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Article

Synthesis, Diagnosis And Evaluation Of The Antibacterial Activity Of New Oxazepane Derivatives

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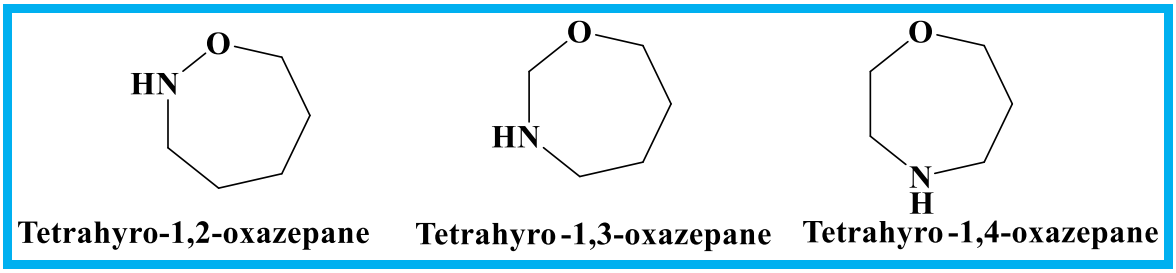
Abstract: The pharmaceutical molecule was prepared, tested, and compared to the antibiotic amoxicillin in terms of efficacy against two different strains of Gram-positive bacteria (Staphylococcus aureus and Gram-negative Escherichia coli). The investigated chemical was created by reacting succinic anhydride, which was obtained from the Schiff base of benzothiazole as a nucleus, with oxazole, which was obtained from a seven-membered ring by sublimation in the presence of dry benzene as a solvent. To confirm the correctness and validity of the results, spectroscopic techniques like proton nuclear magnetic resonance spectroscopy and infrared spectroscopy are employed in addition to physical measurements like product ratio and melting point.

Keywords: Heterocyclic, Oxazapane, Biological Activity.

Introduction

Heterocyclic Compounds are made up of various atoms arranged in ring shapes. like nitrogen, sulfur, or oxygen. These substances are extensively distributed in nature and significant in various sectors, including medicine. Because they include a heterogeneous atom, These substances are present in proteins, enzymes, nucleic acids, carbohydrates and their derivatives, and other biological materials. [1]. Heterocyclic compounds are categorized based on the kind and quantity of atoms in the ring and might have more than one hetero atom [2]. **Oxazapane** is a saturated compound with seven rings. It contains seven atoms consisting of five carbon atoms, one nitrogen atom, and one oxygen atom [3], of which 1,3-oxazapan-7,4-dione can be synthesized by adding anhydrides such as phthalic acid or maleic acid and others. A double bond of Schiff base or isomethylene (C=N) of hydrazine[4]. The two nitrogen atoms and the oxygen atom in the ring are numbered differently in Oxazepane compounds; the nitrogen atom is positioned in position (2, 3, or 4) while the oxygen atom is positioned in position (1)., as shown in the following figure [5]:



Oxazepane compounds have wide biological importance and have received wide attention in the medical field as they have shown antiviral [6], anticonvulsant [7], and antioxidant [8] activities, and exhibit good antifungal and antibacterial activity [9].

Materials and Methods

2.1.Chemicals used: Chemicals prepared from Aldrich, BDH Thomas, Fluka, and Merck, were used.
2.2. Devices used: Melting points were measured with a thermoelectric melter 9300. KBr disk at 400-4000 cm-1 scale, Shimadzu FT-IR 8400S spectrophotometer; Bruker equipment running at 400 MHz for 1H-NMR spectra. Fluka silica gel plates, with a thickness of 0.2 mm, were used in thin-layer chromatography (TLC).

