Synthesis, Identification and Assess the Biological and Laser Efficacy of New Compounds of Azetidine Derived from Benzidine

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Abstract: In this study, new azetidine [A_1-A_5] compounds were prepared by the reaction five of schiff bases compounds with chloroacetyl chloride in 1,4-dioxane. The prepared compounds were characterized by physical properties, UV-Vis, ^{1}H-NMR and FT-IR spectral and C.H.N analysis. TLC checked the purity for these compounds. The antibacterial activities were studied against different kinds of bacteria, namely ***Staphylococcus epidermidis*** and ***Staphylococcus aureus*** Gram (+) ve, ***Eschershia coli***, and ***Klebsiella Pneumonia*** Gram (-) ve. Also, evaluation of laser efficacy is shown for the compounds (A_1-A_5) were laser radiated to (10, 20, 30) seconds. When the melting point and color were measured, it was found that the compounds were not affected or polymerized.

***Keywords*:** Benzidine, Azetidine, Biological activity, Laser Effectiveness.

1. Introduction

Azetidines are present in various natural products (amino acids) [1], and studies have shown that integrating azetidines into pharmaceutically applicable scaffolds can lead to improved pharmacokinetic properties and metabolic stability [2,3]. Methods developed for azetidine synthesis depend on nucleophilic replacement reactions with nitrogen nucleophiles, reduction of β-lactams [4], or ring opening of highly strained azabicyclobutanes [5]. Considering that most of these approaches require functionalized starting materials and involve multistep sequences, aza Paternò–Büchi reactions represent a highly efficient and direct strategy for the synthesis of azetidines [6]. The azetidine group is one of the most important pharmaceutical substructures and is present in a variety of approved drugs such as antibiotics [7] kinases (e.g. baricitinib, cobimetinib) [8], and other groups of compounds (e.g. thrombin inhibitor, azelnidipine) [9]. In addition, the azetidine group reduces the toxicity of the raw materials prepared from it [10] and lead to compounds with an enhanced pharmacokinetic [11]. The enhanced interest in azetidines needs new synthetic methods with varying substitution and stereochemistry to reach desired scaffolds [12]. Substituted azetidines strongly attract chemists because of their importance in catalysis, stereoselective synthesis and medicinal chemistry [13]. Other

techniques achieve the creation of rings and stereocenters simultaneously by uniting two fragments; however, this also leads to difficulties in determining the proper regio- and stereochemistry [14]. The reactions (2+2) were well-explored [15] while the related reactions (3+1) are less common [16].

2. Experimental

2.1. Materials and devices: All chemicals (Chloroacetyl chloride, triethylamine, 1,4-dioxane and dimethyl sulfoxide) had been used were supplied by company Alfa Aesar. The melting points were determined by Electro thermal Melting Apparatus 9300 in open capillary tubes that were uncorrected. Thin-layer chromatography (TLC) was used to track and verify the purity of the reaction. The Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded using FT-IR 8400S Shimadzu Scale (4000-400) cm-1 spectrophotometer. In addition, the UV-Vis. Spectra in ethanol were measured using Shimadzu 800UV in range (200-400) nm. Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectra was recorded on Varian operating at 400 MH_2 instrument using dimethyl sulfoxide (DMSO-d^6) as a solvent. Quantitative analysis of the spectrophotometer elements determined using elements analysis (C.H.N). The prepared compounds were irradiated with helium-neon laser beam (visible laser) of 1 milliwatt and wavelength 600-700 nm, 2010 model.

2.2. Synthesis methods

2.2.1. Synthesis of azetidine [A_1 –A_5]

Mixture of Schiff bases [17] (0.001 mol) with Et_3N (0.004 mol, 0.55 ml) in (10 ml) 1,4-dioxane then chloroacetyl chloride (0.004 mol, 0.3 ml) was added dropwise for a period slowly of 30 min. The reaction mixture was refluxed for (9-12) hrs. So when the reaction was terminated- (TLC identified), the mixture was placed into ice-cold water for solid residue output. Filtering, drying, and recrystallizing of benzene and ether to the effects (50-50) [18]. Physical properties are given in table (1).

