

Study of the biological activity of pyrazoline derivatives with fusidic acid and ZnO as an antibacterial and antioxidant

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Abstract

pyrazoline was synthesized as a derivative, then the polymerization of pyrazoline was carried out with fusidic acid and zinc oxide. All the physical properties affecting the construction process, such as Thin-layer chromatography, solubility, the effect of buffer solutions, and temperatures, were studied. Physical diagnostics such as DSC, TGA, and FT-IR were also investigated. The study of the biological activity of the polymer resulting from the interaction of pyrimidine with fusidic acid and zinc oxide, where the study was conducted against three types of bacteria, and the results of the study were compared with the activity of fusidic acid alone once and with zinc oxide alone again as a control. The results showed that the process of polymerization of pyrazoline with fusidic acid and zinc oxide gives more excellent results against bacteria compared to fusidic acid alone and zinc oxide alone.

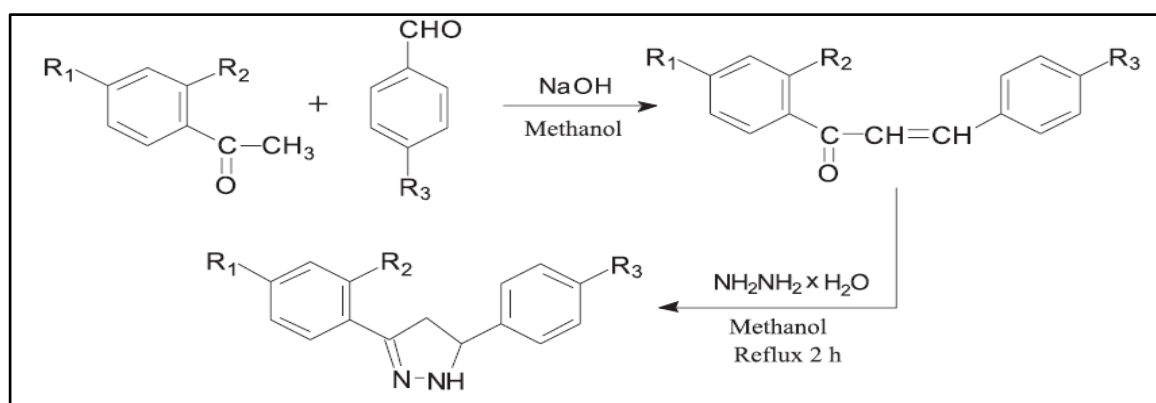
Keywords: pyrazoline derivatives, antibacterial, antioxidant

Introduction

Because of the pyrazoline nucleus' high degree of stability, scientists have used these stable fragments with bioactive moieties to create novel compounds with biologically active properties. Earlier research on substituted pyrazolines showed that they had antibacterial, analgesic, anti-inflammatory, antiviral, antifungal,

anti-arthritic, and antidepressant activities^(1, 2). Several substituted pyrazolines are luminous or fluorescent compounds or have bleaching characteristics. They are helpful as biodegradable agrochemicals as well. After the synthesis of a few new pyrazolines, their antibacterial and anti-inflammatory actions were examined. This was motivated by these findings and continued work on the synthesis, spectrum investigations, and biological characteristics of pyrazolines.^(3, 4)

The synthesized compounds substituted acetophenones and substituted benzaldehydes are used to create substituted chalcones. Acetophenone that had been substituted was dissolved in sodium hydroxide solution. The substituted benzaldehyde was then added while being stirred and left overnight. After being neutralized with hydrochloric acid and filtered, the reaction mixture was placed into ice-cold water. The chalcone and hydrazine hydrate solution in the ethanol was then refluxed. A surplus of ethanol was distilled off, and the remaining substance was then left alone for the night. The filtered and recrystallized crystalline material was made from ethanol. Similar to that, scheme 1, shows how all of the substituted pyrazoline derivatives were created⁽⁵⁻⁸⁾.



scheme (1): Synthesis of pyrazoline derivatives

The Biological activity of pyrazoline derivatives by using the cup-plate agar diffusion technique, all the synthesized compounds were tested in vitro for antibacterial activity against *S. aureus* (MTCC 96), *B. subtilis* (MTCC 619), *E. coli* (MTCC 722), and *P. aeruginosa* (MTCC 424) at doses of 10 g/mL each

124). After the identification of the MICs for each chemical, the screening concentrations were selected. Dimethyl sulfoxide (DMSO), which had been further diluted with water, served as the solvent. The growing medium for the bacterial species was Muller Hinton agar. The control utilized was DMSO. The control had no effect on the utilized strains of microorganisms. A relationship between antimicrobial activity measurement and zone of inhibition diameter was used (mm). By measuring the zone of inhibition in mm at 10 g/mL, the findings were compared to the antibacterial activity ofloxacin, the gold standard medication. The maximal zones of inhibition for the two organisms—one Gram-positive strain and one Gram-negative strain—were 18 mm and 20 mm, respectively. The conventional medication was tested against the other two strains as well⁽⁸⁻¹⁰⁾.