2.3. Preparation of Oxazepane derivatives (F_6-F_{10}).[10]

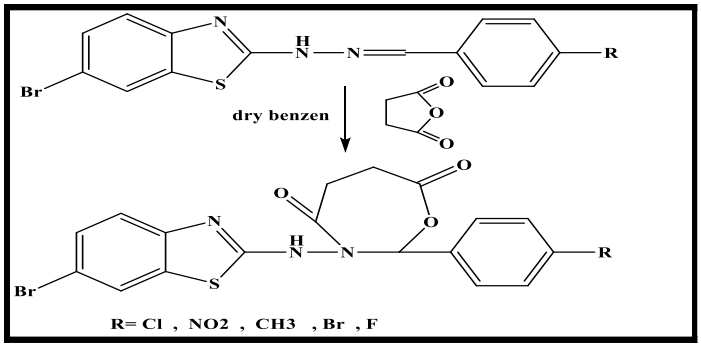
Dissolve equal moles (0.001 mol) of the prepared Schiff base and succinic anhydride in (30 ml) of dry benzene, and heat the mixture for (10-14) hours. Cool, filter the precipitate, and recrystallize. Use the solvent 1,4-dioxane. As shown in Table (1)

.2.4. Biological activity study

Mueller Hinton agar (39 g) was dissolved in 1 liter of distilled water, heated, and stirred with a magnetic stirrer to prepare the medium. It was then sterilized for a duration at 121 °C and 1.5 bar of pressure, cooled to 50 °C, and then poured into a Petri dish and frozen at room temperature [11–15]. The two bacterial isolates that were analyzed and collected by the Advanced Microbiology Research Laboratory, Department of Life Sciences, College of Science, Tikrit University, were Gram-positive [Gr-ve]. [+ve], or Staphylococcus aureus, and Gram-negative [Gr-ve], or E. coli. Using heat-sterilized racks, two colonies were moved from the solid culture medium into test tubes holding five millilitres of distilled water. The tubes were then incubated at 37 °C for sixteen to twenty hours [16–20]. To get an estimated cell count of (1.5 × 108) cells/ml, dilute with saline until the turbidity meets the standard limits. Several of the produced compounds were made into chemical solutions using dimethyl sulfoxide (DMSO) solvent. The concentrations of the solid derivatives (0.1 g) were (0.01, 0.001, 0.0001) mg/ml) of (DMSO) to get a concentration of 0.01 mg/ml. Each compound was diluted in (10). Following that, 9 ml of (DMSO) solvent was added at the same concentration (0.01 mg/ml) to 1 ml of the solution that had been removed. To achieve a concentration of 0.0001 mg/ml, the process entailed first generating a solution with a concentration of 0.001 mg/ml, then extracting 1 ml of DMSO solvent from that solution. [21–26].

Results and Discussion

The diagram shows the series of prepared compounds.



The FT-IR spectrum of compounds (F6-F10) showed two bands at (1704-1688) cm-1, (1659-1643) cm-1 attributed to (C=O) lactone and lactam respectively, two bands at (2972-2925) cm-1, (2927-2881) cm-1 attributed to aliphatic (CH), two bands at (1571-1523) cm-1, (1537-1489) cm-1 attributed to aromatic (C=C), a band at (1296-1287) cm-1 attributed to (C-O), and a band at (1225-1218) cm-1 attributed to (C-N)[27]. as shown in Table 2 and Figure 1.2

The 1H-NMR spectrum of compound F7 showed two triplet signals at (2.85-3.25) ppm for (CH2-CH2) oxazepane, a signal at (7.25) ppm for (CH) oxazepane, signals at (6.88-7.96) ppm for aromatic rings, and a signal at (8.70) ppm for (NH)[28]. as shown in Figure3

The 1H-NMR spectrum of compound F8 showed a signal at (2.31) ppm for (CH3), two triple signals at (2.74-3.15) ppm for (CH2-CH2) oxazepane, a signal at (7.39) ppm for (CH) oxazepane, signals at (7.08-7.99) ppm for the aromatic rings, and a signal at (8.83) ppm for (NH). as shown in Figure 4

The 1H-NMR spectrum of compound F10 showed two triplet signals at (2.62-4.02) ppm for (CH2-CH2) oxazepane, a signal at (7.21) ppm for (CH) oxazepane, signals at (6.99-8.08) ppm for aromatic rings, and a signal at (9.15) ppm for (NH). as shown in Figure 5

