Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.15, 2014

Antibacterial Activity of Aluminum Potassium Sulfate and *Syzygium Aromaticum* Extract Against Pathogenic Microorganisms

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Abstract
Natural products have been used for centuries in treating human disease**.** The present study aimed to determine the antibacterial ability of aluminum potassium sulfate (alum) and *Syzygium aromaticum* (clove) extract against different types of pathogenic bacteria in scientific way. Antimicrobial activity of crude extract from two commonly used medicinally materials, alum and clove were evaluated against four microorganisms over different concentration (10, 20, 30, 40 and 50) w/v %. Minimum inhibitory concentration (MIC) and the diameter of inhibition zone were determined by in vitro bioassay using agar well diffusion method against S. *aureus*, *S. epidermidis*, *E. coli*, *Klebsiella pneumonia*. The two medicinal materials exhibited antibacterial activity and inhibition growth of gram positive and gram negative bacteria isolated from different sites of infection. The inhibition effect of these extracts compared with standard antibiotics cefotaxime. In conclusions, the results of present study revealed that the alum and clove could be used as natural antimicrobial in different ways.
Keywords: Alum, clove, Syzygium aromaticum, S. aureus, S. epidermidis, E. coli, Klebsiella pneumonia, cefotaxime

Introduction

Natural products have been used for a long time in treating human disease and they contain many constituents of therapeutic value. Natural products are environmentally safer, easily available and cheap (Osuala *et al.,* 2009). Alum (aluminum potassium sulfate) the crystallized double sulfates with the formula $KAl(SO_4).12H_2O$ are generally odorless, colorless crystalline solids that turn white in air, which is used in food preservation and antiseptic for many preparation process such as pickling and fermentation and water purification as a flocculants (Bestoon, 2012). Moreover, alum has recommended as category I active ingredient in mouthwashes by the Counter Advisory Panel of U.S. Food and Drug Administration (FDAs) (Olmez *et al.,* 1998).

Cloves or *Syzygium aromaticum* are dried aromatic buds of unopened floral of an evergreen tree 10-20m in height, belonging to the family Myrtacceae, distributed in India, Indonesia, Zanzibar, Mauritius and Ceylon (Aneja and Joshi, 2010). The dried, clove bud contains free eugenol, eugenol acetate and caryophllen. Although these substances made up to 99% of the oil extract from dried flower-buds of *Syzygium aromaticum*, beta-caryophellene, is still one of the main constituents of this oil extract, and it has local anesthetic activity. The essential oil of clove is a colorless or light yellowish extract obtained from dried flower-buds by steam distillation (Ghelaradini *et al.,* 2001). Phenylpropene eugenol is the other most important compound present in *Syzygium aromaticum* due to which it has strong characteristic aroma. Bacteria, Mold, and yeast growth could be inhibited by the addition of clove essential oil (Burt, 2004). The essential oils of clove have been reported to possess numerous antioxidant and antimicrobial properties (Fu *et al.,* 2007).

Material and Methods

Test organism: both gram positive and gram negative microorganisms were used for the test. The gram positive organism include S. *aureus*, *S. epidermidis* (skin), and gram negative bacteria include *E. coli*, *Klebsiella pneumonia* (otitis media). All bacterial strains were maintained on freshly prepared blood agar. The bacterial strains were isolated from skin and otitis media and identified in Microbiology Department, College of Medicine, University of Babylon, Iraq.

Preparation of different concentration of aqueous extracts:

Alum extract:

Different concentration of alum (10, 20, 30, 40 and 50) w/v % was prepared. Alum material was purchased from the local market at Hilla city, Iraq 2014, and was identified in the College of Medicine, Department of Biochemistry, University of Babylon. Crystals of alum $KAl(SO_4).12H_2O$ dissolved completely in hot (100 ml) distilled water at 92 $^{\circ}$C, to prepare final concentration of (10, 20, 30, 40 and 50) w/v % at pH 3.6.

Cloves extract:

Dried plants used in this study were obtained from the local market at Hilla city, Iraq 2014. Distilled water was boiled, dried plants were added to the (100 ml) and left to cool, this content were mixed by the blender and filtered to remove the large un homogenized particles to get clear aqueous extract. Different concentration were prepared (10, 20, 30, 40 and 50) w/v %. The extracts were kept at 4 °C until be used.

In vitro antibacterial activity testing using agar well diffusion assay NCCLs:

The agar well diffusion method was used for the determination of antibacterial activity of aluminum potassium sulfate (alum) and *Syzygium aromaticum* aqueous extract by using bacterial isolates taken from (skin and otitis media) to evaluate its effects on the isolated bacteria. Loop full growth from bacterial isolate was inoculated into nutrient broth incubated at 37 °C for 18 hrs. The bacterial suspension was diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×108 CFU/ml. Muller-Hinton agar was inoculated with 0.1 ml of bacterial inoculum. Using cork borer, wells were made on the cultured media. The aqueous extract was considered as (10, 20, 30, 40 and 50) w/v % concentration. Then, 0.1 ml of aqueous extract were added to wells, then the plates left for 30 min in refrigerator at 4 °C, thereafter, they were incubated at 37 °C for 24 hrs. The inhibition zone was determined to evaluate the activity of aqueous extract by measuring the diameter in millimeter (Crespo *et al.,* 1990).

