Antibacterial Activity of 2- (2-Hydroxy phenylimino) Acetic Acid

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Abstract
The ability of 2-(2-hydroxy phenylimono) acetic acid [2-(2- HP-AA)] to inhibit growth of different types of pathogenic bacteria was investigated.Different concentration were prepared from this chemical compounds(40 mM ,50 mM and 70 mM).
E.coli, staphylococcus aureus and *streptococcus agalactiae* nearly completely inhibited at 70 mM ,while the same concentration did not inhibit the growth of Proteus mirabilis ,*streptococcus mutans, Klebsiella pneumoniae* and *streptococcus pneumonia* .after 24 h incubating in the present study we evaluate that 70 mM considered as the minimum inhibitory concentration.
Key words: Acetic acid, Acetic acid derivatives 2-(2- HP-AA) , Antibacterial effect , Cytoplasmic PH

1. Introduction:
An acetic acid is an organic compound with acidic properties. The most common organic acids are the carboxylic acids ,whose acidity is associated with their Carboxyl group (Dibner and Butin 2002).
Acetic acid is used in food preservation since of their effect on bacteria. The key basic principle on the mode of action of acetic acid is that non dissociated (non ionized) can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that presented as PH- sensitive, meaning that they cannot tolerate a wide internal and external PH gradient (Patanen and Morz,1999).
Certain organic acids involvement acetic acid appear to have toxicity in excess of that which could possibly be due to the PH alone. The consensus of authorities seems to favor the theory that it is the undissociated molecule which is toxic and found that the undissociated acetic acid molecule was toxic (Leon et al.,1993).
The antiseptic action of acetic acid was connected with their influence on surface tension or contributed to the idea that the toxic effect was due to the whole molecule rather than to hydrogen ions alone.
The acetic acid either chemically pure or as Vinegar ,had a marked influence on the growth of different types of bacteria. The same PH, greater inhibition was obtained with an increasing concentration of acetic acid (Beuchat and Golden,1999).
Antibacterial activity of acetic acid is attributed to direct PH reduction of the substrate ,depression of the intracellular pH by ionization of the undissociated acid molecule or disruption of substrate transport by alteration of cell membrane permeability (IKawa,1995) and therefore PH dependent (IFT,1990).

Aim of the study:
The present work were aim to investigate further the antibacterial effect of new organic compound.
2-(2-hydroxy phenylamino) acetic acid [2-(2- HP-AA)] on different types of pathogenic bacteria insulated from different sites of infection.

2. Material and Methods:
2.1 Bacterial Isolates:
Clinical strains of *E.coli,K.pneumoniae,P.Mirabilis,staphylococcus aureus,streptococcus pneumoniae,st.mutans,and St.agalactiae* were obtained from department of microbiology-college of Medicine-Babylon University.Isolates were identified to the species level based on the standards biochemical and microbiological methods (Macfaddin,2000)

2.2 Preparation of different concentration of 2-(2-Hydroxy phenylimino) acetic acid:
The derivative has been synthesized by mousa & sultan (2013) and different concentration of [2-(2-HP-AA)],were prepared in deionized water DDW (40,50,70) mM and all these gradient concentration were tested against the bacterial growth to clarify the minimum inhibitory concentration after filtrated through 0.2 mµ pore size filter (Special communication Prof.Dr.Mufeed Ewadh 2013).

2.3 Minimal inhibitory Concentration (MIC) test :
A minimum inhibitory concentration test was carried out to determine the lowest concentration of [2-(2- HP-AA)] needed to inhibit visible (99%) bacterial growth of fixed concentration of experimental microorganism after an overnight incubation. The MIC value was confirmed based on the inhibition and growth observed on the agar plate which had been spot inoculated. The test was carried out in triplicate and the mean value of MIC was calculated(AL-Bayaty et al,2011).

2.4 McFarland tube standard (0.5):
A barium sulfate turbidity standard solution equivalent to a 0.5 McFarland standard was prepared as described by CLSI (2010).

