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EVALUATION OF ANTIMICROBIAL ACTIVITY OF PRYMIDINE DERVATIVE OF SOME BACTERIA PATHOGENS FROM URINARY TRACT

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Abstract

The current the study is carried out investigate of antimicrobial activity of uracil isophenol compound against some bacteria pathogens causing UTI infection . The urine sample were collecting to hospitals in Najaf of (AL Sadr Medical City) for the period from (2 in July 2013 to 2 in September 2013 up) The results were as follows: The proportion of isolated bacteria is *E.coli* 40% higher and the proportion of bacteria isolated is *Staphylococcus aureus* 32% and isolation of bacteria *Pseudomonas.aeruginosa 28*% in addition to some of the genera were diagnosed. .

In the course of the program directed towards the synthesis of fused nitrogen heterocyclic compound and as an extension of efforts directed towards the development of convenient synthetic approaches for the synthesis one of pyrimidine derivatives called uracil isophenol with an expected broad spectrum of biological activity. Studies have shown that uracil isophenol exhibit various biological activities. In biological study was to demonstrate in trial the new compound "uracil isophenol" in different concentration observed the following ***E.coli*** exhibit sensitivity to all concentration with differ inhibition zone size ; but the same zone size for (10,15,20) mg/ml the inhibition area was reached for 21 mm . in spite of difference in concentration. While ***Staphylococcus aureus*** also sensitive and the zone larger than ***E.coli*** ,and have the same zone size of E.coli of concentration(3)mg/ml was reached for 15 mm . While ***Pseudomonas.aeruginosa*** also exhibit sensitivity but less than others except for (25) mg/ml it have inhibition zone was reached for size of 25 mm . Results were compared with control groups were analyzed statistically by results analysis system completely random ANOVA and LSD**.**

Key word: Uracil Isophenol مشتقات اليوراسيل , اصابات الجهاز البولي UTI infection , *E.coli* , *Pseudomonas.aeruginosa* , *Staphylococcus aureus* , منطقة التثبيط Inhibition Zone

Introduction:

In this article, we have synthesized this compound for study their utility as pharmacological agent due to large number of antibiotics used in the treatment of diseases and bacterial infections, at the present time but the effectiveness of these antibiotics in diminishing with the ability of bacteria to develop the means to defend themselves and to resist the work of antibiotics in many ways making therapeutic useless, and

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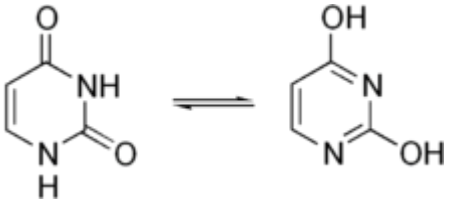
worse, that the possibility of this resistance be transferable from bacterial genus to another, was previously sensitive to a particular counter, and this resistance is spreading in direction proportion to the increase in the use of antibiotics.

The increase in bacterial resistance to anti-factors microorganisms antimicrobial agents such as (*S.aureus, E.coli ,Pseudomonas aeruginosa*) formed a pervasive problem on a global scale, and the form of resistance or model varies according to different regions, which is more common in developing countries due to the use of antibiotic.

Uracil is a common and naturally occurring pyrimidine derivative [1] Originally discovered in 1900, it was isolated by hydrolysis of yeast nuclein that was found in bovine thymus ,spleen, and wheat germ.[2] It is a planar, unsaturated compound that has the ability to absorb light.[3]

Uracil undergoes amide-imidic acid tautomeric shifts because any nuclear instability the molecule may have from the lack of formal aromaticity is compensated by the cyclic-amidic stability.[2] The amide tautomer is referred to as the lactam structure, while the imidic acid tautomer is referred to as the lactim structure. These tautomeric forms are predominant at pH 7. The lactam structure is the most common form of uracil.