In the antioxidants of Pyrazolines are unique among heterocyclic chemicals because of their many biological uses. Pyrazolines are nitrogen-containing five-membered heterocycles. Many pyrazolines derivatives have significant biological actions, spurring study in this area. Pyrazolines and replaced Pyrazolines' intriguing biological activity have garnered attention. Their anti-fungal, anti-bacterial properties depressant, anti-convulsant, anti-inflammatory, anti-bacterial, anti-cancer, antioxidant, antipyretic anti-neoplastic activities, anti-viral, anti-amoebic, anti-cholinergic, antidiabetic, anti-HIV, antimalarial, anxiolytic, antiparasitic, anti-allergic, antimicrobial, anti-tuberculosis, tyrosinase inhibitor, hypoglycemic, hypotensive, immunosuppressive, anti-tumor properties Dihydropyrazole, a five-membered heterocyclic molecule with two nitrogen atoms in neighboring locations and only one endocyclic double bond, is the chemical that makes pyrazoline. Due to its many biological actions, 2-pyrazoline has grown to be the most significant pyrazoline derivative. Many pharmacologically active compounds, including indoxacarb (insecticide), nature (uricosuric), azolid/ tendril (anti-inflammatory), and phenazone aminopyrine/methampyrone (analgesic and antipyretic), include 2-pyrazolines,

which exhibit a wide range of possible pharmacological activity. Moreover, the theory of heterocyclic chemistry has greatly benefited by the usage of pyrazolines, which are also widely utilized in organic synthesis^(11, 12).

The pyrazoline derivative's biological activities such as antimicrobial, antibacterial, and antioxidant, show in figure 1.

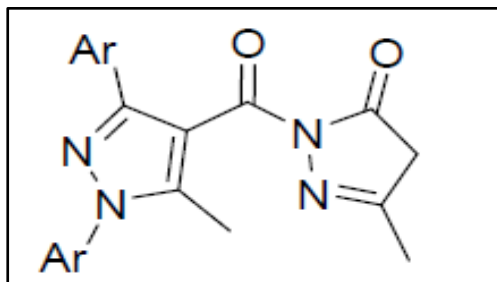


Figure (1): 5-methyl-2-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-2,4-dihydro-pyrazol-3-one and evaluation of their structure activity

Natural antioxidants' effects on pyrazoline-related disorders are directly correlated with their capacity to lessen DNA damage, mutagenesis, carcinogenesis, and limit the development of harmful microorganisms. An indicator of pharmacological utility may be regarded antioxidant activity. So, it is not entirely surprising that many approved medications include antioxidant effects that may support their pharmacological efficacy. As reference antioxidants, catechin, ascorbic acid, and butylated hydroxytoluene (BHT) were utilized⁽¹³⁾.

Chemicals

The chemical substances that were used in this work are listed in Table 1, along with their suppliers.

Table (1): Chemicals and their Suppliers

| No | Material | Company |
|-----------|----------------------------|------------------------------------|
| 1 | Benzyldehyde | Schar-de-hane |
| 2 | Hydrazine | Merk |
| 3 | Zinc oxide | Himedia-lab |
| 4 | Dimethyl formamide | Schar-lab |
| 5 | Dimethyl sulphoxide | Alpha chemika |
| 6 | Absolute ethanol | Ricdol-de-Hanc/ Germany |
| 7 | Chlorophorm | Ricdol-de-Hanc/ Germany |
| 8 | DMF | Schar-lab/ Spain |
| 9 | HCl 5% | Flucka AG/ Switzorland |
| 10 | Methanol | Ricdol-de-Hanc/ Germany |
| 11 | NaOH | Himedia-lab/ India |
| 12 | KOH | Schar-lab/ Spain |
| 13 | DMSO | Alpha chemika/ India |
| 14 | Diethyl ether | Schar-lab/ Spain |
| 15 | Acetone | Ricdol-de-hane/ |

| | | |
|-----------|---|--------------------------|
| | | Germany |
| 16 | SOCl₂(Thionyl chloride) | Carl Roth/Germany |

Synthesis of pyrazoline derivative:

Weight (0.261g,1mmole) of Chalcone and different nucleophile reagents (1mmole of hydrazine) by using sensitive balance. This mixture dissolves in 10 ml of ethanolic sodium hydroxide (C₂H₇NaO₂), 4 gm of NaOH, and 10 ml of ethanol, which is stirred for about 2-3 hours with a magnetic stirrer. This mixture will be poured into a beaker containing 400 ml of cold water and a conscious stirrer for 1 hour. After that, the mixture will be kept in refrigeration for 24 hours. The precipitation obtained will be filtered wash and recyclization mostly by ethanol.

Pyrazoline derivative with fusidic acid and ZnO

(0.288g) of acid chloride of pyrazoline added to (0.516 g) of fusidic acid; preparing this solution three times; adding (0.288g) of Pyrazoline adding to 20 mL of ZnO solution (1 g of ZnO in 20 ml ethanol); preparing this solution three times, separately. Linder sce: cooling (0–5 °C), stirring the reaction mixture for 3 hours for each souluion, then extraction of the mixture to remove all adding added evaporation. and then washed the dry products with distilled water.

Analytical and Instrumentation techniques

Thermal stability: Newly synthesized compounds (pyrazoline) was heat-diagnosed for the purpose of knowing their thermal stability values.

Thermographic analysis (TGA): The analysis of compounds was performed at the analytical laboratory of Basra University, the College of Science, and the Department of Chemistry, using an SDT Q600 V20.9 build 20 thermogravimeter under nitrogen flow.

Differential scanning calorimeter (DSC) study: The analysis of compounds was performed at the analytical laboratory of Basra University/College of Science/Department of Chemistry, using an SDT Q600 V20.9 build 20 thermogravimeter.

