm. The siliquae measure 5 to 6 mm, are elliptical elates from the upper half and glabrous. Racemes are 7 to 15 cm long axillary and terminal; flowers are white or pale pink; pedicels are 3-5 mm long [9].

Tetracycline antibiotics were one of the first classes developed following the introduction of penicillin G and sulphonamides. Doxycycline was developed by Pfizer and is a semi-synthetic derivative of oxytetracycline that first became available in 1967. It has the advantage over other members of the tetracycline family of improved oral absorption and a prolonged serum half-life [10]. Doxycycline has activity against a very broad range of Gram-positive, Gram-negative and atypical bacteria as well as some protozoa such as malaria. Doxycycline inhibits bacterial protein synthesis by reversibly binding to the 30S ribosomal subunit and preventing the association of aminoacyl-tRNA with the bacterial ribosome. Further inhibition of protein synthesis occurs in mitochondria through binding to the 70S ribosomes [11]. The purpose of the current work was to evaluate the antibacterial efficacy of alum and *Lepidium sativum* seeds against *V. cholera* compared to tetracycline and doxycycline in vitro.

Material and methods

In this cross-sectional study, *Vibrio cholera* O1serotype ogawa were isolated from fifteen Iraqi patients in Al- Shomali General

Hospital, Babylon city, Iraq from March to November 2022. Isolates were obtained from watery diarrheal stool then cultured in peptone water for 8 hrs and were overnight cultured on blood agar, MacConkey agar and TCBS Agar (HiMedia, India) to distinguish between different species. They were biochemically tested by using oxidase, indole, urease, simmons citrate, kligler iron agar, Gram staining, string test and antisera polyvalent ogawa and inaba (Hardy Diagnostics, USA). Isolates were confirmed by VITIK-2 [12].

Alum solution concentration was 2%, prepared by dissolving 2grams of white alum in 100 ml distilled water then putting the solution in autoclave at 120 °C for 20 min. The antibiotic susceptibility testing was done by the standard well diffusion method on Mueller-Hinton agar. Turbidity standard protocol was followed in order to have homogenized bacterial inoculum suspension, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [13].

Lepidium sativum seeds extract concentration was 2%, prepared by crushing 2grams of seeds then putting it in 100 ml distilled water and then it was autoclaved at 120 °C for 20 min.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of alum and planets extracts; the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm is punched aseptically with a sterile a tip, and a volume (50 µL) of the alum was introduced into the well. Then, agar plates were incubated under suitable conditions. The disc diffusion method used to evaluate the activity of tetracycline 30 µg and doxycycline 30 µg [13].

For ethical approval, the first stage was acceptance, in which a patients' consents to participate in research and permit the collection of information and their medical history. The Babylon Health Directorate approved of the ethical stance. A professional safety protocol was applied throughout samples handling.

For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. T-test was applied to examine measurement data, *p* value < 0.05 was considered significant.

Results

For 15 isolated *V. cholera*, the antibacterial activity of 2% alum was 100 % active against *V. cholera*, while 80% of isolates were sensitive to tetracycline and the activity of doxycycline was 86%. *Lepidium sativum* seeds showed no activity against isolates (Figure 1).

The comparison between the antibacterial activity of alum (28.47± 1.80 mm) and tetracycline (19.27± 6.96 mm) showed significant differences (p=0.00004). As well as the comparison of the antibacterial activity of alum (28.47±1.80 mm) and

doxycycline (22.00± 5.76 mm) was statistically significant (p=0.0003) (Table 1).

The correlation between the three agents as shown in Table (2), there was a significant positive correlation between alum and tetracycline (r= 0.56, p= 0.02). While there were no significant differences between alum and doxycycline (r= 0.38, p= 0.15).

Table 1. Comparison between the means of different antimicrobials.

