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ORIGINAL ARTICLE

Synergistic antibacterial interaction between an alum and antibiotics on some microorganism

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ABSTRACT

Objective: To determine the antibiotic resistance, antimicrobial effects of Alum and coalescence between alum and antibiotics to killing microorganisms.

Methods: The tested bacteria used in this study were collected from different acute infection sources (wound, burn, axillary, and eye swab, sputum, and diarrhea) after that isolation and identification by using standard bacteriological methods. Detection antibiotic sensitivity pattern of isolates was determined by disc diffusion method, detection the antimicrobial effect of alum of isolates was determined by agar well diffusion method, and estimation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of alum also detection the combination of alum and antibiotic on isolated.

Results: In this study found all isolates resistant to several antibiotics tested, all the isolates considered multi drug resistant. Antibacterial activity by inhibition zone (mm) estimation for alum on tested isolates, the inhibitory effect very observed very strong inhibition , was highest on *E. coli* and *C. albicans* (resistance to most antibiotics) in this results observed sensitive to alum in concentration 16 % and 20% in *E.coli* , but in *C. albicans* sensitive in concentration 8%, 16%,and 20%, and other isolates sensitive to 20% alum concentration, this results showing the bacteria was highest sensitive alum than antibiotics, the minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC), all microorganisms (MOs) experiment in 20% was MIC and MBC except *K. pneumoneae* 20% only MIC but in *C. albicans* the 10% was MBC and MBC and in *E. coli* the 10% was MIC only.

The Combination between antimicrobial agents and inhibition zone (IZ) in mm, this result know the Combination of alum with antibiotics (tetracycline and cefotaxime) highly effect than antibiotic combination, and this combination very strong in killed and destroy microorganisms and effect of alum with antibiotic the best effect than a single effect. For example, effect alum alone the IZ (38 mm) on *E. coli* but IZ (46 mm) combination with cefotaxime but with tetracycline IZ (40 mm), and etc.

In this study, Synergistic antibacterial interaction between alum and antibiotics on all isolates.

Conclusion: This study found that the antibacterial effect of Alum increases with its concentration from 8% to 20% and over concentration, the alum very high effect than antibiotics and the high effect increased with combination antibiotics. The results of agar well diffusion method similar with MIC and MBC. In summary, the showed a wide spectrum antibacterial activity. These results suggest that alum have antibacterial actions against some pathogens and may be used for the treatment and prophylaxis against diseases.

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INTRODUCTION

Alums are utilizable for a range of industrial processes. They are soluble in dihydrogen monoxide; have an astringent, acid, and sweetish taste; react acid to litmus; and crystallize in conventional octahedral. When heated they liquefy; and if the heating is perpetuated, the dihydrogen monoxide of crystallization is driven off, the salt froths and swells, and at last an amorphous powder remains. Potassium alum is the mundane alum of commerce, albeit soda alum, ferric alum, and ammonium alum are manufactured[1]. Alum is withal utilized in purification of imbibing dihydrogen monoxide in industries. In a holding tank, some alum (phitkari) is integrated to the dihydrogen monoxide so that the negatively charged light colloidal components

cohere and get heftily ponderous (flocculate) when alum makes the colloidal particles neutralized by making its aluminum ions get loaded with the colloidal components. When the colloidal components get cumbersomely hefty they can be facilely dissevered from the tank for further chlorination of dihydrogen monoxide in the process of purification of dihydrogen monoxide[2]. In medicine alum is utilized in many subunit vaccines as an adjuvant to enhance the body's replication to immunogens. Such vaccines include hepatitis A, hepatitis B and DTaP. Alum in powder or crystal form, or in styptic pencils, is sometimes applied to cuts to obviate or treat infection. Powdered alum is commonly cited as a domicile remedy for canker sores. Preparations containing alum are utilized by pet owners to stem bleeding associated with animal injuries caused by infelicitous nail clipping. Alum is listed as an ingredient of some brands of toothpaste or toothpowder[3]. Aluminum is a naturally occurring metal that is found in the earth's crust. In its pure form, it is a soft, gray, shiny metal that is mined. Metallic aluminum is used as a structural material in the construction, automotive and aircraft industries, as well as in cookware, soft drink cans and aluminum foil. Aluminum salts are used as coagulants to purify municipal water that is drawn from lakes or reservoirs. Aluminum compounds are also found in some antacids, food additives, and antiperspirants[4].