Uracil tautomers: Amide or lactam structure (left) and imide or lactim structure (right) . Uracil can be used for drug delivery and as a pharmaceutical. When elemental fluorine is reacted with uracil, 5-fluorouracil is produced. 5-Fluorouracil is an anticancer drug (antimetabolite) used to masquerade as uracil during the nucleic acid replication process.[1] Because 5-Fluorouracil is similar in shape to, but does not perform the same chemistry as, uracil, the drug inhibits RNA replication enzymes, thereby eliminating RNA synthesis and stopping the growth of cancerous cells.[1] Uracil can also be used in the synthesis of caffeine. [A Novel Method]

Uracil's use in the body is to help carry out the synthesis of many enzymes necessary for cell function through bonding with riboses and phosphates[1] Uracil serves as allosteric regulator and coenzyme for reactions in the human body and in plants [4] UMP controls the activity of carbamoyl phosphate synthetase and aspartate transcarbamoylase in plants, while UDP and UTP requlate CPSase II activity in animals. UDP-glucose regulates the conversion of glucose to galactose in the liver and other tissues in the process of carbohydrate metabolism.*[4]* Uracil is also involved in the biosynthesis of polysaccharides

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and the transportation of sugars containing aldehydes.[4]

Uracil can be used to determine microbial contamination of tomatoes. The presence of uracil is an indication of lactic acid bacteria contamination in the fruit.[5] Uracil derivatives containing a diazine ring are used in pesticides. [6] Uracil derivatives are more often used as antiphotosynthetic herbicides, destroying weeds in cotton, sugar beet, turnips, soya, peas, sunflower crops, vineyards, berry plantations, and orchards.[6]

It was the discovery of antibiotics significant impact on the low rate of inflammatory diseases, which has encouraged the production of these antibiotics and industrial quantities to meet the needs of the world, it seemed the perfect solution to get rid of the problem of germs final.

Material and methods

Gram's stain: This stain was used to differentiate Gram-negative from Gram-positive bacteria and was carried out according to [7].

Identification of Bacteria: A single colony was taken from each primary positive culture on blood agar and on MacConckey agar and it was identified depending on its morphology(colony shape, size, colour, , and texture) and then it was examined by the microscope after being stained with Gram's stain. After staining, the biochemical tests were done on each isolate to complete the final identification Including IMVC test, sugar fermented urease test ,iron production ,H2S formation , and some enzyme production . [7][8] [9][10][11].

Antibiotics sensitivity methods: A. The Kirby–Bauer standardized single disk method was carried out [12] 1. -Mueller–Hinton medium was employed for this experiment. The medium was cooled to 45–50°c and autoclaved appropriate volume was poured in the Petri dishes. **2. -**With a sterile wire loop, the tops of 4–5 pure colonies were transferred to a tube containing 5 ml of BHI broth. -The broth was incubated at 37°c until it's turbidity standard. This usually required at least 4–6 hours incubation. The cells density was estimated as 1.5×10^8 cell /ml by comparison with McFarland standard tube No.0.5 . **3.** sterile cotton swab on a wooden applicator stick was dipped into the standardized bacterial suspension. The excess fluid was removed by rotating the swab with firm pressure against the inside of the tube above the fluid level. 4: With a sterile forceps, the selected disks were placed on the surface of medium and pressed firmly but gently into the agar with sterile forceps. Within 15 minutes the inoculated plates were incubated at 37°c for 18 hours in an inverted position. 5. After incubation the diameters of the complete inhibition zones were noted measured by using a ruler. The end point, measured to the nearest millimeter was compared with zones of inhibition determined by NCCLs and to decide the susceptibility of bacteria to antimicrobial agent, whether being resistant or sensitive.

Experimental prymidine derivative : A solution of aryldiazonium salt of a substituted aniline (0.01 mol) was slowly added to a well cooled, stirred mixture of pyrimidine derivative (0.01

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mol) in 10% aqueous NaOH (10 ml) containing excess sodium acetate. The mixture was kept at room temperature for one day. The precipitate was filtered off, washed with water , dried and recrystallized from DMF/water.

