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Turbidimetric Method for Measuring of Antibacterial Effect of 2-Methyl-4-(3,4 dihydroxy Phenyl)-Thiazole on the Growth Activity of Escherichia Coli and Pseudomonas Aeruginosa

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Abstract

Thiazoles are heterocyclic nitrogenous compounds, which were given a great attention due to their antibacterial effect, antihypertensive, stimulating glucose absorption (like antibiotics), acts as cytoprotective agent. The present work is conducted to evaluate the degree of antibacterial activity on the growth activity of *Escherichia Coli* (*E.Coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) by incubation with 2-methyl-4-(3,4 dihydroxy phenyl)-Thiazole compound (MDHPT).

Optical density (O.B) was measured by turbidmetric method, any increase in O.D would represent the increase in the bacterial growth .The results showed that there was a 75.7 % inhibition when using 190.4 µg/ml (MDHPT) solution and 64.3% was obtained when using concentration of 369 µg/ml. It has been concluded that (MDHPT) compound shows a bacterial biological activity.

Key words: Thiazoles, E.Coli, P. aeruginosa, incubation medium, incubator, minimum inhibitory concentration (MIC)

Introduction

The study of new derivative of Thiazole has been given a great attention, due to their antibacterial activity [1, 2, 3] antihypertensive [4] and stimulation of glucose absorption like antibiotics [5], lowering cholesterol [2], shows bacteria static activity [3] and acts as cytoprotective agent by induction of glutathione-S-transferases (GST) represent not only cell detoxification and survival but also cancer prevention [6,7].

Several attempts were carried out to elucidate the effect of different antibiotics related to this compound on the growth activity of pathogenic Staphylococcus aureus [2,3,8,9,10,11,12,13,14] This work is related to the action of 2-Methyl-4-(3,4 dihydroxyphenyl)-thiazole (MDHPT) on the growth activity of Escherichia Coli (*E.Coli*) and ***Pseudomonas aeruginosa*** (***P. aeruginosa***) either in solid or in liquid media .

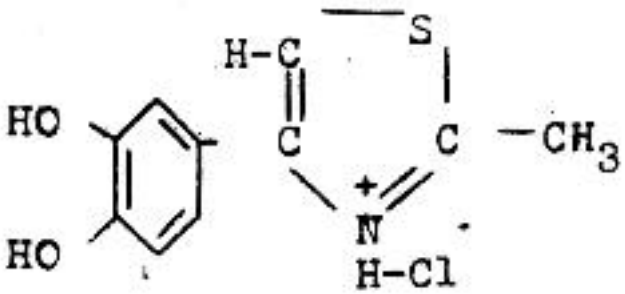
Both bacteria are of normal flora of the intestine and they cause different diseases especially in weak people [15].

The clinical records revealed that 83% of patients with leukemia having infection with Pseudomonas aeruginosa [16].This bacteria is also resistant to many antibiotics which may be attributed to alteration in cell membrane transport system. [17].

The aim of this study is to evaluate the degree of the antibacterial inhibitory effect of 2-Methyl-4-(3,4 dihydroxyphenyl)-Thiazole on the growth activity of *E.Coli* and P.aeruginosa and to find the effective dose required .

Materials and Methods

2-Methyl-4-(3,4 dihydroxyphenyl)-Thiazole hydrochloride, was synthesized by professor Shukri, J, Department of chemistry, College of Science , University of Baghdad [1].



(2-Methyf-4-(3,4 dihydroxyphenyl)-Thiazole)

A stock solution was prepared by dissolving (4×10^3 µg/ml) in water with few drops of glycerol.

To prepare a starting culture of bacteria, a loop full of stock culture from each type of bacteria was added to 50 ml of tryptose phosphate broth medium (29.5 g/liter, pH 7.3) and incubated in shaking water bath for 20-24 hours at 37° C and at 200-250 rpm (rotation / minute).

Experimental flasks of 250 ml with side arm, each contains 20 ml of sterilized culture medium. Each flask was inoculated with 1.0 % (V/V) of both used bacteria suspension which contain 2.4×10^7 cell / ml [18]. The number of bacterial cells was calculated by using Neuber heamocytometer [19]. (MDHPT) solution was added to the inoculated media at the beginning of cultivation, the flasks were closed with cotton plugs under sterilized conditions. A control was run under the same conditions without the addition of thiazole solution. The flasks were put in the shaking incubator at 200-250 rpm to ensure adequate aeration at 37° C for 24 hours.