2.3. Antibacterial Activity

The measurement of antibacterial activity of compounds [A_1-A_5] against gram positive and negative such as ***Eschershia coli, Klebsiella Pneumonia*** **are Gram (-) ve and** ***Staphylococcus epidermidis, Staphylococcus aureus*** **are Gram (+) ve** by using the disk diffusion method. They sprayed the disks with a DMSO. Then, incubate them overnight at 37 ºC. The negative omission was DMSO. The maximal inhibition zone against the development form of test microorganism was observed and calculated for analysis. ***Ampicillin***, ***amoxicillin***, and ***Ciprofloxacin*** were used as control samples at three concentrations [19, 20].

3. Results and Discussion

In this research, five compounds were prepared including azetidine [A_1-A_5] as in the scheme (1) and Identification by UV-Vis, ^{1}H-NMR, FT-IR Spectra and C.H.N analysis.

3.1. Characterization of azetidine [A_1-A_5]

The azetidine [A_1-A_4] were prepared by the reaction (1 mole) schiff base with (2 mole) of chloroacetyl chloride in 1,4-dioxane.

The UV spectra showed the transitions n→π* and π→π* indicated the existence of unbonded pair electrons on nitrogen, oxygen atoms, and aromatic (double bond) framework.

The FT-IR spectrum showed azetidine disappearance of band azomethine (C=N) group (1622-1672) cm^{-1}, beside new bands appear at (2906-2988) cm^{-1} attributed to the (CH) aliphatic as well as the emergence of band at (1650-1666) cm^{-1} due to (C=O) for azetidine. Besides other bands at (3369-33463) cm^{-1} for (OH) and at (3033-3082) cm^{-1} for aromatic (C-H), in addition, at (1573-1598) cm^{-1} and at (1469-1498) cm^{-1} due to (C=C) aromatic ring, and at (1421-1444) cm^{-1} for azo (N=N), and at (840-880) cm^{-1} for (C-Cl), as shown in fig. (1,2,3). U.V and FT-IR spectrum are given in table (2) [21].

In addition, the ^{1}H-NMR spectrum of [A_1] fig. (4) displays simple single signal at δ= 9.40 ppm attributed to (OH) (A) and a multiple signal in the range δ= (9.05-7.10) ppm for the aromatic protons (B,C,D,E,F), and doublet signal at δ= (6.47 & 6.45) ppm attributed to (CH-Cl) (G), and doublet signal at δ= (6.09 & 6.07) ppm attributed to (CH-N) (H) in addition to singlet signal at δ= 2.50 ppm attributed to DMSO-d^6 (I).

In addition, the ^{1}H-NMR spectrum of [A_3] fig. (5) displays simple singlet signal at δ= 10.15 ppm attributed to (OH) (A) and doublet signal at δ= (9.23 & 9.24) ppm attributed to (N=CH) (B) in the ring and a multiple signal in the range δ= (8.51-7.85) ppm for the aromatic protons (C,D,E,) and doublet signal at δ= (7.50 & 7.48) ppm attributed to (CH) (F) and doublet signal at δ= (6.19 & 6.18) ppm attributed to (CH-Cl) (G), and doublet signal at δ= (5.99 & 5.95) ppm attributed to (CH-N) (H) as well as singlet signal at δ= 2.50 ppm attributed to DMSO-d^6 (I). [22-24].

3.2. Antibacterial activity

The effect of the prepared compounds **[A_1-A_5]** on the growth of bacteria, namely ***Eschershia coli, Klebsiella Pneumonia*** **Gram (-ve),** ***Staphylococcus epidermidis*** and ***Staphylococcus aureus*** **Gram (+ve)** was determined. Antibacterial activity of the prepared compounds were studied and the results showed that some of the prepared compounds possess good antibacterial activity. The results of inhibition zone (IZD) in millimeters are shown in table (3) [25], see fig. (6,7) and see scheme (2–5).