Using a sterile cotton swab, Mueller Hinton agar (MHA) medium is inoculated into test tubes containing diluted bacterial growth. Excess inoculum is then removed by pushing the swab against the test tube's inner wall and wiping it [29–34]. Give the plate ten to fifteen minutes to enable the medium to dry and the culture to soak before redistributing the inoculum uniformly. The agar diffusion technique was used to assess the synthesized compounds' antibacterial activity. Using the cylindrical measurement method (per USP 35), holes are created in the Petri dish following the inoculation of the culture medium with the bacterial isolates [35–40]. In each well, prepare three concentrations of the chemical (40 µl), and then incubate the plate for twenty-four, twenty-four, and forty-eight hours at 37°C [41–47]. The data are interpreted after a few hours to demonstrate the sensitivity of the derivative employed, which is based on the inhibitory diameter found in the Petri dish around the well utilized. A higher inhibitory diameter corresponds to a higher bioavailability of the drug that was created. The World Health Organization's tests, laboratories, and statistics [48–52] all provide the inhibitory diameter of common antibiotics (such as amoxicillin) used in solution form. As shown in Table 3 and Figure 6.7

Table (1): Some physical properties of for Prepared compounds (F6-F10).

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
F6	4-Cl	C18H15BrClN3O3S	211-209	67	Red
F7	4-NO2	C18H15BrN4O5S	235-237	65	Brown
F8	4-CH3	C19H18BrN3O3S	256-254	72	Light Yellow
F9	4-Br	C18H15Br2N3O3S	243-241	70	Orange
F10	4-F	C18H15BrFN3O3S	217-216	79	Blue

Table (2): FT-IR absorption results for Prepared compounds (F6-F10)

Comp. No.	R	ν(C-H) Arom.	ν(C-H) Aliph.	ν(C=O) Lactone Lactam	ν(C-O) ν(C-N)	ν(C=C) Arom.	Others
F6	4-Cl	3062	2972 2927	1691 1647	1296 1222	1571,1537	ν(C-Cl) 754
F7	4-NO2	3041	2925 2881	1693 1657	1290 1220	1561,1489	ν(NO_2) *asy*. (1520) *sym*. (1361)
F8	4-CH3	3060	2950 2906	1697 1659	1287 1225	1552,1497	--
F9	4-Br	3029	2943 2867	1704 1643	1296 1218	1558,1519	ν (C-Br) 590
F10	4-F	3036	2942 2903	1688 1655	1293 1223	1523,1494	ν (C-F) 937

Table (3): Biological efficacy of produced substances and control methods (measured in millimeters of inhibition).

Comp. No.	E. Coil Conc. mg/ml			Staph. Aureus Conc. mg/ml		
	0.01	0.001	0.0001	0.01	0.001	0.0001
F6	18	15	3	21	16	10
F7	17	13	10	23	20	15
F8	15	15	5	18	15	10
F9	15	10	8	15	10	5
F10	18	10	5	30	15	8
Amoxicillin	23	20	18	25	23	20