Natural products have been utilized for centuries in treating human diseases and they contain components of therapeutic value. Natural products are environmentally safer, facilely available, and frugal[5]. Alum (Aluminum potassium sulfate), the crystallized double sulphates with the formula KAl(SO4)2.12H2O, are generally odourless, achromic crystalline solids that turn white in air, which is utilized as an astringent and antisepsis in sundry aliment preparation processes such as pickling and fermentation[6], and as a flocculants for water purification among other things. The chemical material as alum has many benefits as it has antibacterial effect on bacteria, also, it has anti yeast effect that inhibits the growth of *Candida albicans* in which its effect on the budding process.

MATERIALS AND METHODS

Tested bacteria

The tested bacteria used in this study were collected from different acute infection sources (wound, burn, axillary , and eye swab, sputum, and diarrhea) after that isolation and identification by using standard bacteriological methods according to MacFaddin, (2000), while the final identification was performed with the automated VITEK-2 compact system using Gram-negative identification cards (GN-ID), these are *E. coli* , *Klebsiella pneumonia*, *Pseudomonas aeruginosa* , *P. fluorscen* , *Proteus vilgaris* and *C. albicans*, isolates had been stored in 5% glycerol solution -20˚C.

Alum aqueous extract

Fifty gram of alum material was purchased from the local market at AL-Najaf AL-Ashraf City, Iraq, and was identified in the College of science department of biology, Kufa University, crystals of alum KAl(SO4)2·12(H2O). Dissolved 50 gm by 100ml distilled water completely, to obtain a final concentration of 50 % at pH 3.4[7].

Cefotaxime and tetracycline solution

This solution was prepared by dissolving 5mg cefotaxime in 5ml with injection distilled water in same vial, and dissolving 250 mg tetracycline capsule in 1 ml (0.1 N HCL) after that added 4 ml injection distilled water the stock solution concentration 250 mg/5 ml.

In vitro Antimicrobial activity testing using Agar well diffusion assay by NCCLS[8]

The agar well diffusion method was used for the determination of antibacterial activity of aluminum potassium sulphate (alum) aqueous extracts by using bacterial isolates to evaluate its effects on the isolated bacteria. Loopfull growth from bacterial isolate was inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were 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 0.1ml of bacterial inoculum .Using corked borer, wells were made on the cultured media. The alum aqueous extracts were considered as the 50% concentration prepared four concentration 4%, 8%, 16%, and 20% . Then, 0.1ml of each concentration alum aqueous extracts were added to wells, and placing some antibiotic disc behind the well distance about 2 cm to know the interaction between antibiotics disc and alum aqueous extracts wells, then the plates left for 30 min in refrigerator at 4˚C, thereafter, they were incubated at 37˚C for 24 hrs. The activity of alum aqueous extract was determined by measuring the diameter of inhibition zone in millimeter[9]. Estimation of the minimum inhibitory (MIC) and maximum bactericidal concentration (MBC) of alum aqueous extract according[10].

Muller-Hinton agar plates which had been flooded separately with different tested bacteria isolates, were allowed to dry at 37˚C for 30 min. before, placing conventional antibiotics discs shown in Table 1 by disc diffusion technique on it, the plates were incubated at 37˚C for 24 hr. based on the method of[11], the diameter zones of inhibition was measured and recorded according to the[12].

Combination of alum with cefotaxime

Depending on results antibiotics disc with alum well, selected antibiotics to combination with alum aqueous extracts, in this study cefotaxime and tetracycline selected, the concentration of cefotaxime 10mg/0.1ml (500 mg /5 ml cefotaxime stock solution) and tetracycline 5mg/0.1ml (250 gm /5ml tetracycline stock solution) were added to wells to each isolate, placing behind the well alum aqueous extract, the distance between them about 2 cm to know the interaction between (cefotaxime and alum) and (tetracycline + alum) , then the plates left for 30 min in refrigerator at

4˚C, thereafter, they were incubated at 37˚C for 24 hrs. The activity of (cefotaxime + alum aqueous extracts) and (tetracycline + alum aqueous extracts) was determined by measuring the diameter of inhibition zone in millimeter.