Analytical and spectral techniques:

Infrared spectrum: Infrared spectra of the synthesized compounds (pyrazoline) were recorded by an FT-IR 8400S Shimadzu Spectrophotometer (Japan) using a KBr disk in the range 4000-400 cm at the department of pharmaceutical chemistry/college of pharmacy, University of Basrah.

Ultraviolet spectra: The ultraviolet spectra of the synthesized compound (pyrazoline) were recorded by a Cecil 7200 spectrophotometer at the department of pharmaceutical chemistry, college of pharmacy, and University of Basrah.

Thin-layer chromatography: Thin-layer chromatography of the resultant compounds was carried out with the appropriate eluents (ethanol and hexane). UV Tran's illuminators and iodine vapour were used to detect the spots.

Study the effect of solvents on pyroazolines

Prepare 0.005g of Pyrazoline in 10 ml of each solvent (acetone, dichloromethane, chloroform, DMSO, ethanol, and methanol) at room temperature and show the effect of the solvent on the stability of complexes by UV spectroscopy.

Study the effect of pH by using a phosphate buffer: Prepare all compounds (0.005 gm of Pyrazoline with 0.005 gm of fusidic acid and 0.005 gm of ZnO, respectively) in ethanol at room temperature and show the effect of pH by UV spectroscopy.

Biological activity

In vitro study of pH effect

The in vitro interaction of pyrazoline was carried out in the same set of dissolution mediums with fusidic acid and ZnO. In this experiment, fusidic acid and ZnO, were added at zero time to the dissolution medium already maintained at 37 °C, while pyrazoline were added after 15 minutes, and the absorbance was measured every 15-minute interval for 3 hours at pH (1, 4, 7, 4, and 9). Aliquots were withdrawn and assayed for both drugs. The absorbance of each mutule versus time was calculated in each set of experiments.

Antibacterial study of pyrazoline derivative with fusidic acid

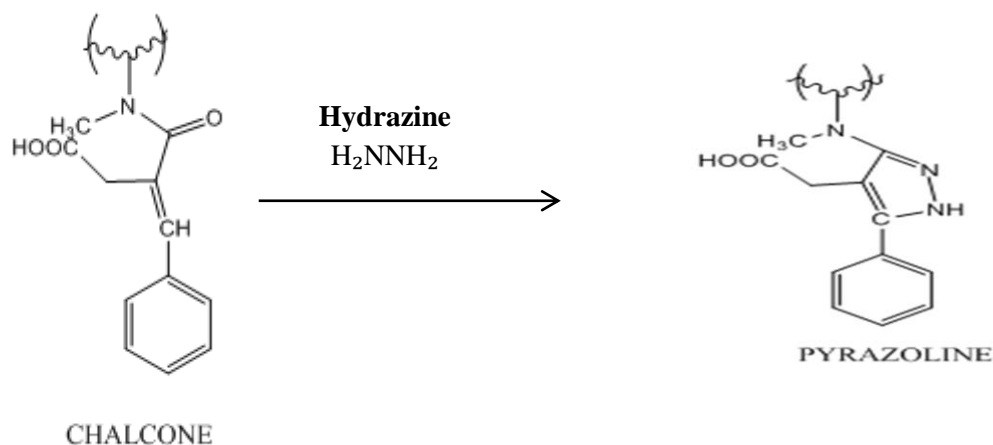
The antibacterial potential of the prepared Samples (Pyrazoline with fusidic acid) was investigated against Gram-negative and Gram-positive bacterial strains using an agar well diffusion assay.

antioxidant study of Pyrazoline with zinc oxide

The biological activity effect of polymer prodrugs: Pyrazoline with zinc oxide as antioxidant was studied.

Results and Discussion

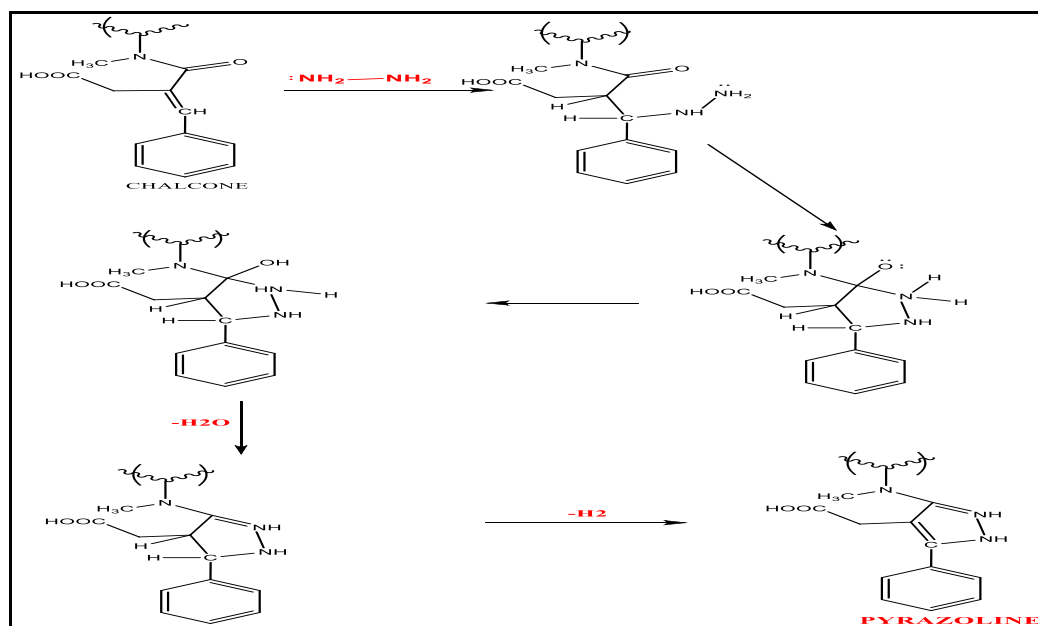
synthesis of Pyrazoline: Cyclization of alpha, beta unsaturated ketone with (Hydrazine H_2NNH_2), Scheme 2 shows the synthesis of a Pyrazoline.