2.5 Detection of bacterial growth by optical density:
The optical density of each tube was measured at a wave length of 750 nm against the standard medium ,and the measurement being performed every 1 to 2 hrs. During the logarithmic phase to growth .the OD results were collected as the means of three measurements (Bnyan,2013).Fig (1)

2.6 Screening of organic compound –acetic acid effect in bacterial growth :
Organic compound_acetic acid [2-(2- HP-AA)] effect in different concentration was analyzed for inhibition activities against indicator bacteria by agar –well diffusion Muller-Hinton agar seeded with bacterial isolates. The inoculums to be used in this test were prepared by adding (5) isolated colonies grown on blood agar plate to (5) ml of nutrient broth and incubated at $37C^0$ for 18 hrs. and compared with (0.5) Mc farland tube. A sterile swabs was used to obtain an inoculums was streaked on Muller-Hinton agar plate and left to dry. Wells (5) mm were hollowed out in agar using a sterile cork borer, a volume of (50) µl of tested organic compound-acetic acid were dropped separately in each well, and incubated at 37^0 C for 24 hrs. and inhibition zone around the wells were measured and recorded in millimeter after subtraction 5 mm (well diameter).

3. Results:
In the present study investigation of antibacterial effect of the organic compound-acetic acid against different Gram negative and Gram positive bacteria was recorded.(Table 1).
Bacterial isolates were subjected to study the effect of organic compound –acetic acid in different concentration on their growth these results showed in table (2).It was found that this chemical compound have the ability to inhibit the growth of E.coli, staphylo coccus aureus and strepto coccus agalactiae,and the best inhibitory concentration was determined as 70 mM as showed in table (3).The mean optical density OD_{750} reading of E.coli, S.aureus and st.agalactiae were subjected to [2-(2- HP-AA)] in different concentrations.The presence of this compound in the growth of K.pnenmoniae, P.mirabilis, St mutaris ,St.pneumonial ,did not cause substantial inhibition of growth. However {2-(2- HP-AA)} nearly completely inhibited growth of E.coli , S.aureus and St.agalactiae (Fig.1).40 mM and 50 mM did not show an inhibitory effect on all bacterial isolates. While 70 mM considered as minimum inhibitory concentration of [2-(2- HP-AA)].

4. Discussion:
Organic acids are generally recognized as safe (GRAS) antimicrobial agents,and the dilute solutions of organic acids(1,3 %) are generally without effect on desirable senseroy properties when used to prewserve many products from contaminated pathogenic bacteria(Smulder and geer,1998).Previous studies focused on limited treatments for controlling bacteria in which results were in consistent because of the extensive variations in conditions of experiments.
From our results addition of organic compound-acetic acid to the growth medium of E.coli, S.aureus and st.agalactiae nearly completely inhibited growth of tested strains.These results were similar to results obtained by other investigators . Raftari et al., (2009) reported that the number of bacterial growth were decreased when the affected meat with E.coli and S.aureus treated with acetic acid as spray wash.Also Roe etal., (2002) explain the inhibitory effect of acetic acid on strain of E.coli that organic acid toxicity is multifunctional and includes the ability of the undissociated acid to diffuse freely across lipid bilayers and liberate protons in the cytoplasm lowering the cytoplasmic PH(Booth,1995);the interaction of the undissociated acid in to the lipid bilayer at low external PH (Stratford and Anslow,1998);and the consequence of anion accumulation consistent with these modes of action,the inhibition provoked by these compound is PH dependent (Russell and Diez ,1998).
The PH is one of the important factors which influences the growth of bacteria.It has been well established that most microorganisms grow best at PH values around 7 (Jay etal ., 2005),therefore, PH reduction is one of the

inhibitor factors, which can limit the growth of bacteria (Dubal etal.,2004) .It was indicated that direct bacteriocidal action of acetic acid result from PH decrease within bacterial cell resulted in reduction of E.coli population (Malicki etal.,2004).Morever our resuls similar to another study that found the bacteriostatic effect of acetic acid against e.coli was proportional to PH decrease in culture medium (Shin etal., 2002).

Bornemeier etal., (1997) tested the ability of acetic acid against staphylococcus aureus and listeria monocytogenes and their results indicate the effectiveness of acetic acid to inhibit the growth of two bacterial species.Another study referred to as the effect of acetic acid in the growth of staphylococcus aureus and activity of production of their entero toxins.(Rode etal., 2010).