RESULTS

Table 1 show all isolates found to be resistant to several antibiotics tested, all the isolates considered multi drug resistant.

Table 1: **Antimicrobial activity (Inhibition zone in mm) of conventional antibiotics on M.Os**

NO.	Antibiotics	*S. aureus*	*K. pneumoneae*	*P. vilgaris*	*Ps. aeruginosa*	*Ps. flourecence*	*E. coli*	*C. albicans*
1	Cip	20	32	34	28	16	28	R
2	CTX	R	R	R	36	R	R	R
3	M	R	R	R	R	R	R	R
4	CLR	R	R	28	R	R	R	R
5	Te	20	R	R	R	R	R	R
6	TMP	30	10	32	R	R	R	R
7	Gm	R	R	3	R	2.4	0.6	R
8	KF	R	R	R	R	R	R	R
9	Ci	R	R	24	36	R	R	R
10	Amox	R	R	16	R	R	R	R
11	Cef	R	R	R	R	R	R	R
12	AK	10	R	14	R	R	16	R

Antibiotic concentration disc(µg/disc): Gm: gentamicin(10), M: metronidazole(5), TMP: trimethoprime(5), Amox: Amoxicillin(20), Te: tetracycline(30), CLR: clarithromycin(15), SXT: Co-trimoxazole(1.25/ 6.25), CTX: cefotaxime(30), AK: amikacin(30), CFX: cefixime(5), Ci: ceftriaxon(30), S: streptomycin(10), CF: cephalothin(30), Cip: ciprofloxacin(5), R: resistance.

Table 2 show the Antibacterial activity by inhibition zone (mm) estimation for Alum on tested bacteria, the inhibitory effect very observed very strong inhibition , was highest on *E. coli* and *C. albicans* **,** resistance to most antibiotics but sensitive to alum in 16 %and 20% in *E.coli* , but in *C.albicans* sensitive in 8%, 16%,and 20%, and other bacteria sensitive to 20% alum concentration, this results showing the bacteria was highest sensitive alum than antibiotics.

Table 2: **Antibacterial activity by inhibition zone (mm) estimation for Alum on tested bacteria**

Tested bacteria	Alum concentration				
	Control	4%	8%	16%	20%
S. aureus	0	0	0	0	26
K. pneumoneae	0	0	0	0	28
P. vilgaris	0	0	0	0	30
Ps. aeruginosa	0	0	0	0	34
Ps flourecence	0	0	0	0	30
E. coli	0	0	0	22	38
C. albicans	0	0	20	34	44

Table 3 showing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), all isolates experiment in 20% was MIC and MBC except *K. pneumoneae* 20% only MIC but in *C. albicans* the 10% was MIC and MBC and in *E. coli* the 10% was MIC only.

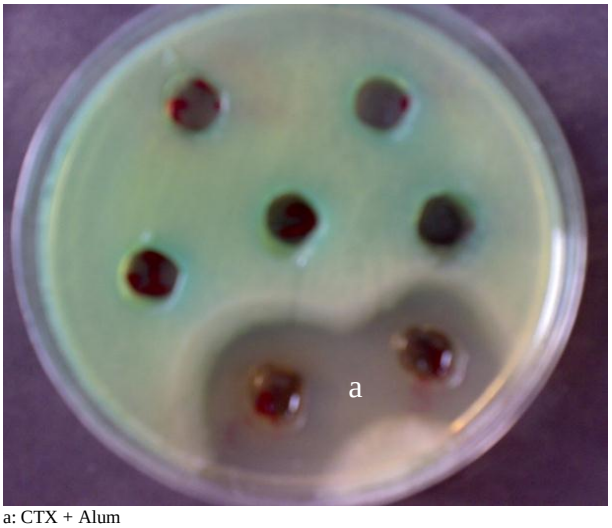
Table 3: **MIC and MBC of Alum on microorganisms (MOs)**