Scheme 2: synthesis of pyrazoline

Mechanism of pyrazoline synthesis:

The Mechanism of pyrazoline shows in Scheme 3.



Scheme 3: Mechanism of pyrazoline derivative

Schmidt condensation, we can be show in this reaction. Reaction of chalcone with hydrazin in the presence of NaOH or KOH. The first reaction step involves attacking the electrons of nitrogen in hydrazine compound to the carbon atom of the double bond in the chlcone compound. The second step when intermediate compound formed and this intermediate is unstable. The electrons of second "N" attack the carbon of carbonyl group wich it is good nudeohilic center and alkoxide ion formed. The third step, the hydrogen will be transferred to the oxygen alkoxide to form hydroxy pyrazoline. The fourth step involves dehydration reaction and formed pyrazoline formula ^(14, 15).

Thermal stability:

Differential Scanning Calorimetry (DSC) is one of the thermal decomposition techniques used in studying what happens to the samples to be examined in terms of changes in their condition and thermal transformations resulting from the emission and absorption of heat as a result of the cooling and heating processes. This technique can be applied to many metals and ceramics. And polymers, organic and inorganic materials, pharmaceuticals, and foods by determining their stability and degree of purity . Differential scanning calorimetry (DSC) is a technique used to investigate the response of polymers to heating. DSC can be used to study the melting of a crystalline polymer or the glass transition. The DSC set-up is composed of a measurement chamber and a computer. Two pans are heated in the measurement chamber. The sample pan contains the material being investigated. A second pan, which is typically empty, is used as a reference. The computer is used to monitor the temperature and regulate the rate at which the temperature of the pans changes. A typical heating rate is around 10 °C/min ^(16, 17).

Differential scanning calorimeter (DSC) study:

A study of the decomposition of the prepared compounds was carried out using the differential calorimetric decomposition curve (DSC), which showed results shown in the table for the compounds showing the starting temperature (T_i) and the ending temperature (T_f). And its type—whether it emits or absorbs heat—and the shapes of the curves are shown in the table (2) . The compounds in the DSC were also given decomposition results shown in the table. Each stage has a type of state, whether it is absorbent or heat-emitting at the maximum temperature, which is consistent with the gravimetric decomposition.

Table (2): Thermal Decomposition Results (DSC)

| Compounds | $T_i/^\circ\text{C}$ | $T_f/^\circ\text{C}$ | Maximum temperature point $^\circ\text{C}$ | Type |
|------------|----------------------|----------------------|--|-------------|
| pyrazoline | 76.654 | 129.639 | 106.397 | endothermic |
| | 272.125 | 379.846 | 324.698 | exothermic |

Thermographic analysis (TGA):

Thermo gravimetric analysis (TGA) of polymers is conducted to measure weight changes as a function of temperature and time. The weight changes of polymeric materials can be caused by decomposition and oxidation reactions as well as physical processes such as sublimation, vaporization, and desorption⁽¹⁸⁾. Thermo gravimetric (Tg) is the study of the relationship between a sample's mass and its temperature. It can be used to study any physical (such as evaporation) or chemical process (such as thermal degradation) that causes a material to lose

volatile gases. Polymers have different thermal stabilities and thus the qualitative “fingerprint” afforded by TG in terms of temperature range, extent and kinetics of decomposition provides a rapid means to distinguish one polymer from another using only milligram quantities of material ⁽¹⁹⁾. Table 3 gives results showing compatibility with the proposed general formula of the ligand and some of its complexes. The table also shows information for each stage of gravimetric decomposition that the complex goes through, as follows:

T_i = temperature at which decomposition begins in one step

T_f = temperature at which decomposition ends in one step

T_{max} = maximum weight loss temperature

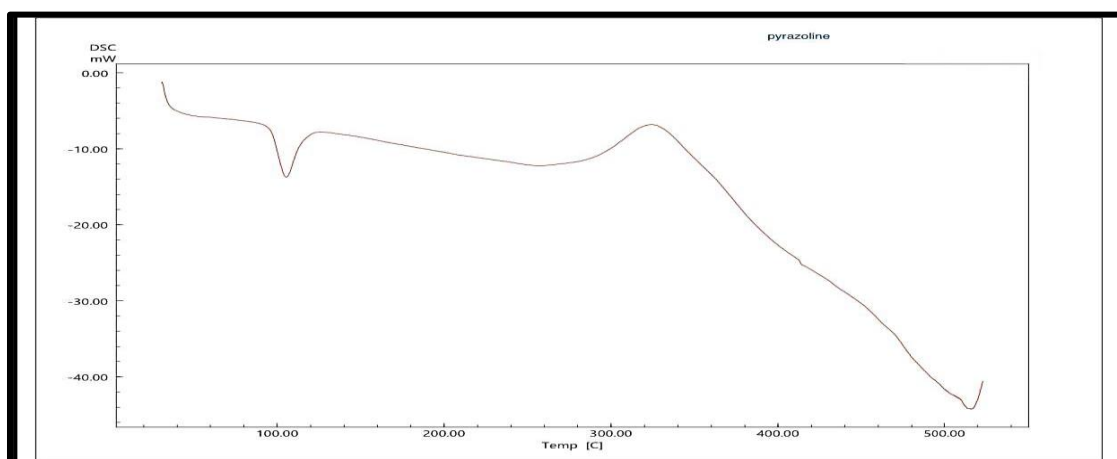
Table (3): Data of the gravimetric pyrolysis curve

| Complexes | Step | $T_i/^\circ\text{C}$ | $T_f/^\circ\text{C}$ | $T_{DTG_{max}}$ | Weight mass loss% found | | |
|---|------|----------------------|----------------------|-----------------|-------------------------|--|--|
| pyrazoline | 1 | 22.601 | 61.362 | 37.672 | 11.2691 | | |
| | 2 | 69.934 | 121.025 | 93.968 | 30.2610 | | |
| | 3 | 164.462 | 596.693 | 337.678 | 58.3741 | | |
| <p>Thermal stability of the material at a maximum temperature = 236.746 °C</p> | | | | | | | |

In light of the results of the thermo gravimetric decomposition of the prepared compounds, it gave thermal stability values of (37.672, 93.968, and, 337.678) for compounds pyrazoline, respectively, so the Thermal stability of the material at a maximum temperature = 236.746 °C. The first compound has undergone three stages of decomposition, during which water from crystallization is lost in the

first two stages and the material begins to completely decompose at a temperature of (11.2691, 30.2610, and 58.3741).

While the second compound has been given five stages of decomposition, it loses during the first three stages water of crystallization, and during the fourth and fifth stages, the total weight loses to 500.116 and the percentage of loss by practical weight is 100.8494. The third compound has been given three stages of decomposition. In the first two stages, through which water of crystallization is lost, and in the third stage, it begins with the loss of the total substance until it ends at 596.693, A percentage of loss by practical weight was given as 99.904.