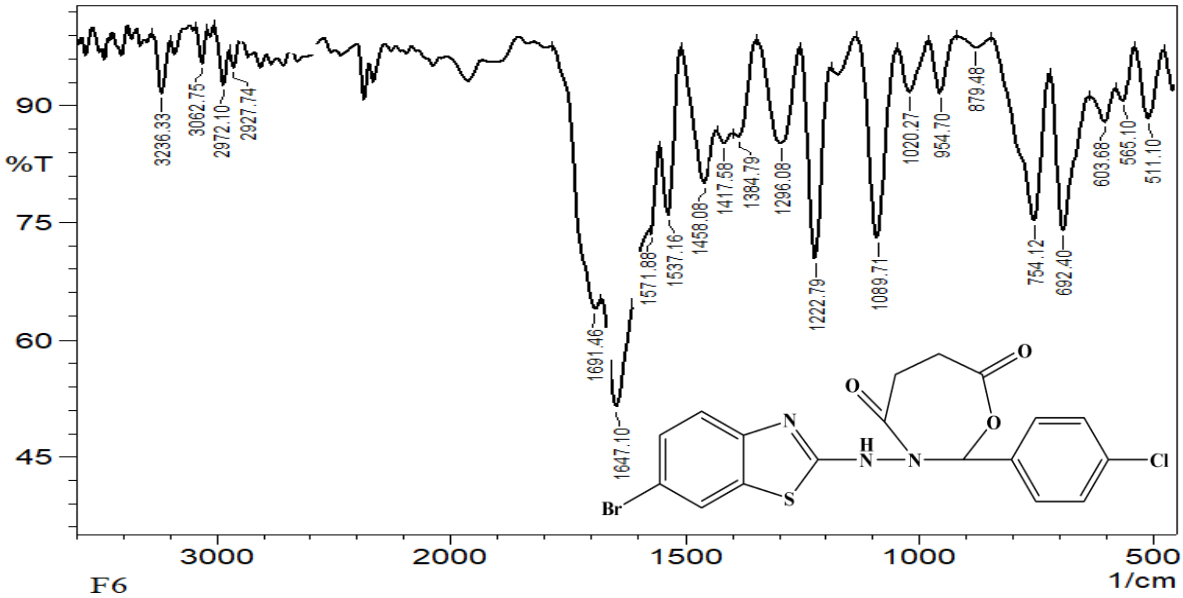


Figure (1): The compound's FT-IR spectra (F6).

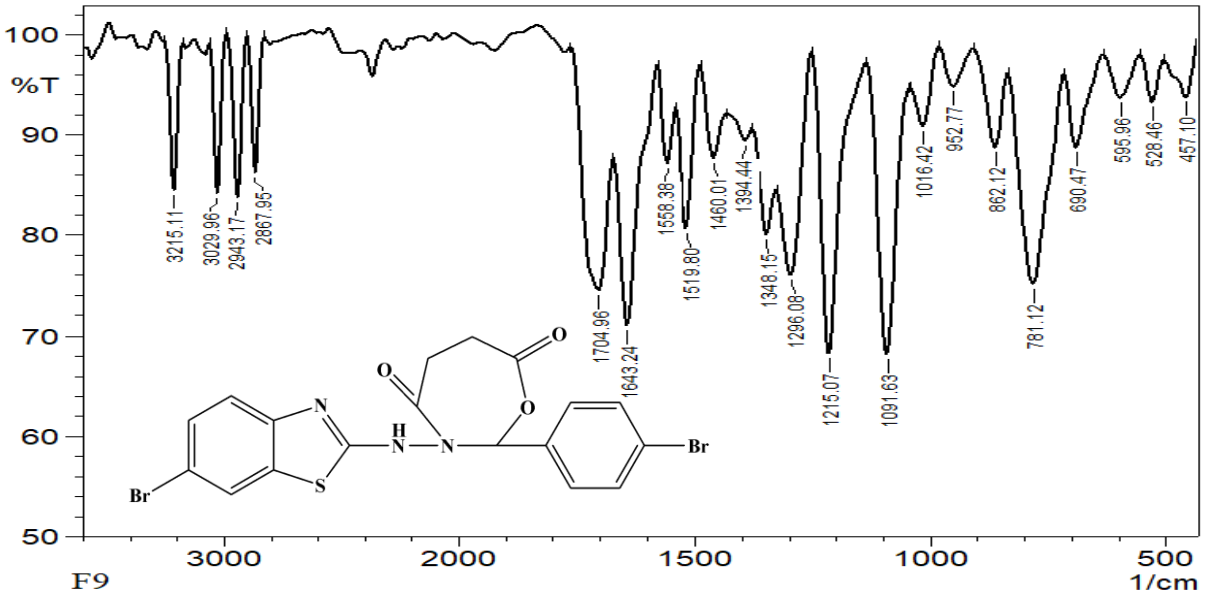


Figure (2): The compound's FT-IR spectra (F9).