MOs.	Control	Alum concentration						
		20%		10%		5 %	2.5 %	1.25 %
		MIC	MBC	MIC	MBC			
S. aureus	0	-	-	+	+	+	+	+
K. pneumoneae	0	-	+	+	+	+	+	+
P. vilgaris	0	-	-	+	+	+	+	+
Ps. aeruginosa	0	-	-	+	+	+	+	+
Ps flourecence	0	-	-	+	+	+	+	+
E. coli	0	-	-	-	+	+	+	+
C. albicans	0	-	-	-	-	+	+	+

Table 4 Showing the Combination between antimicrobial agents and inhibition zone (IZ) in mm, this result know the Combination of alum with antibiotics (tetracycline and cefotaxime) highly effect than antibiotic combination, and this combination very strong in killed and destroy microorganisms and effect of alum with antibiotic the best effect than a single effect. For example, effect alum alone the IZ (38 mm) in 20% concentration on *E. coli* but IZ (46 mm) combination with cefotaxime and with tetracycline IZ (40 mm), and etc., this result observed in Figure 1.

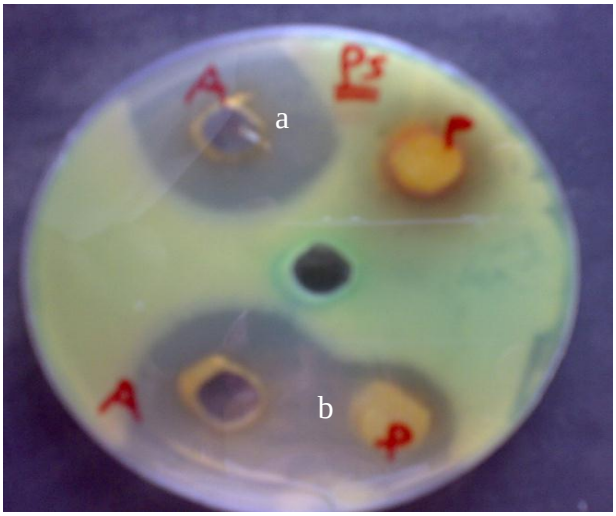
Table 4: **Combination between antimicrobial agents and inhibition zone (IZ) in mm.**

MOs.	Synergism between antibiotic		Combination (inhibition zone IZ in mm) Of alum with antibiotics	
	Combination	IZ	Cefotaxime + Alum (IZ)	Tetracycline + Alum (IZ)
S. aureus	Gentamycin + Cefotaxime	40	60	40
K. pneumoneae	Ciprofloxacin + Cefotaxime	32	62	42
P. vilgaris	Cefotaxime + Ttetracycline	38	45	55
Ps. aeruginosa	Tetracycline + Cefotaxime	36	50	37
Ps flourecence	Clarithromycin + Gentamycin	42	40	36
E. coli	Gentamycin + Ceftriaxone	30	46	40
C. albicans	Tetracycline + cefotaxime	30	50	38



a: CTX + Alum

A



a: Mixture of Alum+Te ; b: Synergism (clear zone)

B

Figure 1: **The Antibacterial activity of alum with antibiotics on *Ps aeruginosae* : A- Senergism effect between cefotaxime and alum B- Senergism effect between tetracycline (Te) and alum.**

Figure 1 A: showing the synergism effect of cefotaxime and alum presented high inhibition zone indicated antimicrobial activity on virulence bacteria *Ps aeruginosae* , Figure 1 B showing two state the once state synergism effect of tetracycline and alum each one in well-presented high inhibition zone indicated antimicrobial activity and second state mixture between tetracycline and alum in well and in a well only tetracycline, the mixture Alum +Te highly antimicrobial activity against bacteria than tetracycline in alone.

Discussion

Antibiotic resistance is a major clinical and public health quandary which forces researchers to probe for alternatives culls. Natural chemical compounds are among these alternatives. In this study, alum salt was tested against isolates. To the best of our erudition, this is the first study on antimicrobial effects of alum against. The preliminary results from this study could lead to future effort to investigate how alum salt inhibits growth of the bacteria and potential utilization of alum salt. In the present study the isolates expressed high resistance to antibiotics.