Biological Activity Testing of Prepared Chemical Compounds : By using[13]. method for the test of biological activity of the prepared chemical compounds which includes the following steps:

1. Prepare bacterial suspension and compare with McFarland tube 0.5 .
2. Spread bacterial suspension on (Muller Hinton Agar) homogeneously (0.1 ml) to cover the whole surface. Make holes in the agar by using 6 mm diameter cork piercing.
3. Prepare diluted solutions (5,10,15,20,)mg/ml for each compound at physiological pH(7).
4. Put the prepared solutions in holes to investigate their biological activity .
5. Incubate the petri-dishs at 37C. for 24 hours.
6. Measure the diameter of inhibition zone for each hole by the ruler to determine the effectiveness of each compound and compare with the standard limits of sensitivity of the same species of bacteria against antibiotics .

Statistical Analysis: The analysis of the results was according to the model testing process and the design was higher of accuracy and tested incorporeal results by using a least significant difference(L.S.D.) below the level of probability P≥0.05 based [14].

Results:

Isolation of bacteria: This table (1) shows a higher incidence of bacteria *E.coli* among people with inflamatory infections where the incidence of 40 % and is ranked second bacterium , *Staph aureus* where the rate of 32 %, and the rate of *pseudomonas aurogenosa* 28% . where he stated [15] in study on urinary tract infection that among the pathogens that infect the urinary system *E.coli* bacteria were the most frequent injury in terms of the percentage was 88.2% and the incidence of bacteria *Klebsiella pneumoniae*% 66 is due to the bacteria *E.coli* bacteria are located in naturally in the urinary tract, so the injury to be higher among the types of bacteria, and that the injury to the urinary system disease pathogens among the most common injuries in other organs because of the urinary system that direct communication to the external environment [16].

Table(1):The Number and Percentage of Isolated Bacteria

Total number of sample(68)	*Bacteria*		
	E.coli	*Staph aureus*	*pseudomonas aurogenosa*
no. of bacteria	27	22	19
percentage of bacteria	40%	32%	28%

Antibiotics sensitivity test :

Conducted examining the sensitivity of isolates of bacteria that cause inflammation of the urinary tract to the 13 types of antibiotics and showed the results shown in the table (2) that there is variation in the resistance of the isolates under study to the antibiotics used, the results show

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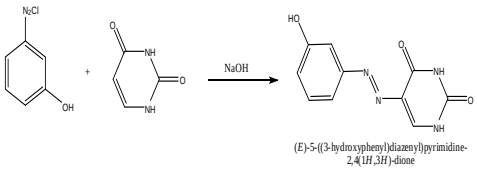
that isolates E.coli were resistant by the antibiotic Ampicillin, Streptomycin as it was sensitive to the antibiotic Nalidixic acid, Polymyxin, but fell to the antibiotics used for the other, while varied in their resistance and sensitivity to bacteria *Ps. aeruginosa* by antibiotics Streptomycin, Neomycin, Gentamycin, Polymyxin , But Staphylococcus aureus bacteria was showed resistance to the antibiotic Nalidixic acid, Polymyxin and sensitive to antibiotics Ciprofloxacin, varied sensitivity and resistance to other antibiotics. According to a study [17] [18] strains belonging to the genus *Ps. aeruginosa* is characterized by resistance to antibiotics Ampicillin, Trimethoprim and their sensitivity to Ciprofloxacin. The study reported that all isolates *Ps. aeruginosa* were resistant to Ampicillin this resistance is related to the genes carried on plasmid or chromosome [19]، . While the marked bacteria Staphylococcus aureus resistant to Erythromycin and its sensitivity to antibiotics Gentamycin, Cephalothin, [20].