3.3. Influence of lasers on prepared compounds [A_1-A_5]

A laser system with a power of (5) milliwatt that gives laser rays in continuous waves in the visible region of the spectrum at a wavelength (600-700) nm. Laser radiation of compounds [A_1-A_5] lasered for (10, 20, 30) seconds. It has been found the compounds have not been affected. When the melting point and color are determined, they did not disintegrate or polymerize. This denotes that the laser beams used did not affect the compounds. Since they are stable, as shown in the table (4) [26].

Table (1): Physical properties and elemental analysis of prepared compounds [A_1-A_5]

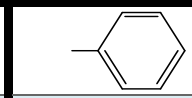
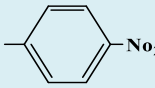
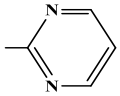
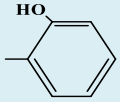
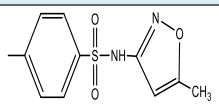
Comp. No.	Ar	Molecular Formula M. Wt.	Color	M.P (C^0)	T. hr.	Y. %	R.f. MeOH	found / (calc.) %		
								C%	H%	N%
A_1		$C_{42}H_{30}Cl_2N_6O_4$ 753.64	Brown	199-200	11	73	0.90	66.85 (66.94)	4.07 (4.01)	11.07 (11.15)
A_2		$C_{42}H_{28}Cl_2N_8O_8$ 843.63	Black	164-166	12	81	0.72	59.71 (59.80)	3.36 (3.35)	13.24 (13.28)
A_3		$C_{38}H_{26}Cl_2N_{10}O_4$ 757.59	Brown	257-258	9	72	0.88	60.20 (60.25)	3.51 (3.46)	18.42 (18.49)
A_4		$C_{42}H_{30}Cl_2N_6O_6$ 785.64	Dark brown	237-239	9	43	0.68	64.15 (64.21)	3.89 (3.85)	10.66 (10.70)
A_5		$C_{50}H_{38}Cl_2N_{10}O_{10}S_2$ 1073.93	Gray	210-212	12	57	0.73	55.90 (55.92)	3.61 (3.57)	12.98 (13.04)

Table (2): FT-IR and UV/Vis data of prepared compounds [A_1-A_5]

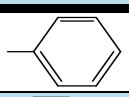
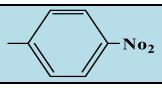
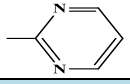
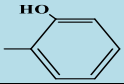
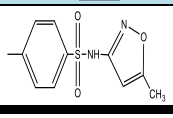
Comp. No.	Ar	$\lambda_{1\ max}$ nm $\lambda_{2\ max}$ nm	IR (KBr) cm^{-1}						
			ν O-H	ν C-H Arom.	ν C-H Aliph.	ν C=O	ν C=C	ν N=N	ν C-Cl
A_1		227 394	3463	3072	2941	1652	1579 1483	1421	840
A_2		246 395	3369	3033	2925	1656	1598 1496	1444	858
A_3		257 336	3401	3064	2906	1650	1587 1875	1439	871
A_4		263 397	3386	3058	2920	1666	1573 1465	1429	854
A_5		206 383	3426	3082	2988	1659	1591 1498	1438	880