Changes in the concentration of viable bacteria during lag, exponential and stationary phases were measured by changes in the optical density at 607 nm, using EEL colorimeter (Evans Electroselenium LTD. Halstead, Essex, England).

Results

Different concentrations of 2-Methyl-4- (3,4- dihydroxyphenyl)-thiazole ranging from (19.9 μg/ml (8.1 x 10^{-2}) - 190.4 μg/ml (7.8 x 10^{-1} μM)) were added to *E. Coli* culture media (after six hours of incubation). The results showed an inhibition of (24.3, 37.8, 48.6 and 75.7 %) in the growth rate of the bacterial culture, at concentrations of (19.9 μg/ml (8.1^{-2} μM), 59.1 μg/ml (2.4 x 10^{-1} μM), 97.5 μg/ml (4 x 10^{-1} μM), and 190.4 μg/ml (7.8 x 10^{-1} μM)) respectively; Table 1, Fig. l.

Inoculation with *P. aeruginosa* required higher (MDHPT) concentrations than that used with *E. Coli* at the same conditions. Concentrations of (135.2 μg/ml (5.5 x 10^{-1} μM), 244.4 μg/ml (l.0 μM), 313.4 μg/ml (1.28 μM) and 369.6 μg/ml (1.49 μM)) showed an inhibitory effect of (16.13, 38.7, 48.39, and 64.3%) respectively in comparison to control activity for *E.Coli* and *P. aeruginosa*. At the same experimental conditions Table 2, Fig 2. The results were the mean of ten experiments with $P< 0.05$.

All data are expressed as a mean of ten experiments with $P< 0.05$.

Discussion

(MDHPT) was studied with Staphylococus aureus results show bacteria static activity of 70% inhibition (MIC 200 μg/ml = 0.82 μM) [2,3]. In this study a bacteria static activity of 75.7 % was achieved with a concentration of, 190.4 μg/ml (8.1uM) inoculated, with *E.Coli* in liquid medium after 6 hours of incubation time. This results were inconsistent with what was reported by (Faraid, Al-Chalabi et al, and Shukri, J. et al), while a higher concentration solution of (MDHPT) 313.4 μg/ml (l28 μM), was required to achieve nearly the same rate of inhibition (64.3 %) as with *E. Coli* [2, 3].

Bacteria static activity was achieved, this may be explained by the presence of potent group(s) that achieved the inhibitory action on the growth activities of each of gram- negative *E.Coli* and *P.aeruginosa*, and also gram-positive *Staphylococcus aureus* (*St. aureus*). The presence of active Methyl group ($-CH_3$) at position 2 of the Thiazole ring which can react with carbonyl groups of the peptidoglycan or lipopolysaccharide (LPS) of the bacterial cell membrane [2, 20, 21].

The dissociation of phenolic groups present in the compound (MDHPT) may cause a release of excess protons (H^+) to the medium which may be picked up by the ($-NH_2$) of the peptidoglycan of the bacterial cell membrane forming excess ($-NH^+_3$) which may create an ionic attraction leading to a cell wall damage[3,19].

The cell wall of gram-negative bacteria has distinctly layered appearance, the inner region consists of monolayer of peptidoglycan, while the outer cell wall bilayer inward facing lipids are macromolecules called lipopolysaccharides (LPS), half the mass of the outer membrane consists of Braun proteins which covalently linked to peptidoglycan layer, adjacent outer lipopolysaccharides are held together by electrostatic interaction with divalent ions (Zn^{+2}, Ca^{+2}, Mg^{+2}), while gram-positive cell wall is multi-layered network which appears to be continually-growing by the addition of new peptidoglycan linear hetropolysaccharide chain cross-linked by short polypeptides at the interface with concomitant outer surface .[21,22,23].