Effects of four different concentrations of aqueous alum extract on six bacterial isolates and one *C. albicans* isolate isolated from different acute infection sources are presented in Table 2 , this designate the inhibition zone incremented with the incrementation of concentration of alum solution from 8%-20%, this denotes that alum has an antibacterial action, this action increases with incremented alum concentration which concurs with the findings of Putt and Kleber[13] who found that the protective effect of the 100 PPM Alum solution was less than solutions containing 1000 PPM Alum or more, and accede with Mohammed[14] found that 60% alum concentration gives IZ of 29mm diameter on *Ps. 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. Utilizing 50% alum + 20% hydrogen peroxide coalescence gives 42mm diameter inhibition zone on *Ps.aeruginosa*[15] and accede with the results that the alum gives positive results on all bacterial isolates and the maximum inhibition zone 42 mm was observed for 100% concentration on *Enterococcus durans* and the minimum inhibition zone 9 mm was observed for 3.125% concentration on *Enterobacter cloacae*[16].

Predicated on the broth dilution assays, the MIC of 10-20 mg/mL appeared as optimal concentration of alum against isolates. Precedent studies have revealed that alum is efficacious against a wide variety of microbial pathogens[17,18] including *S. aureus*, *E. coli* and *K. pneumoniae*[19,20]. In 2014, Bnyan *et al*.[26], additionally observed a paramount bactericidal effect of alum against *S.aureus*, *S. epidermidis*, *E. coli* and *K.pneumoniae*[20]. However, the mechanism of bactericidal effect of alum is not prominent[21]. Some postulations attribute the antibacterial effect of alum to reduction in acidity or deleterious effects on bacterial cell wall. Furthermore, histological studies substantiate the safety of alum salt for mammalian consumption[22]. It cannot be directly absorbed due to its negatively charged molecule, which are unable to pass through the cell membranes and ergo alum remain an innocuous substance[23]. However higher concentration of alum might cause nephrotoxicity and intestinal bleeding[24]. Alum salt is used in cosmetics as antiperspirant to reduce axillary odor by blocking sweat ducts and preventing sweat secretion[24]. Alum crystals are highly soluble in water and when used under arm, they are dissolved by the body's sweat leaving a dry thin layer on the skin's surface which prevents sweat to come in contact with odor-causing bacteria[25]. Further studies are required to investigate the safety, allergy and efficacy of alum on human skin when used as antiperspirant. According to this study a concentration of 10 or 20 mg/mL could be considered appropriate for formulation of deodorant lotion, cream and gel this concentration less when mixed with cefotaxime or tetracycline. From this study it can be concluded that

alum has excellent antimicrobial inhibitory effects microorganisms especially commixed with antibiotics.