Figure(2): DSC of pyrazoline

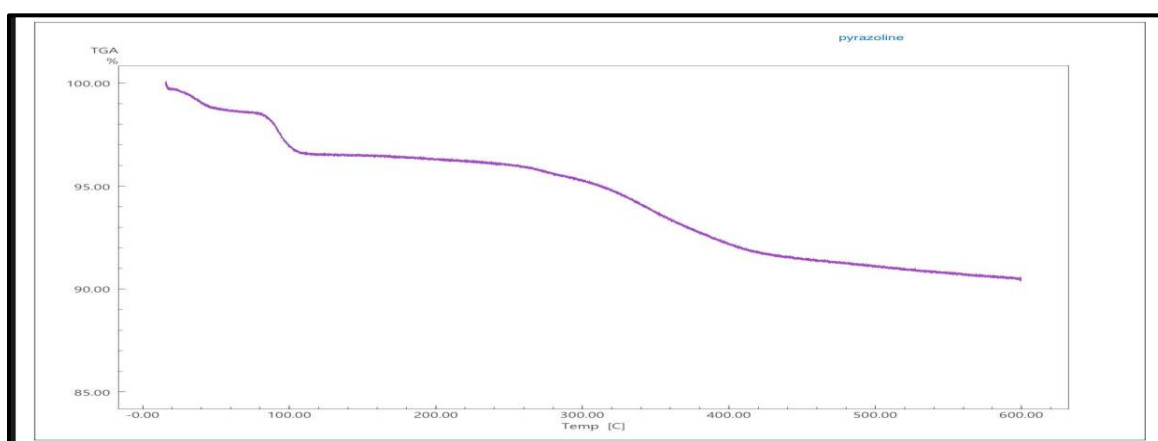


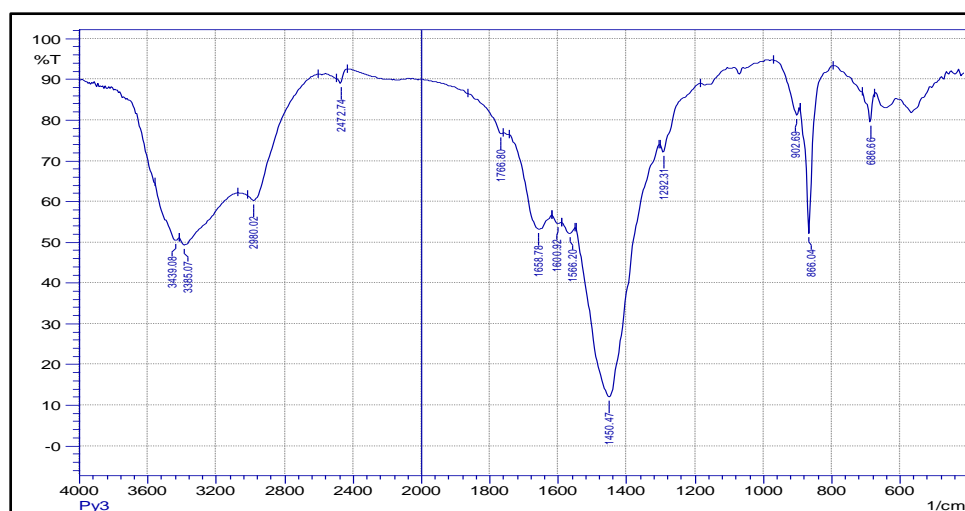
Figure (3): TGA of pyrazoline

Analytical and spectral techniques:**FT-IR Spectrum of Pyrozoline:**

Refer to N-H for a strong band display at 3385cm^{-1} . The middle band exhibits C=N at 1658cm^{-1} . The mid-band signal at 2980cm^{-1} is C-H aliphatic. Weak band signals at 1566 signify conjugated C-C=C. Pyrazoline showed a distinctive band in the infrared spectrum at $(3296-3346)\text{cm}^{-1}$ due to N-H stretching. It also showed a strong band at $(1594-1606)\text{cm}^{-1}$ for the vibration of C=N stretching⁽²⁰⁾.

Table (3): FT-IR spectra of synthetic Pyrozoline:

| Comp.name | C-N | C=N | N-H | O-H | C-H ali. | | |
|--------------------------|------|------|------|------|-------------|--|--|
| Pyrazoline derivative | 1292 | 1600 | 3439 | 3385 | 2980 | | |

**Figure (4):** FT-IR spectra of pyrazoline

biological activity:

3.5.1. antimicrobial effectiveness

Fusidic acid, one of the most significant antibacterial compounds, requires ongoing evaluation of its mechanism of action and rate of resistance to aid in the creation of novel treatment strategies⁽²¹⁾. Fusidic acid has a modest antibacterial effect on the majority of gram-positive and gram-negative bacteria at different dosages. (Figs. 5 and 7). Hence, the potential for synergistic effects of the antibiotics pyrazoline, against *Staphylococcus*, *Streptococcus*, and *Pseudomonas* has been assessed and shown meaningful efficacy at various doses⁽²²⁾. Also, promoting the proper use of this antibiotic in conjunction with other medications is crucial for promoting the prevention of future occurrences of identical spots by emphasizing patient and clinician education. as compared to the effectiveness of the several antibiotics that were evaluated. Different antibacterial activities were detected in the outcome.^(23, 24).

The concentration of all antibiotics (500 g/ml) had a greater influence than other concentrations on antibacterial activity as a control against the majority of gram-positive and gram-negative bacteria (Figs. 8 to 10). The antibiotic (pyrazoline plus fucidic acid) exhibits more antibacterial activity on *Streptococcus pyogenes* and *Staphylococcus aureus* than *Pseudomonas aeruginosa*.

The most important scaffold is pyrazoline, which is used in a variety of pharmaceutical processes. In the cell walls of gram-negative bacteria like *Pseudomonas*, LDs (Lipopolysacchaxeds) serve as the outer membrane. Low fluidity is provided by hydrocarbon chains in the minor area of LPs. Antibiotics and other organic compounds are extremely effectively blocked from passing through the outer membranes⁽²⁵⁾. Plasmids, which have the capacity to transmit antibiotic and other antibacterial chemical resistance, are also a part of the genetic composition of *Pseudomonas*⁽²⁶⁾. This pyrazoline might cure bacteria due

to its extensive action. Its use with other antibiotics may boost their antibacterial effectiveness and prevent topical antibiotic resistance. Intermediate-dose in vitro inhibition has unknown therapeutic consequences. Yet, these effects may help create combinations of drugs that improve therapeutic efficacy and reduce adverse effects⁽²⁷⁾.

Fucidic acid as control

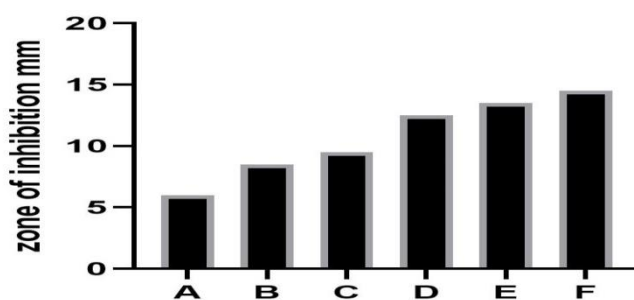
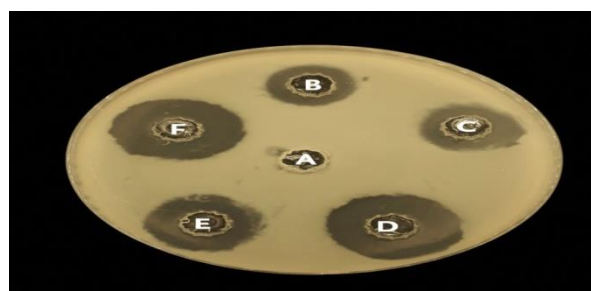


Figure (5): Antibacterial activity of (Fu) against *Streptococcus*. A, control. B, 50 microgram/ml. C, 100 microgram/ml. D, 200 microgram/ml. E, 400 microgram/ml. F,500 microgram/ml .