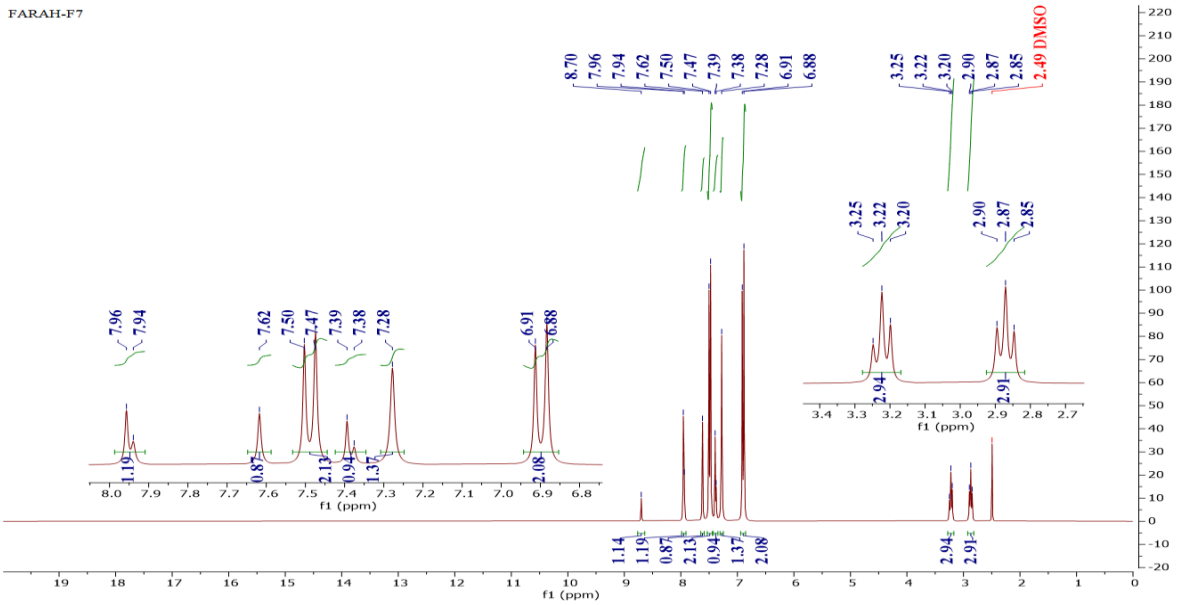


Figure (3): 1-H NMR spectra of the substance (F7).

