INFLUENCE OF SOME WEAK ACIDS, WEAK BASES AND SALTS AGAINST SOME PATHOGENIC MICROORGANISMS

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ABSTRACT:

This study was designed to investigate of antibacterial activity of many chemical compounds includes : 4 % imidazole, 2 % imidazole, 1% imidazole , 2.5% sodium citrate, 5% sodium citrate, 5% sodium acetate,2.5% acetic acid, 5% acetic acid, 5% citric acid, 5% magnesium chloride ($MgCl_2.6H_2O_2$), 5% magnesium sulphate ($MgSo_4$), 5% sodium carbonate (Na_2Co_3), 5% potassium dichromate ($K_2Cr_2O_7$), 5% potassium permanganate ($KMnO_4$) and 5% sodium hydrogen carbonate ($NaHCO_3$) have been carried out against (*Escherichia coli*, *Pseudomonas aeruginosa* , *Klebsiella oxytoca* , *Proteus mirablis* and *Serratia liquefaciens*) that diagnosed by API 20 E technique and *Staphylococcus aureus* , Pathogenic fungal strains (*Aspergillus niger*, *A. flavus*, *A. fumagatus*, *Fusarium sp.* and *Candida albicans*). The results showed 4 % imidazole, 2 % imidazole, 1% imidazole, sodium acetate , 2.5% acetic acid, 5% acetic acid, 5% citric acid , 5% Na_2CO_3, $K_2Cr_2O_7$ have different antibacterial activity according to types of bacteria, whereas 4 % imidazole, 2 % imidazole , 2.5% acetic acid, 5% acetic acid and 5% Na_2Co_3 only have antifungal activity, and showed compounds: 4 % imidazole & $K_2Cr_2O_7$ have antimicrobial activity more than standard positive control antibiotic.

INTRODUCTION :

The effects of organic acids and their salts as antibacterial agents in reducing the bacterial colonies during storage in food industries, especially in meat industries such as beef, poultry and pork have been largely studied (Bogaert & Naidu, 2000).

As an alternative to antimicrobial therapy, acetic and boric acid have been suggested for local treatment of bacterial infections. Both acids have been shown to exert antibacterial effects on different bacterial species, including staphylococci (Houlsby *et al.*, 1986; Russel & Diez-Gonzalez, 1998). Sodium salts of the low molecular weight organic acids; such as acetic, lactic, and citric have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food systems including meat (Maca *et al*., 1997;Sallam & Samejima, 2004), poultry (Williams & Phillips, 1998), and fish (Boskou & Debevere, 2000).

A considerable number of pyrazolo[3,4-*d*]pyrimidines are known to be bioactive. They display antibacterial (Kern *et al* .,1985), antifungal (Hasobe *et al* ., 1986) , antimicrobial (Kitahara *et al* 1986) , antitumor (Anderson *et al* 1990), antiviral (Petrie *et al* ., 1985) , and antipyretic (Elnagdi *et al*., 1987) activities.

Carbonic acid salts, such as sodium carbonate and sodium bicarbonate, widely

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used in the food industry, are food additives allowed with no restrictions for many applications under European and North American regulations (Lindsay ,1985; Multon ,1988). The antimicrobial activity of these chemicals has been described *In vitro* and in a wide range of substrates as well (Corral *et al*., 1990).

Individually, hydrogen peroxide (H_2O_2) and sodium bicarbonate ($NaHCO_3$) are known to possess antimicrobial activity against oral micro-organisms (Miyasaki *et al*., 1984; Newbrun *et al*., 1984). The present work was aimed at some weak acids, weak bases and salts to have antimicrobial activity against some pathogenic microorganisms.

Material & Methods

Microorganisms:

Eleven pathogenic microorganisms (*Escherichia coli, Pseudomonas aeruginosa , Klebsiella oxytoca , Proteus mirablis* and *Serratia liquefaciens*) that diagnosed by API 20 E technique and *Staphylococcus aureus* , Pathogenic fungal strains (*Aspergillus niger, A. flavus, A. fumagtus, Fusarium sp.* and *Candida albicans*) were obtained from Microbiology laboratory of Medicine college-University of Thi-Qar that were diagnosis by Traditional chemical tests .