Determination of minimal inhibitory concentration (MIC) of aqueous Alum and clove extracts:

To determine the lowest concentration of antimicrobial agent, the minimum inhibitory test was done to fixed the best concentration that inhibit visible bacterial growth of experimental microorganisms for an overnight incubation period (Pandey and Singh, 2011). The MIC of aqueous extract of alum and cloves were determined by both dilution methods. A two fold serial dilution of aqueous extract of alum and clove were prepared.

McFarland tube standard (0.5):

A barium sulfate turbidity standard solution equivalent to 0.5 McFarland standards was prepared as described by (Bnyan, 2013).

Results

Antibacterial activity of aqueous extract of alum and clove in different concentrations.

In the present study investigation antimicrobial effects of aqueous extract by agar well diffusion assay, in the present different concentration (10, 20, 30, 40 and 50) w/v % of both alum and clove and inhibition zone increased by increases of concentration. The minimum inhibition zone was observed in alum and clove aqueous extracts, (40, and 33) mm respectively and the minimum were in 20% for each extract 20 and18 respectively. Table 1 and Table 2.

Table 1 Antibacterial Activity of Aqueous Extract of Alum in Different Concentrations.

Pathogens	Zone of inhibition (mm)					
	cefatxime	Alum				
		10%	20%	30%	40%	50%
S. aureus	0	Negative	20	25	30	35
S. epidermidis	18	Negative	22	28	35	40
E. coli	20	Negative	20	23	28	36
Klebsiella pneumonia	16	Negative	23	27	30	37

Table 2 Antibacterial activity of aqueous extract of clove

Pathogens	Zone of inhibition (mm)					
	cefatxime	clove				
		10%	20%	30%	40%	50%
S. aureus	0	Negative	16	23	28	33
S. epidermidis	18	Negative	18	22	27	32
E. coli	20	Negative	20	23	29	33
Klebsiella pneumonia	16	Negative	19	21	25	30

The antimicrobial study by agar well diffusion methods shows that the alum and clove has antimicrobial activity comparable to that of commercial antibiotic cefotaxime.

The antibacterial activity of aqueous extract of alum with time of incubation.

The antibacterial activity of aqueous extract of alum was tested against different type of gram positive and gram negative microorganisms with time of incubation and was shown inhibition time of 24 hours. Figure 1.

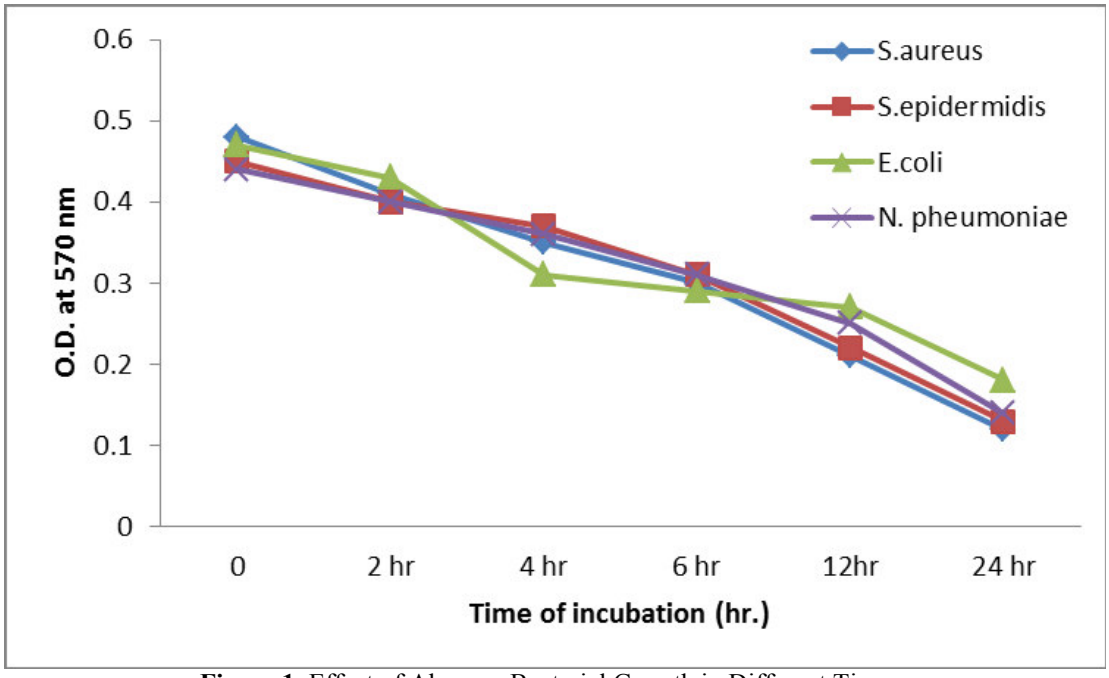

Figure 1 Effect of Alum on Bacterial Growth in Different Times.