REFERENCES

1. Greenwood N.N. and Earnshaw A."Chemistry of the Elements (2nd Edn.)". Oxford:ButterworthHeinemann. ISBN 0-7506-3365-4.1997. DOI: 10.1002/(SICI)1099-0739(199812)12:12<880::AID-AOC755>3.0.CO;2-C.
2. Picon M. "L'alun des oasis occidentales d'Egypte: researches sur terrain et recherches en laboratiore in Bogard". 2005
3. Al-Husainy I.A."Effect of aqueous solution of aluminum potassium sulphate on Candida albicans". Higher Diploma thesis, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Baghdad University. 2004.
4. Mohammed B."Study effect mix of chemical (alum, hydrogen peroxide) with extract citrullus colocynthis on bacteria Pseudomonas aeruginosa isolated from contamination hospital". M.Sc. thesis, College of Dentistry, Baghdad University. 2004.
5. Osuala F.I., Ibidapo obe M.T., Okoh H.I., Aina O.O., Igbasi U.T. and Nshiogu M.E. "Evaluation of the efficacy and safety of Potassium Aluminium Tetraoxosuiphate in the treatment of tuberculosis". European J. Biol. Sci. 2009; 1:10-14.
6. Al Ani M.N."Evaluation of some prepared solutions from some medical plants and chemicals on Pseudomonas aerugenosa, Higher Diploma thesis". Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Baghdad University. 2004.
7. Macfaddin J.F."Biochemical tests for identification of medical bacteria"; (3rd ed.), Lippincott Williams and wilkins, U.S.A. 2000.
8. National Committee for Clinical Laboratory Standards (NCCLS)."Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically". Approved Standard M100-S12. Wayne PA; 2002.
9. Barry A.L."The antimicrobic susceptibility test: principles and practices". Lea and Febiger, Philadelphia. 1976.
10. Working party of the British society for antimicrobial chemotherapy."A guide to antibiotic sensitivity testing". J. antimicrob. Chemother. 1991; 27.(suppl. D.):1-50.
11. Crespo M.E., Jimenez J., Gomis E. and Navarro C. "Antimicrobial activity of essential oil of Thymus serpylloides subspecies gardorensis". Microbios. 1990; 61:181-184.
12. National Committee for Clinical Laboratory Standards (NCCLS)."The performance standards for Antimicrobial susceptibility testing methods". (7th ed.). 2003; 22(1): USA.
13. Putt M.S. and Kleber C.J. "Effect of aluminum concentration on dental caries formation in the rat". Oral Dis. 1995; 1(2): 80-5.
14. Mohammed B."Study effect mix of chemical(alum, hydrogen peroxide)with extract citrullus colocynthis on bacteria Pseudomonas aeruginosa isolated from contamination hospital". M.Sc. thesis, College of Dentistry, Baghdad University. 2004.
15. Al Ani M.N."Evaluation of some prepared solutions from some medical plants and chemicals on Pseudomonas aeruginosa". Higher Diploma thesis; Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Baghdad University. 2004.
16. Mohammed I.N., Amina N.A. and Ismail A.A. "Effect of Nigella Sativa (Black seed), salvadora persica (siwak) and Aluminum potassium sulphate (alum) Aqueous extracts on isolated bacteria from teeth root canal". Iraqi J. Biotech. 2010; 9(1): 99-104.
17. Dutta S., De S.P. and Bhattacharya S.K. "In vitro antimicrobial activity of potash alum". Indian J. Med. Res. 1996; 104:157-9.
18. Mourughan K. and Suryakanth M.P. "Evaluation of an alum-containing mouthrinse for inhibition of salivary streptococcus mutans levels in children--a controlled clinical trial". J. Indian Soc. Pedod. Prev. Dent. 2004; 22(3):100-5.
19. Bestoon M.F. "Evidence for feasibility of aluminum potassium sulfate (alum) solution as a root canal irrigant". J. Coll. Dent Uinv. Bagh. 2012; 24(1):1-5.
20. Bnyan I.A., Alta'ee A.H. and Kadhum N.H. "Antibacterial Activity of Aluminum Potassium Sulfate and Syzygium Aromaticum Extract Against Pathogenic Microorganisms". J. Nat. Scie. Res. 2014; 4(15):11-4.
21. Arul P.F., Babu G.D., Lavanya M., Vidhya K.S. and Devasena T. "Toxicity Studies of Aluminium Oxide Nanoparticles in Cell Lines". Int. J. Nanotechnol. Appl. 2011; 5(2):99-107.
22. Osuala F.I., Ibidapo-obe M.T., Okoh H.I., Aina O.O., Igbasi U.T., Nshiogu M.E., Onubogu C.C., Egbuna K.N., Ajibaye O. and Orok A.B. "Evaluation of the efficacy and safety of Potassium Aluminium Tetraoxosulphate (Vi) (ALUM) in the Treatment of tuberculosis". Eur. J. Biolo. Scie. 2009; 1(1):10-5.
23. Alzomor A.K., Moharram A.S. and Al Absi N.M. "Formulation and evaluation of potash alum as deodorant lotion and after shaving astringent as cream and gel". Int. Curr. Pharm. J. 2014; 3(3):228-33. DOI: http://dx.doi.org/10.3329/icpj.v3i2.17512 .
24. Pitten F.A., Rudolph P., Below H. and Kramer A. "Assessment of the activity of antiperspirants added to surgical hand disinfectants: methodological aspects and first observations". J. Hosp. Infect. 2001; 48 Suppl A: S29-32.
25. Stop using "Natural" deodorants until you read this [Internet]. 2010. http://articles.mercola.com/sites/articles/archive/2010/02/16/aluminum-lurks-in-crystal-deodorants.aspx. Accessed 16 February 2010.
26. Bnyan I.A., Alta'ee A.H. and Kadhum N.H. "Antibacterial Activity of Aluminum Potassium Sulfate and Syzygium Aromaticum Extract Against Pathogenic Microorganisms". J. Nat. Scie. Res. 2014; 4(15):11-4.