Table (2) Antibiotics sensitivity

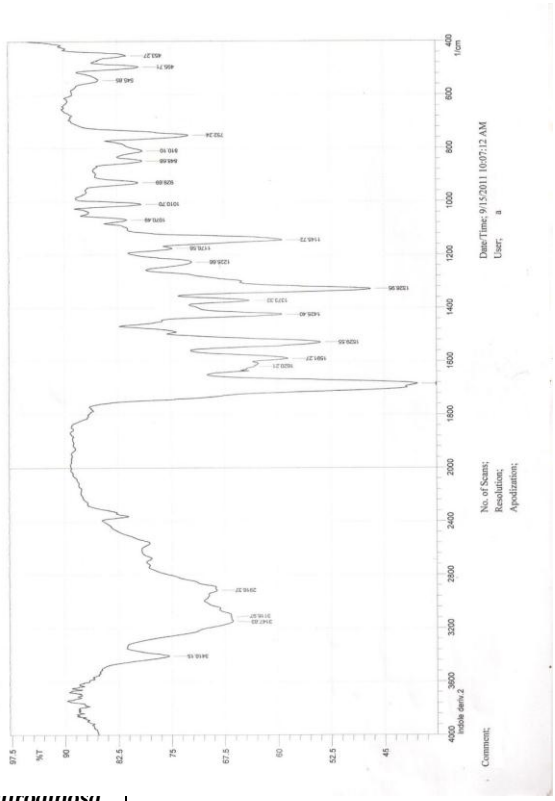
Antibiotics	Abstract	Content (µg)	Inhibition zone (		
			S.aureus	***E. coli***	***P. aurouginosa***
Gentamicin	CN	10	S	R	R
Ciprofloxacin	CIP	5	S	R	S
Azithromycin	AZM	15	S	R	R
Nalidixic acid	NA	30	R	R	R
Cephalothin	KF	30	R	R	R
Vancomycin	VA	30	R	R	R
Trimethoprim	TMP	5	S	R	R
Amoxicillin	AX	30	R	R	R
Erythromycin	E	15	R	S	S
Norfloxacin	NOR	10	S	S	R
Amoxcillin	AMC	30	R	S	R
Metronidazole	MET	5	R	R	R
Ampicillin	AM	10	R	R	R

The synthesis one of pyrimidine derivatives called uracil isophenol :

In this paper, we describe a rapid and convenient method for the synthesis of phenyl diazenyl pyrimidine under catalyzed conditions bases by NaOH (Scheme 1).



Scheme 1. Synthesis of 5-((3-hydroxyphenyl)diazenyl)pyrimidine-2,4(1H,3H)-dione



biological activity to model compound uracil isophenol on bacteria

The results showed that *Ps. aeruginosa, E. coli* and *Staph. aureus* were sensitive to the new compound "uracil isophenol" from prymidine derivative, and increasing the compound concentration increases the inhibition zone in different concentration observed the following

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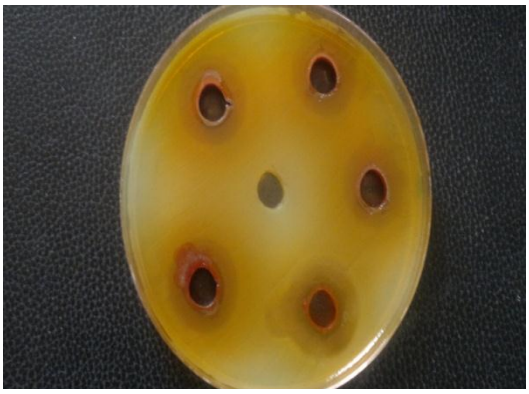
E.coli exhibit sensitivity to all concentration with differ inhibition zone size ;but the same zone size for (10,15,20) mg/ml in spite of difference in concentration. While ***Staph.aureus*** also sensitive and the zone larger than ***E.coli*** ;but have the same zone size for(10,15)mg/ml,& have the same zone size of ***E.coli*** of concentration (3)mg/ml. While *Ps. aeruginosa* also exhibit sensitivity but less than others except for (25) mg/ml it have the same zone size of ***E.coli*** as shown in table below . this results maybe back to capacity this compound in effective on bacteria , this different back to biological activity of this compound , such as *Staphylococcus aureus* was found resistant in vitro. it has effecting groups working on bacteria metabolism, the compound model was capable to combine with chemical material in cell after that become complex inside the cell, the bacterial will become dehydrated and die .this result applicator with [21] used the prepare compound 2-[4-(dimethylamino)-3-[(4-methoxyphenyl)diazenyl]phenyl]-3-(aryl)-2-hydrobenzo[e]-1,3-oxazepine-4,7-dione) and also used prepare DBHBED found was effect to E.coli this compound effect to my be on metabolism of this bacteria [16] .This might be due to the change in the genetic properties of the living cells