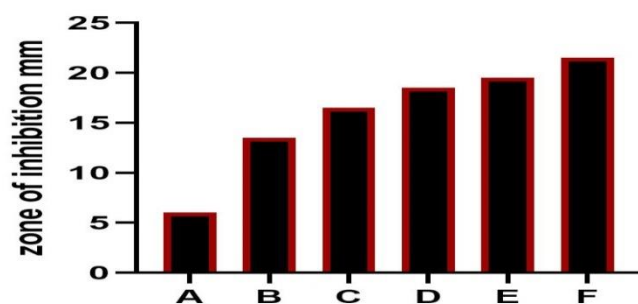


Figure (6): Antibacterial activity of (Fu) against *Staphylococcus*. A, control. B, 50 microgram/ml. C, 100 microgram/ml. D, 200 microgram/ml. E, 400 microgram/ml. F,500 microgram/ml .

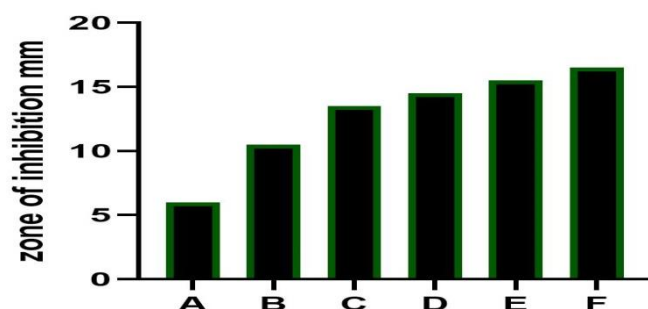


Figure (7): Antibacterial activity of (Fu) against *Pseudomonas*. A, control. B, 50 microgram/ml. C, 100 microgram/ml. D, 200 microgram/ml. E, 400 microgram/ml. F,500 microgram/ml .

Anti-bacterial activity of pyrazoline with fucidic acid

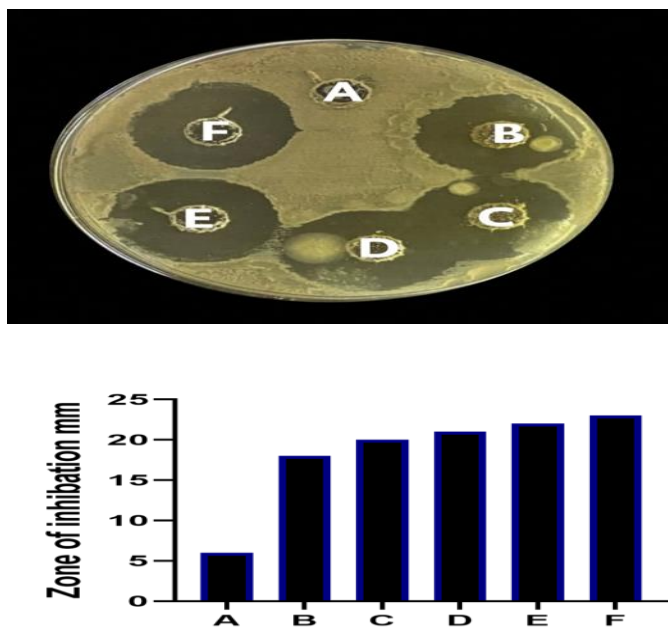


Figure (8): Antibacterial activity of (pyrazoline plus fucidic acid) against *S. aureus* A, control. B, 50 microgram/ml. C, 100 microgram/ml. D, 200 microgram/ml. E, 400 microgram/ml. F, 500 microgram/ml

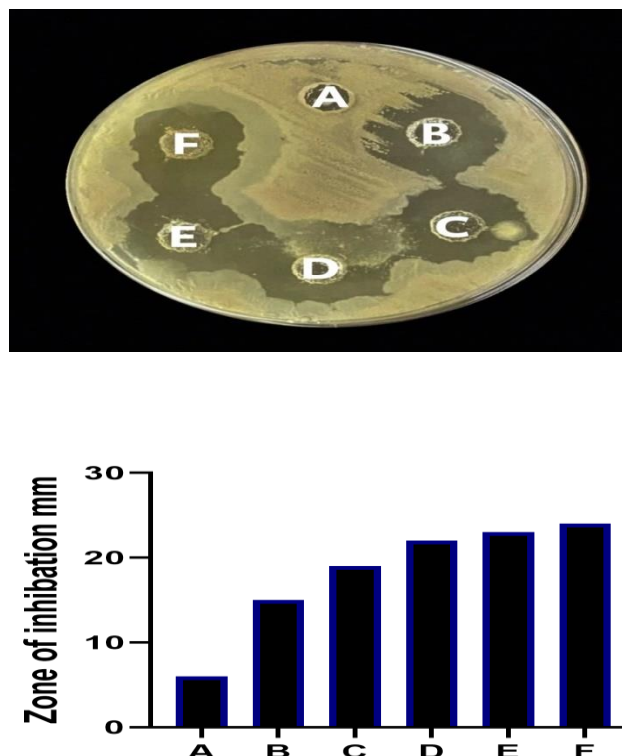


Figure (9): Antibacterial activity of (pyrazoline plus fucidic acid) against *Streptococcus pyogenes*. A, control. B, 50 microgram/ml. C, 100