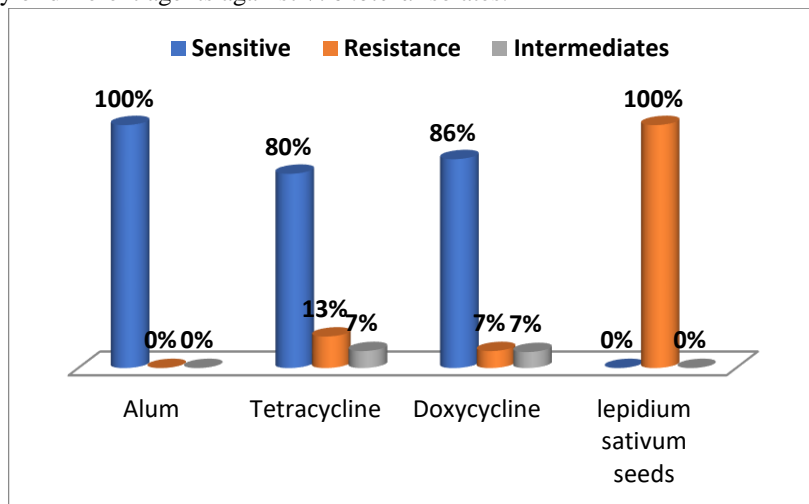
Variables	No.	Mean per mm	Std. Deviation	p value*
Alum	15	28.47	1.80	0.00004
Tetracycline	15	19.27	6.96	
Alum	15	28.47	1.80	0.0003
Doxycycline	15	22.00	5.76	

* P value significant at the 0.05 level. No = number of samples.

Table 2. Correlation between antibacterial activity of different agents.

Variables	N	Correlation	Significance.
Alum & Tetracycline	15	0.56	0.02
Alum & Doxycycline	15	0.38	0.15

Figure 1. Activity of different agents against *V. cholera* isolates.



Discussion

Cholera, an acute diarrheal disease, poses a serious threat to public health globally particularly in developing countries causing 28,000–142,000 deaths worldwide from a total 1.4–4.3 million reported cases [2]. In this study, the antibacterial activity of 2% alum was 100 % active against *V. cholera*. This finding agreed with another study

reported activity of alum concentration 0.25 to various bacterial growths [14]. In a study using alum against *Staphylococcus* species from wounds, it was 100% effective at 20 % alum concentration [15].

Alum at concentration 5 gm/100 ml revealed high inhibition effect against bacteria including *Staph. aureus* compared with ceftriaxone and tobramycin antibiotics. As well as the 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) [16].

Another study mentioned that antibacterial activity by inhibition zone (mm) estimation for alum on tested isolates, the inhibitory effect was very strong, highest sensitivity of *E. coli* and *C. albicans* observed to alum in concentration 16 % and 20% in *E. coli* [17]. Antibacterial activity of alum against isolates at different concentrations (w/v %), produced zone diameters of inhibition ranging from 9.3 - 21.2 mm [18].

A study found that 60% alum concentration revealed that inhibition zone of 29 mm diameter on *Pseudomonas aeruginosa*, while 50 and 40% alum concentrations were 25- and 22-mm diameter, respectively. Consequently, alum is utilized in wound and burns disinfection and in treatment of ulcers in the oral cavity [19].

Another study also mentioned that alum susceptibility trend was as *K. pneumoniae* > *S. aureus* > *E. faecalis* > *E. coli* > *E. faecium* at highest tested concentration (35mg/ml) after 24hr of incubation [20]. Alum solution showed excellent inhibitory effects on all bacterial strains at concentrations (7.5– 18.75 mg/mL). All tested bacteria showed no growth with alum at concentrations of 7.5, 10, 15 and 20 mg/mL [21]. Alum at concentration 0.025 showed good activity against *Vibrio cholera* [14].

In the current study, *Lepidium sativum* seeds extracting was no effecting on *V. Cholera*. Another study mentioned that *Lepidium sativum* seeds was active on Gram negative and Gram positive bacteria (*Klebsiella pneumoniae*, *Proteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans*) [22-23].

Another study mentioned that antibacterial activity of *Lepidium sativum* seeds extracts was determined using pathogen Gram positive and Gram-negative bacteria by disc assay method. Extracts exhibited both antioxidant (54.66 % of β -carotene/linoleic bleaching assay) and antibacterial activities (20 mm with *Salmonella Enteritidis*) [24].

Conclusions

Alum is promising agent for *V. cholera* treatment compared to both doxycycline and tetracycline. *Lepidium sativum* seeds revealed no activity against *V. cholera*.

Limitations of the study

The main limitation of this study was the absence of genotyping study for the bacteria. The current study took into consideration the phenotypic properties only. Small number of specimens may not reflect all population in the Babylon city.

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Conflicts of interest

There are no conflicts of interest.

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