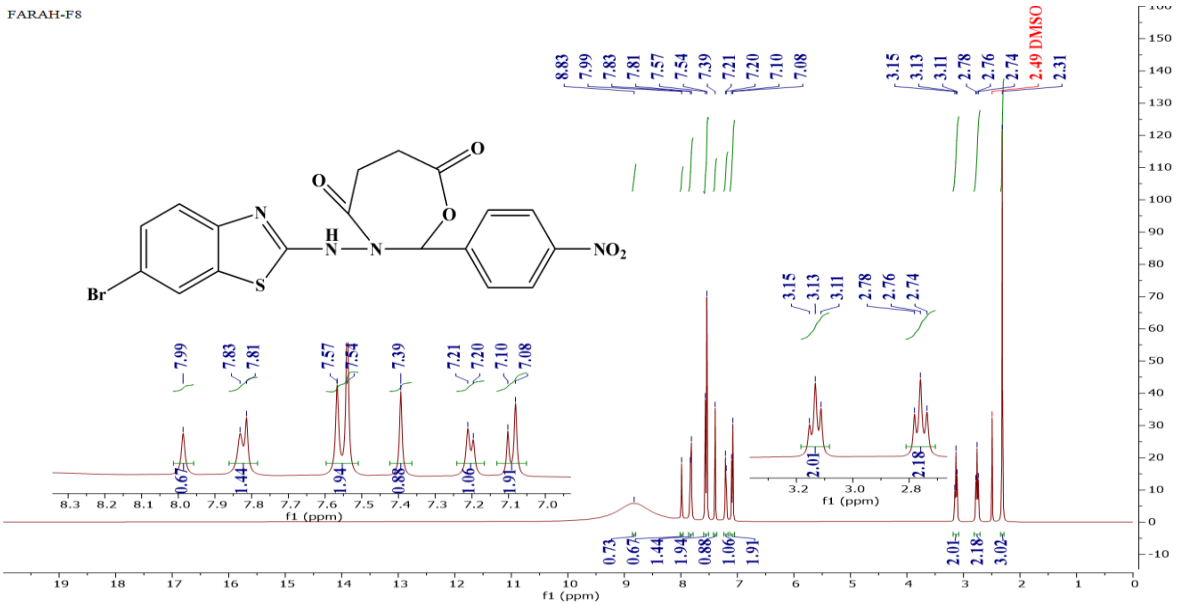


Figure (4): 1-H NMR spectra of the substance (F8).

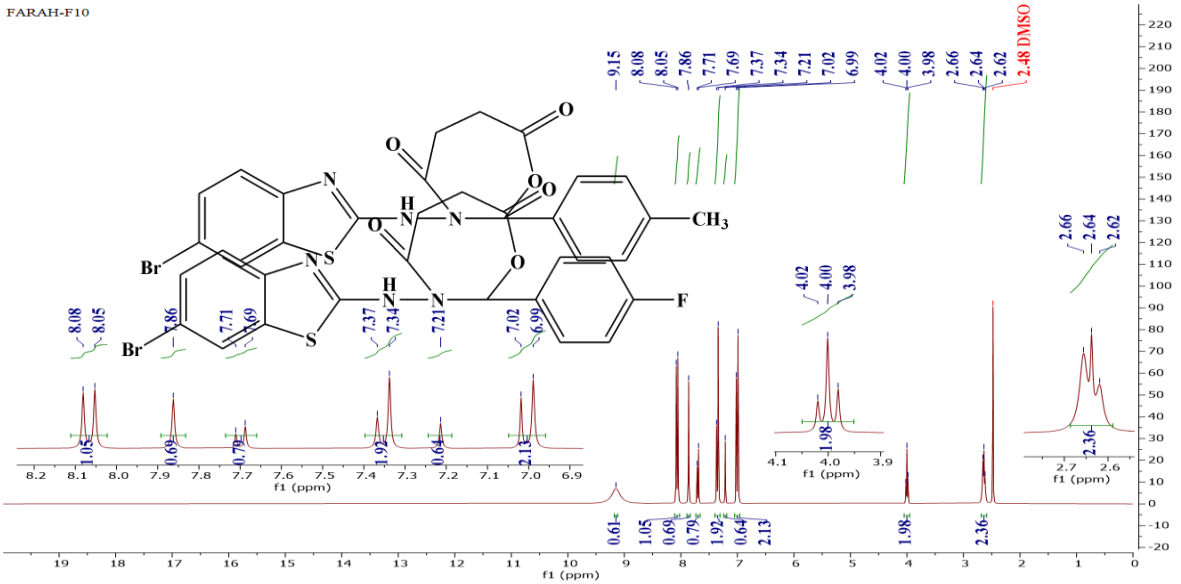


Figure (5): 1-H NMR spectra of the substance (F10).