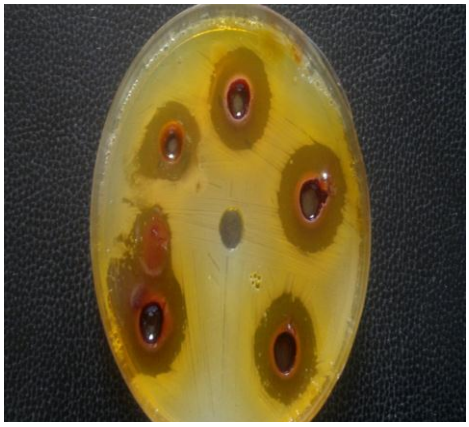
Table (3) biological activity to model compound *(uracil isophenol*) on some genus of bacteria

bacteria	**Inhibition zone**					**LSD** P≥0.05
	3mg/ml	*10mg/ml*	*15mg/ml*	*20mg/ml*	*25mg/ml*	
E.coli	15	21	21	21	25	1.3
Staph aureus	15	22	22	26	27	1.4
Ps. aeruginosa	12	18	20	22	25	1.6

Pictures of biological activity to model compound :



Eeffect of model compound on *Staph aureus*



Effect of model compound on *E.coli*

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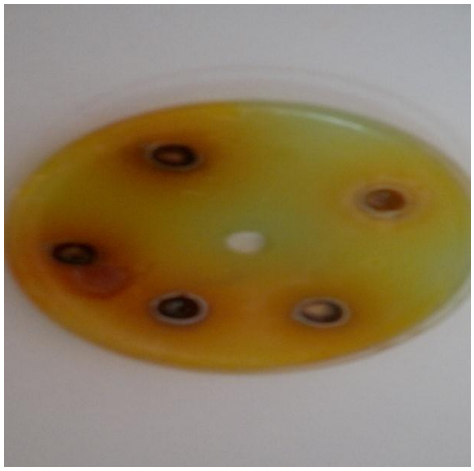

Effect of model compound on *Ps. aeruginosa*

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تقييم فعالية المضادات الميكروبية لمشتقات البريميدين على بعض الممرضات البكتيرية المسببة لإصابات المسالك البولية

الخلاصة:

تحدد هذه الدراسة الكشف عن الفعالية التضادية لمشتقات البريميدين (يوراسيل ايزوفينول) ضد بعض الاجناس المعزولة من اصابات الجهاز البولي والمأخوذة من فترة (2تموز 2013 الى 2 ايلول 2013) من مدينة الصدر الطبية في محافظة النجف الاشرف . اظهرت النتائج ان نسب عزل بكتيريا *E.coli* نسبة وجودها 40 % وهى الاعلى مقارنتا ببكتيريا *Staphylococcus aureus* والتي بلغت نسبة العزل لها في عينات الادرار 32% ,بينما اوضحت النتائج ان بكتيريا *Pseudomonas.aeruginosa* هي الاقل وبلغت 28% .تم تصنيع

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مركب جديد من مشتقات البريميدن والمسمى مركبات الايزو فينول uracil isophenol المحتوية على حلقات من النتروجين المهمة وتم تشخيص القمم الحاوية على الجذور ذات الفعالية البيولوجية . اظهرت نتائج الفعالية التضادية للمركب uracil isophenol فعالية بايلوجية على جميع الاجناس البكتيرية المأخوذة في البحث حيث بينت تاثير المركب بدرجه عاليه على بكتيريا *E.coli* حيث كان تاثير المركب طريق قياس منطقة التثبيط حوالي 21 ملم لأعلى تركيز مأخوذ الا وهو 20 µg/ml . وكان تاثير المركب على بكتيريا *Staphylococcus aureus* 15 ملم في التركيز اعلاه . بينما بكتيريا *Pseudomonas.aeruginosa* كان 15ملم في تركيز 20 µg/ml. حللت النتائج احصائيا تبعا لتحليل التام العشوائية ANOVA واستخرجت قيمه LSDحسب القيمة الجدولية $P \geq 0.05$