There may be another possibility to explain the inhibitory effect it-may-be that the activity of (MDHPT) is due to its ability to chelate essential metal ions e.g. (Zn, Mg^{+2}) from bacteria which cause a disturb of these interaction of gram-negative (*E.Coli & P.aerugenosa*) by binding to the divalent ions in the cell wall which .cause the disintegration. Also chelation may occur to those ions that required for enzymes activation

processes occurred in bacterial cell wall. [20,21,22]. Variation in the results obtained with both bacteria under study could be due to high variation in amounts of the peptidoglycan present in the gram-negative or gram positive bacteria cell wall membrane [21,24].

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Table (1): Represents counts of viable E.Coli **cells and % inhibition when 2-Methyl-4-(3,4 dihydroxyphenyl) -Thiazole compound was added to inoculation medium compared to control. Mean of ten experiments P< 0.05**

Time hours	Test No. of viable E.Coli Cells Added 1.99 µg/ml (0.8 µM) (MDHPT)	% Inhibition	Control
0	2.4×10^{7}	0.0	2.4×10^{7}
2	2.6×10^{7}	24.3	3.6×10^{7}
4	7.2×10^{8}	32.8	3.6×10^{9}
6	3.5×10^{9}	48.6	8.8×10^{9}
24	4.9×10^{12}	75.7	7.0×10^{12}

Table (2): Shows counts of viable P. aeruginosa cells and % inhibition when 2-Methyl-4-(3,4 dihydroxyphenyl)-Thiazole compound was added to inoculation medium, compared to control. Mean of ten experiments P< 0.05

Time hours	Test No. of viable P. aeruginosa Cells Added 369.6 µg/ml (1.49 µM) (MDHPT)	% Inhibition	Control
0	2.4×10^{7}	0.0	2.4×10^{7}
2	1.5×10^{8}	16.13	8.6×10^{7}
4	1.3×10^{9}	38.7	$8{,}0 \times 10^{9}$
6	3.9×10^{10}	48.39	8.0×10^{10}
24	5.8×10^{11}	64.3	9.0×10^{11}

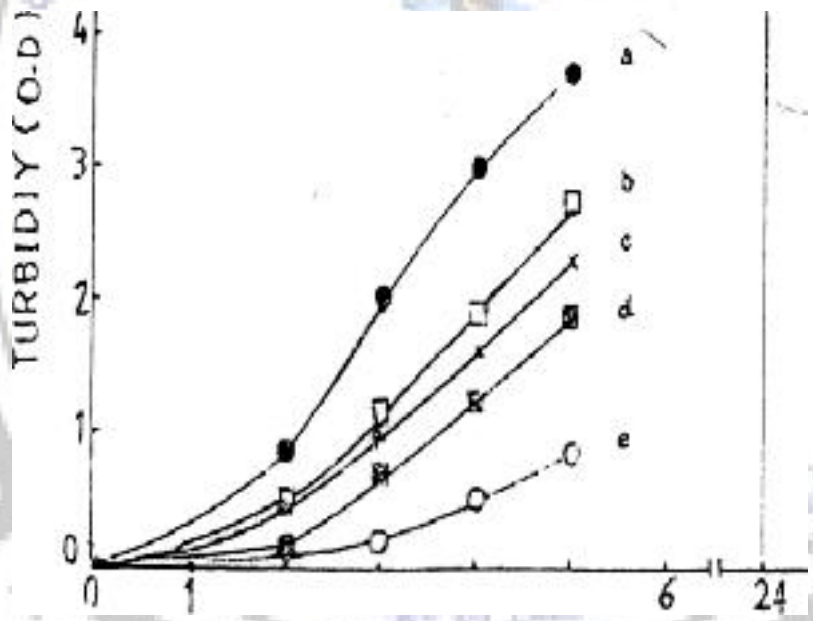

Fig. (1): Effect of various concentrations of 2-Methyl-4 3,4 dihydroxyphenyl) 4- Thiazole hydrochloride on the growth activity of E. Coli

a- Control bacterial growth of E.Coli, b-Inoculation medium contains 8.1×10^{-2} uM of (MDHPT). c- contains 2.4×10^{-1} µM (MDHPT), d- (MDHPT) 4×10^{-1} µM e-(MDHPT) $7.8 \times \times 10^{-1}$. Experimental conditions were described in the method. Data are expressed as a mean of ten experiments , P<0.05,