microgram/ml. D, 200 microgram/ml. E, 400 microgram/ml. F, 500
microgram/ml

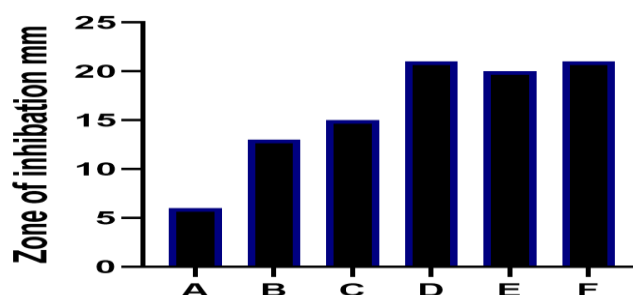


Figure (10): Antibacterial activity of (pyrazoline plus fucidic acid) against *P.aeruginosa*. A, control. B, 50 microgram/ml. C, 100 microgram/ml. D, 200 microgram/ml. E, 400 microgram/ml. F, 500 microgram/ml

antioxidant performance

Assay for 'DPPH Scavenging

In Figure (11), the scavenging capacity of the (pyrazoline) was assessed utilizing a steady DPPH (2,2-diphenyl-1-picrylhydrazyl) technique (Sigma– Aldrich, USA) (Sigma– Aldrich, USA). The volume was increased to 2 mL using 100% ethanol after 500 L of DPPH and 500 L of the produced compounds were combined. At 517 nm, the absorbance of each substance was measured ⁽²⁸⁾.

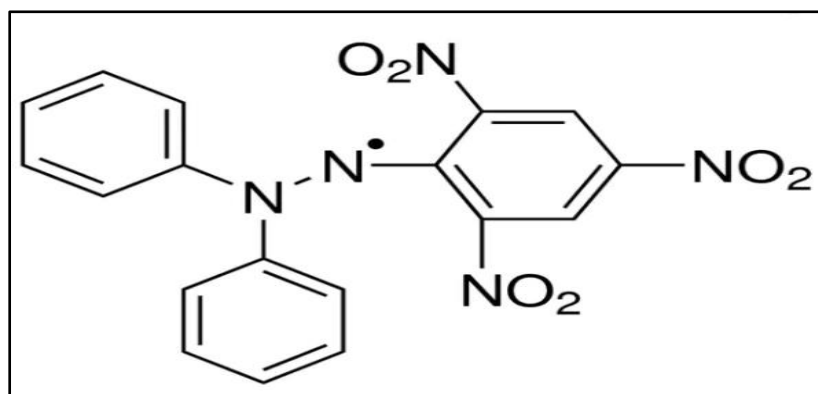


Figure (11): Chemical structure of DPPH

Statistical Analysis

The data was statistically analyzed using GraphPad Prism 6. which were displayed as the mean and standard deviation of three replicates for each experiment .

RESULTS

In Figure (12), the antioxidant activity of each Zn, and Zn-3 was investigated using DPPH assay. When an electron is spare, DPPH (2,2-diphenyl-1-picrylhydrazyl) has a dependable free radical associated with it. The outcomes are shown in Figure 12. The results demonstrated that the Zn, Zn-3 possess antioxidant action through their capacity to neutralize free radicals. The results is concentration dependent manner Zinc's capacity to slow down oxidative processes has been acknowledged and researched. Zn's antioxidation mechanism can generally be split into acute and long-term effects. Chronic effects are caused when a biological system is exposed to zinc over an extended period of time, which induces the production of another chemical that is the ultimate antioxidant, such as metallothioneins. Prolonged zinc deficiency typically increases vulnerability to several forms of oxidative stress Two mechanisms contribute to the acute effects: either protein sulfhydryls are protected, or z OH production from H_2O_2 is reduced due to the antagonistic action of redox-active transition⁽²⁹⁾. metals like iron and copper. One of three processes is hypothesized

to reduce sulfhydryl reactivity in order to protect protein sulfhydryl groups:zinc's direct interaction with the sulfhydryl, attaching to a different protein location adjacent to the sulfhydryl group causes steric hindrance, or a conformational change as a result of interaction to another protein location ^(30, 31).

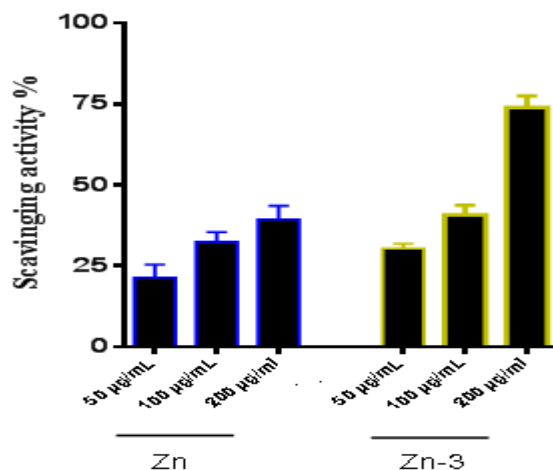


Figure (12): Activity against free radicals in Zn and its compounds. The results are shown as mean standard deviation.

Conclusion

Synthesis of polymer prodrug and testing its activity as an antibacterial against different strains of bacteria for topical preparation. Synthesis of polymer prodrug and testing its activity as an antioxidant for topical preparation. Some prepared polymer prodrugs exhibited medium to good antibacterial activity against *Pseudomonas aeruginosa*, *staphylococcus aureus*, and *streptococcus pyogenes* when compared with the parent agent. All prepared polymer prodrugs exhibited good antioxidant activity when compared with the parent agent.

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