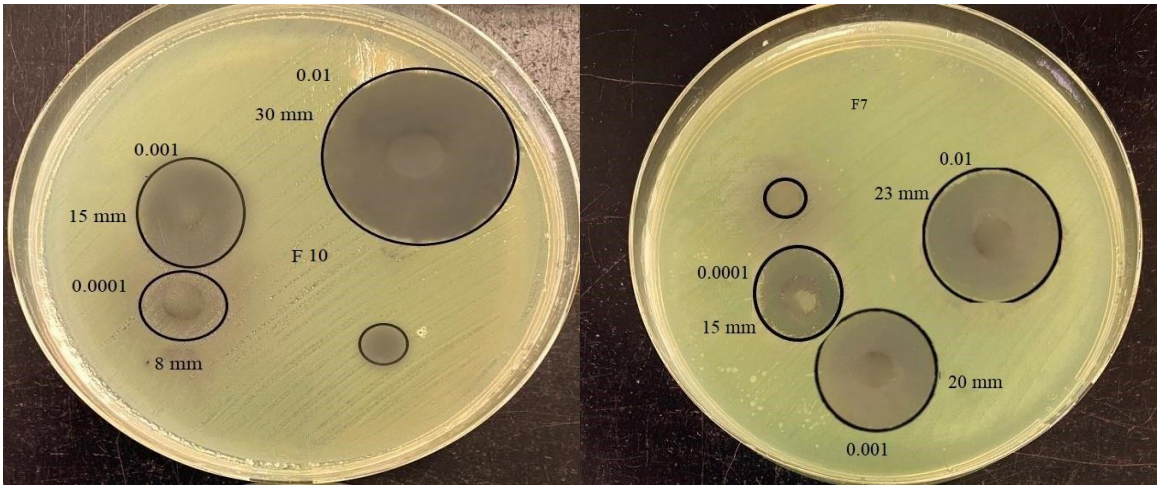


Figure6: Biological effectiveness of the compound F7,F10against becteral Staph. aurous

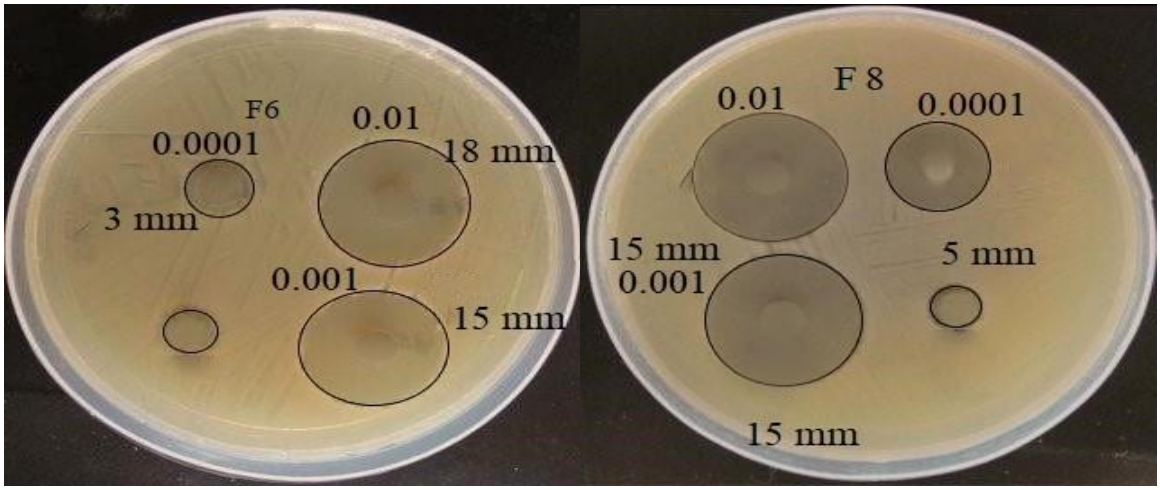


Figure 7: Biological effectiveness of the compound F6,F8 against becteral E.Coli

Conclusion

The reaction of (C=N) with succinic anhydride always gives a seven-membered ring called oxazepane. The prepared compounds showed high purity when measured by FT-IR and H-NMR spectra and gave a good product percentage. The prepared compounds can also be used as pharmaceutical compounds due to the high inhibition they showed against the two types of bacteria used in the study compared to the antibiotic. Compound F10 showed an inhibition percentage of up to)30 mm(against Staphylococcus aureus bacteria, and compound F6 showed the highest inhibition against E.Coli bacteria at a rate of)18 mm(. The inhibition increased with increasing concentration.

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