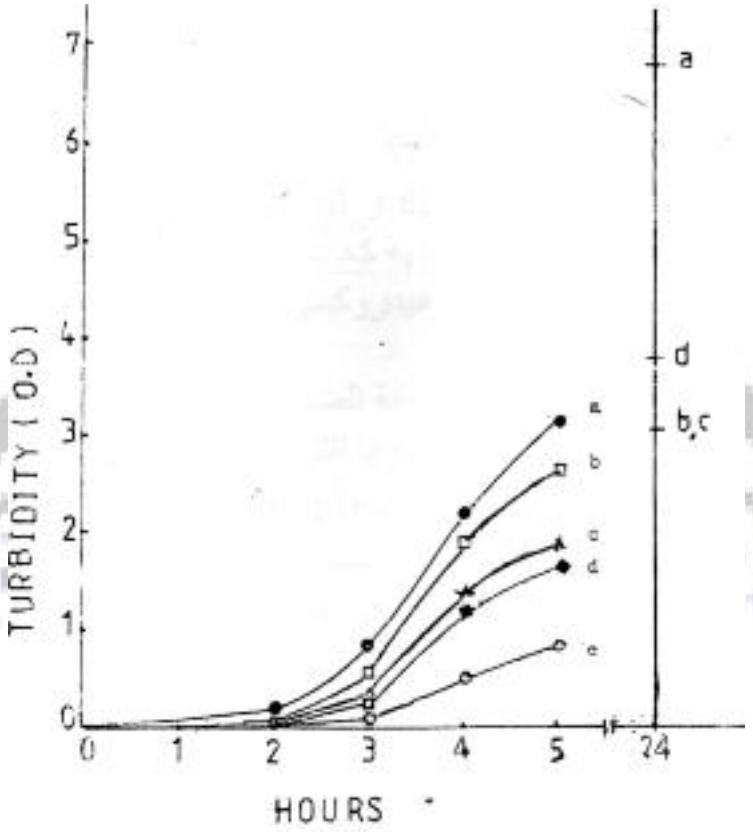

Fig. (2):Effect of various concentrations of 2-Methyl-4-(3,4 dihydroxyphenyl) - Thiazole hydrochloride (MDHPT) on the growth activity of P. aeruginosa

a-Control bacterial growth, b- (MDHPT) 5.5 x 10^{-1} µ M.

c- (MDHPT) 1.0 µM. d- (MDHPT) 1.2 µM e- (MDHPT) 1.49 µM.

Experimental conditions as in Fi g 1. P< 0.05.

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تأثير مضادات البكتريا لمركبات الثايوزول في مستوى النمو للبكتريا

(Escherichia coli و Pseudomonas aeruginosa)

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استلم البحث في : 26 نيسان 2009 قبل البحث في: 18 آب 2009

الخلاصة

تعد مركبات الثايوزول العضوية من المركبات التي أعطيت أهمية كبيرة في ضوء خصائصها بوصفها مضادات بكتيرية وحيوية فضلاً عن كونها محضرات لعملية امتصاص سكر الكلوكوز وتقدير فعالية التثبيط لنمو البكتريا للأنواع (Pseudomonas aeruginosa و Escherichia coli) باستعمال مركبات الثايوزول العضوية حاضنات فقد استعمل المركب العضوي ثنائي المثيل – 4 – (3,4 ثنائي هيدروكسي فنيل) – ثايوزول (MDHPT) لهذا الغرض.

أظهرت النتائج من خلال قياس الكثافة الضوئية تعني ازدياد تثبيط نمو البكتريا التي تم اختيارها في هذه الدراسة. كما أثبتت النتائج بأن تركيز مادة الثايوزول (190.4 µg/ml) لها فعالية تثبيط بنسبة (75.5%) بينما كانت النسبة المثبطة للنمو (64.3%) عند التركيز (369 µg/ml) وفي ضوء النتائج التي عد المركب (MDHPT) ان له تأثيراً كبيراً في نمو البكتريا.

الكلمات المفتاحية : مركب الثيازول، وسط حراري (حاضن)، حاضن ، أقل تركيز تثبيطي (MIC)

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