Chemical compounds : 4% imidazole, 2% imidazole, 1% imidazole, 2.5% sodium citrate, 5% sodium citrate, sodium acetate, 2.5% acetic acid, 5% acetic acid, 5% citric acid, magnesium chloride $MgCl_2.6H_2O_2$, 5% magnesium sulphate $MgSo_4$, 5% sodium carbonate Na_2Co_3, potassium dichromate $K_2Cr_2O_7$, potassium permanganate $KMnO_4$ and sodium hydrogen carbonate $NaHCO_3$ marked as 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15 were used to test the biological potential against many pathogenic microorganisms.

Antimicrobial assay

In vitro antimicrobial activity of chemical compounds were determined by agar well diffusion technique (Perez *et al*.,1990). This agar plates were incubated at 37 ˚C for 24 hours for bacteria and (25-30) ˚C for (1-3) days for fungi . Three replicates were formed for every bacterium (Cruickshank *et al* .,1975). The test solutions were prepared in distilled water. The inhibition zones were measured after 24h for bacteria and 1-3 day for fungi. Penicillin (P), Streptomycin (S) were used as standards positive control for bacteria, and Fluconazole (20 mg/ml) for fungi.

RESULTS :

Table (1) showed that 4% and 2% imidazole have high antibacterial activity were found on all bacterial strains ,also showed signification variation in the inhibition zones, whereas 1% imidazole was effected on all bacterial types expect *P. aeruginosa*. At compound sodium acetate , the highest activity was recorded through 32 mm inhibition zone against *Serratia liquefaciens* but this compound no has effect against *P. aeruginosa, Proteus mirablis* & *S. aureus*. Acetic acid at 2.5% and 5% concentration have high activity against all bacterial types. Citric acid has activity against all bacterial types expect *Serratia liquefaciens* and *Klebsiella oxytoca.* Five percentage Na_2Co_3 has antibacterial activity with inhibition zone ranging 12-15 mm against *Klebsiella oxytoca, S. aureus* and *Proteus mirablis*, whereas compound $K_2Cr_2O_7$ has high activity against *E. coli* and *P. aeruginosa* only. 2.5% sodium citrate , 5% sodium citrate, $MgCl_2.6H_2O_2$, 5% $MgSo_4$, $KMnO_4$ and $NaHCO_3$ no have antibacterial activity

against any types of bacteria, when compare the antibacterial activity of 15 chemical compounds with two antibiotic discs as control positive show the inhibition zones of some compounds more than inhibition zones of penicillin and streptomycin.

Table 2 showed 4% imidazole, 2% imidazole, 2.5% acetic acid, 5% acetic acid & 5% sodium carbonate Na_2Co_3 only have effectiveness against fungal types with different inhibition zones, whereas other compounds no have activity against fungi.

DISCUSSION :

In the present study we investigated the antimicrobial activity of 15 compounds against eleven pathogenic microorganisms.

The results showed 2.5% and 5% acetic acid have high effects against all bacterial and fungal types, this results approach with (Sloss *et al* ., 1993) showed the topical use of acetic acid at concentrations between 0.5 to 5% eliminated *P. aeruginosa* from the burns and soft tissue wounds of 14 out of the 16 patients within two weeks treatment. (Nagoba *et al*., 2008) Particulars of wound infection, susceptibility pattern of *P. aeruginosa*, concentration of acetic acid used for the treatment, (Muhsin *et al*.,2010) showed the 2% acetic acid has antifungal activity against fungal types isolated from otomycosis.

From tables (1&2) showed sodium citrate has no effect any types of microorganisms whereas Blaszyk & Holley,1998 showed activity of 0.2 and 0.4 % of sodium citrate , monolaurin, eugenol (phenolic compound) on the growth of six organisms including common meat spoilage (*Lactobacillus curvatus, Lactobacillus sake, Leuconostoc mesenteroides, Brochothrix thermosphacta*) and pathogenic (*Escherichia coli* and *Listeria monocytogenes*) were more effective than each component separately.

$NaHCO_3$ no has influence against using microorganisms whereas (Kyung *et al* ., 2010) showed Natural apo-ovotransferrin has little antibacterial activity, but ovotransferrin added with 50mM $NaHCO_3$ has antibacterial activity against *E. coli* and *L. monocytogenes* .

All concentration of imidazole have high antimicrobial activity against most bacterial and fungal types, Pyrazolopyrimidine bearing imidazole, pyrazolopyrimidine having benzenesulfonamide, and pyrazolopyrimidine bearing *N*-(4,6-dimethylpyrimidine) moiety were found to be the most active compounds against Gram-positive bacteria *S. aureus* and *B. subtilis*. On the other hand, pyrazolopyrimidine containing benzensulfonamide showed high activity against Gram negative bacteria. In addition, pyrazolopyrimidine having propionic acid and benzenesulfonamide 12 and 16 and exhibited good antifungal activity against *A. fumigatus* , while compound 12 and 16 revealed remarkable activity against *A. flavus*. Also, compounds 3b and 3c showed promising activity against *Penicillium species*(Ghorab *et al*., 2004).