The antibacterial activity of aqueous extract of clove with time of incubation.

Also, the antibacterial activity of aqueous extract of clove was tested against different type of gram positive and gram negative microorganisms with time of incubation and was shown inhibition time of 24 hours. Figure 2.

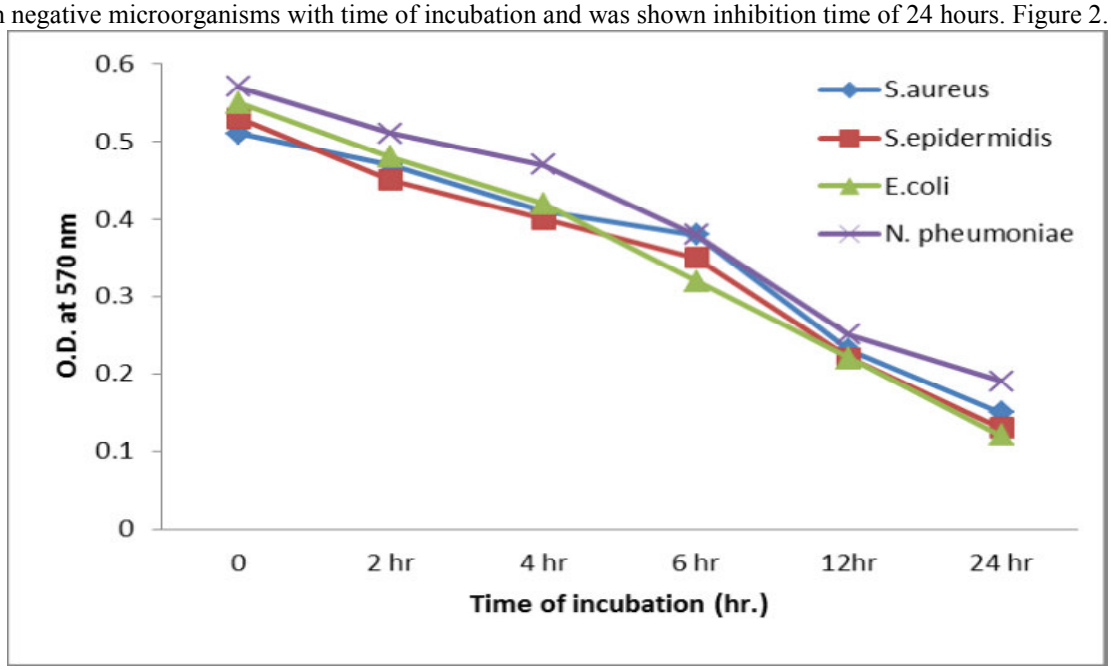

Figure 2 Effect of Clove on Bacterial Growth in Different Times.

Discussion

Aluminum potassium sulfate and *Syzygium aromaticum* are valuable materials source of medicinally useful compounds had been traditionally used for several applications. Aqueous extracts were being good sources for the bioactive compounds that exhibited good antimicrobial properties. Different concentrations of alum were tested against four bacterial isolates and the results showed that the 20% of alum consider as the MIC. Also, from the results, it was observed that the bacterial growth inhibition was increase when the alum concentration increase. Since the body doesn't absorb aluminum, alum could be consider as a harmless material and has a low toxicity in laboratory animals. But it has been documented that the ingestion of 30 grams could kill an adult humans. High level of alum solution could caused distraction of gum tissue, kidney damage and high mortality rate due to intestinal bleeding (Mallinckreod chemical. J. Bakr. Inc., 2009). The bacterial resistance to different anti-microbial agents consider as a major problem in medicine and public health. Nowadays studies focused with

new antibacterial targets which attenuating virulence factor and capacity of certain microorganism to eliminate the infection (Oneda *et al.,* 1994). The uses of alum as combinations with chlorhexidine or erythromycin increase its activity as antibacterial agents than uses as single salt (Cheong *et al.,* 2008). However more studies about alum and its physical and antioxidant properties if there, as well as toxicity are necessary. In the present study the aqueous extract of clove have been studied against some gram positive and gram negative bacteria namely *S. aureus*, *S. epidermidis*, *E. coli* and *Klebsiella pneumonia*. The screening was performed by agar well diffusion method. The aqueous extract of clove was active against all types of bacteria isolated from skin and otitis media and effective against a large number of other bacteria (Cail, 1996) and (Cuman, et al. 2009).
(Chaieb *et al*., 2007) have reported the inhibitory effect of clove extract against *Staphylococcus aureus* bacteria. Antibacterial compound of clove effective against gram positive and gram negative bacteria .

Conclusions

The preliminary study of this project revealed that the alum and clove can be used as natural antimicrobial in different ways like natural deodorants (alum) since is a very natural and healthy alternative to all the chemical based deodorants. Spices (clove) can be used as natural antibacterial in food products due to their less lethal effects as compared to synthetic chemical additives.

Acknowledgements

Authors would like to thank the presidency of Microbiology and Biochemistry Departments in the College of Medicine, University of Babylon, Iraq, due to their facilities to accomplish the task of this study.

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