Our results showed that strepto coccus agalactiae affected by high concentration of acetic acid .It was refered that the bacteria differ greatly in their sensitivities to weak acids, certain bacteria and lactobacilli are able to grow rapidly in low PH but E.coli and some strains of streptococcus are not resistant (Russell,1991).this study intiated to test what extent acetic acid inhibitis the growth of different types of certain pathogenic bacteria and our results showed the degree of inhibition depend on the acid concentration .The undissociated molecule of the acid is known to be the active antimicrobial and also to be responsible for PH value (IFT,1990), (Mc Donald etal.,1990),fathermore it is known that the action of acetic acid as antimicrobial agents is generally improved by anions which interferes with dissociation of acid molecule, however certain specific cations may also significantly increase the effectivness of organic acids by increasing the solubility of the acid in the microbial cell membrane (Roe etal.,1998).

Conclusions :

This study showed the possibility of novel role of [2-(2- HP-AA)] inhibiting the growth of three different types of bacteria isolated from different sites of infection .The concentration of 70 mM of this derivatives can be used as antibacterial in different ways. Upon passive diffusion of acetic acid into the bacteria, where the PH is near or above neutrality, and the acid will dissociate and lower the bacteria internal PH,leading to situation that will impair or stop the growth of bacteria. On the other hand ,the anionic part of the organic acid that can not escape the bacteria in its dissociated form will accumulate within the bacteria and disrupt many metabolic functions, leading to osmotic pressure increase incompatible with the survival of the bacteria.

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Table (1): Bacterial isolates and site of infection

Types of bacteria	Site of isolation
E.Coli	Urine
K.pneumoniae	Urine
P.mirabilis	Urine
S.aureus	Blood
St.agalactiae	Vaginal swab
St.pneumoniae	Blood
St.mutans	Oral cavity

Table (2) : Growth media used in the experiments in all isolates

Control medium	Test Medium
BHI	BHI + 40 mM
BHI	BHI + 50 mM
BHI	BHI + 70 mM

Table (3) : Effect of different concentration of organic derivatives on bacterial isolates

Bacteria Types	Organic derivatives of acetic acid Concentration		
	40 mM	50 mM	70 mM
E.Coli	-ve	-ve	23 mm
K.pneumoniae	-ve	-ve	-ve
P.mirabilis	-ve	-ve	-ve
S.aureus	-ve	-ve	20 mm
St.agalactiae	-ve	-ve	18 mm
St.pneumoniae	-ve	-ve	-ve
St.mutans	-ve	-ve	-ve

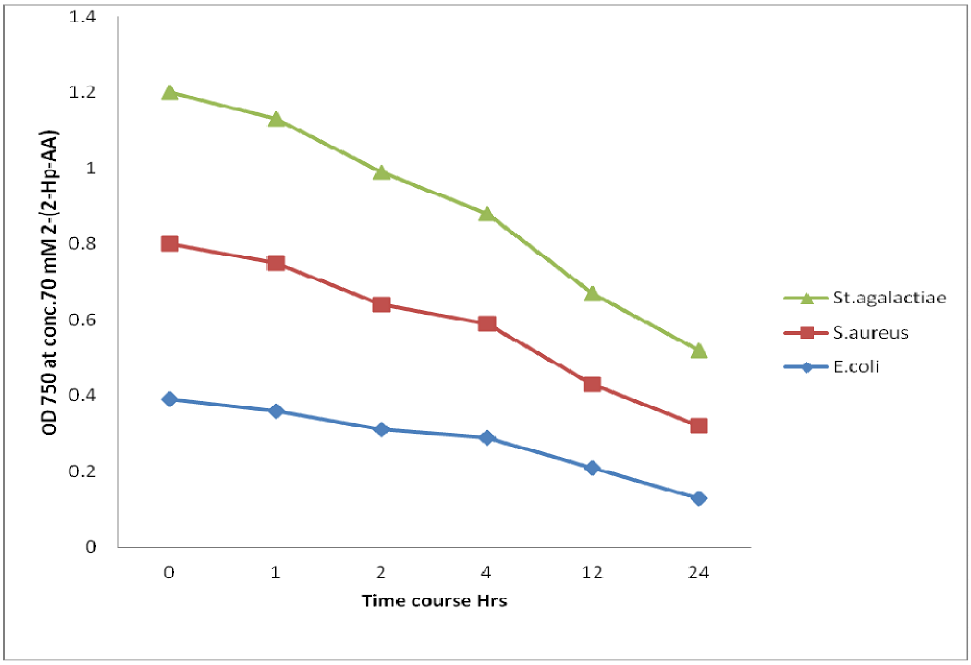

Fig.(1): Time course of bacteria growth inhibition by 70 mM [2-(2- HP-AA)]