Sodium acetate has high activity was against *Serratia liquefaciens* with inhibition zone 32mm but this compound has no effect against *P. aeruginosa, Proteus mirablis* & *S. aureus*, this results may be approach with (Sallam, 2007) showed sodium acetate, sodium lactate, and sodium citrate can be utilized as safe organic preservatives for fish under refrigerated storage and these salts have antibacterial activity against bacterial types causing spoilage such as: *P. aeruginosa*, lactic acid bacteria, and

Enterobacteriaceae. Citric acid has activity against all bacterial types expect *Serratia liquefaciens* and *Klebsiella oxytoca*, but this compound do not effect against fungal types, whereas 5% sodium carbonate Na_2Co_3 has activity against *A. flavus* and *C. albicans*.

In conclusion these results indicated that the biologically active compounds: 4% imidazole, 2% imidazole , sodium acetate, 2.5% acetic acid, 5% acetic acid and 5% magnesium sulphate $MgSo_4$ were almost potent more than the standard antibiotic Penicillin and Streptomycin as positive control, and these compounds were the best alternative when infection is caused by multiple antibiotic resistant strains.

Tables

Table 1. Antibacterial activity (the test solution was prepared in distilled water).

Compound	*E. coli*	*P. aeruginosa*	*K. oxytoca*	*S. liquefaciens*	*P. mirablis*	*S. aureus*
1	22	17	24	22	17	14
2	16	22	18	16	12	19
3	8	-	10	9	10	11
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	22	-	14	32	-	-
7	11	13	14	8	11	15
8	16	16	17	11	10	21
9	10	18	-	-	20	13
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	15	-	-	15	12
13	29	30	-	-	-	-
14	-	-				-
15	-	-	-	-	-	-
P	-	-	-	-	9	15
S	14	19	15	-	28	-

Table 2. Antifungal activity (the test solution was prepared in distilled water)

Compound	*A. niger*	*A. flavus*	*A. fumagatus*	*C. albicans*	*Fusarium* sp.
1	15	13	13	30	20
2	12			16	13
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	13	-	15	11	23
8	16		12	10	21
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	25	-	10	-
13	-	-	-	-	-
14	-	-	-	-	
15	-	-	-	-	-
Fluconazole	26	22	27	20	21

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تأثير بعض الحوامض الضعيفة والقواعد الضعيفة والأملاح تجاه بعض الحياء المجهرية الممرضة

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المستخلص:

صممت هذه الدراسة لملاحظة الفعالية المضادة للجراثيم لعدد من المركبات الكيميائية المتضمنة: 4 % imidazole و 2 % imidazole و1% imidazole و2.5% sodium citrate و 5% sodium citrate و sodium acetate و2.5% acetic acid و 5 % acetic acid و 5% citric acid و $5\%MgCl_2.6H_2O_2$ magnesium chloride و 5%sodium carbonate و 5% magnesium sulphate و $K2Cr_2O_7$ 5%potassium dichromate و 5%potassium permanganate $KMnO_4$ و $NaHCO_3$ 5%hydrogen carbonate sodium تجاه الجراثيم: *Escherichia coli* و *Pseudomonas aeruginosa* و *Klebsiella oxytoca* و *Proteus mirablis* و *Serratia liquefaciens* التي تم تشخيصها باستخدام تقنية API 20 E و *Staphylococcus aureus*، الفطريات المرضية: *Aspergillus niger* و *A. flavus* و *A. fumagatus* و *Fusarium sp.* و *Candida albicans*. بينت النتائج أن المركبات : 4 % imidazole و2% imidazole و1% imidazole و sodium acetate و2.5% acetic acid و 5 % acetic acid و sodium carbonate و $K_2Cr_2O_7$ تمتلك فعالية حيوية مختلفة اعتمادا على النوع البكتيري، في حين نجد أن 4 % imidazole و 2 % imidazole و2.5% acetic acid و 5 % acetic acid و sodium carbonate٥% تمتلك فعالية فطرية ،كما بينت النتائج أن 4 % imidazole و $K_2Cr_2O_7$ تمتلك فعالية أكثر من السيطرة الموجبة .

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