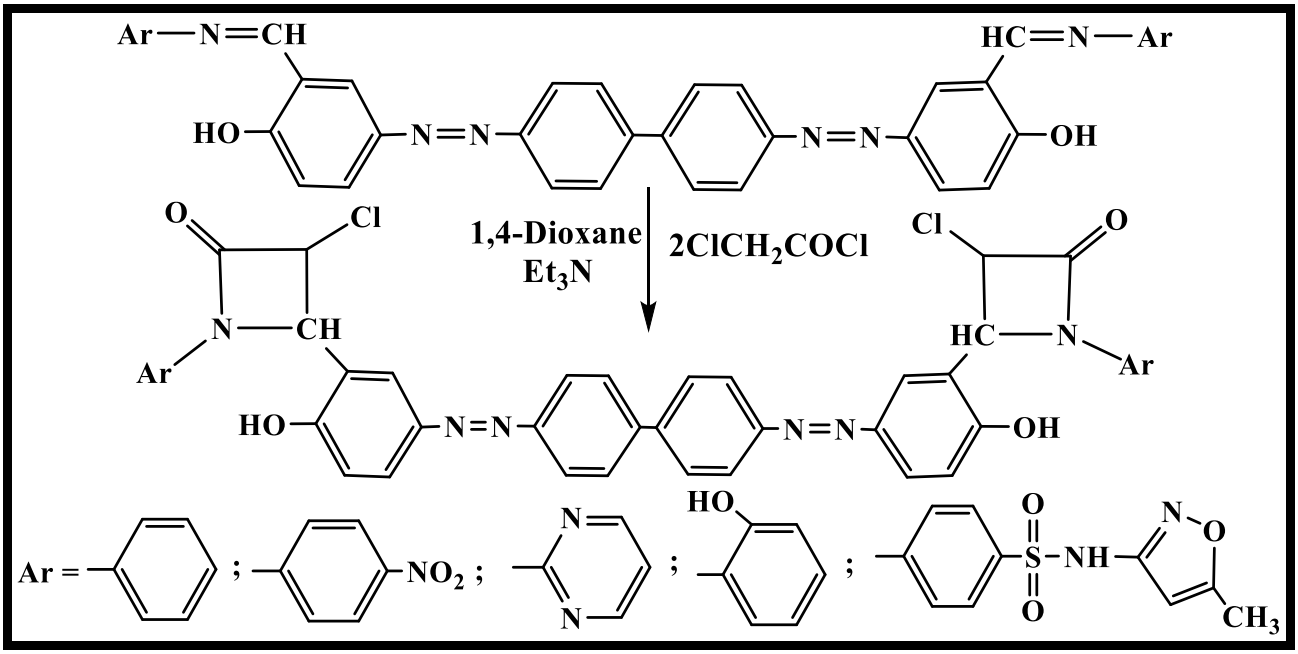
Table (3): Antibacterial activity of the prepared compounds [A_1-A_5] and control antibiotic

Comp. No.	*E. Coil* Conc. mg/ml	*K. Pneumonia* Conc. mg/ml	*S. Aureus* Conc. mg/ml	*S. Epidermidis* Conc. mg/ml

	25	50	100	25	50	100	25	50	100	25	50	100
A_1	2	3	4	1	3	5	2	4	5	0	2	3
A_2	2	3	5	2	2	4	0	1	2	1	3	4
A_3	1	3	4	2	4	4	1	2	3	1	2	3
A_4	0	2	3	2	3	5	0	1	2	1	3	4
A_5	3	5	5	2	3	4	2	4	5	2	3	4
Amoxicillin	2	3	4	2	4	4	2	3	4	1	2	3
Ampicillin	2	4	4	2	3	3	2	3	4	2	2	3
Ciprofloxacin	2	3	3	2	3	4	1	2	3	1	3	4
Blank disk	0	0	0	0	0	0	0	0	0	0	0	0

Table (4): The results of the irradiation of the compounds by laser beams

Comp. No.	10 S		20 S		30 S	
	M.P. ^{0}C	Color	M.P. ^{0}C	Color	M.P. ^{0}C	Color
A_1	199-200	Brown	199-200	Brown	199-200	Brown
A_2	164-166	Black	164-166	Black	164-166	Black
A_3	257- 258	Brown	257- 258	Brown	257- 258	Brown
A_4	237-239	Dark brown	237-239	Dark brown	237-239	Dark brown
A_5	210-212	Gray	210-212	Gray	210-212	Gray



Scheme (1): Route of prepared compounds [A_1-A_5]

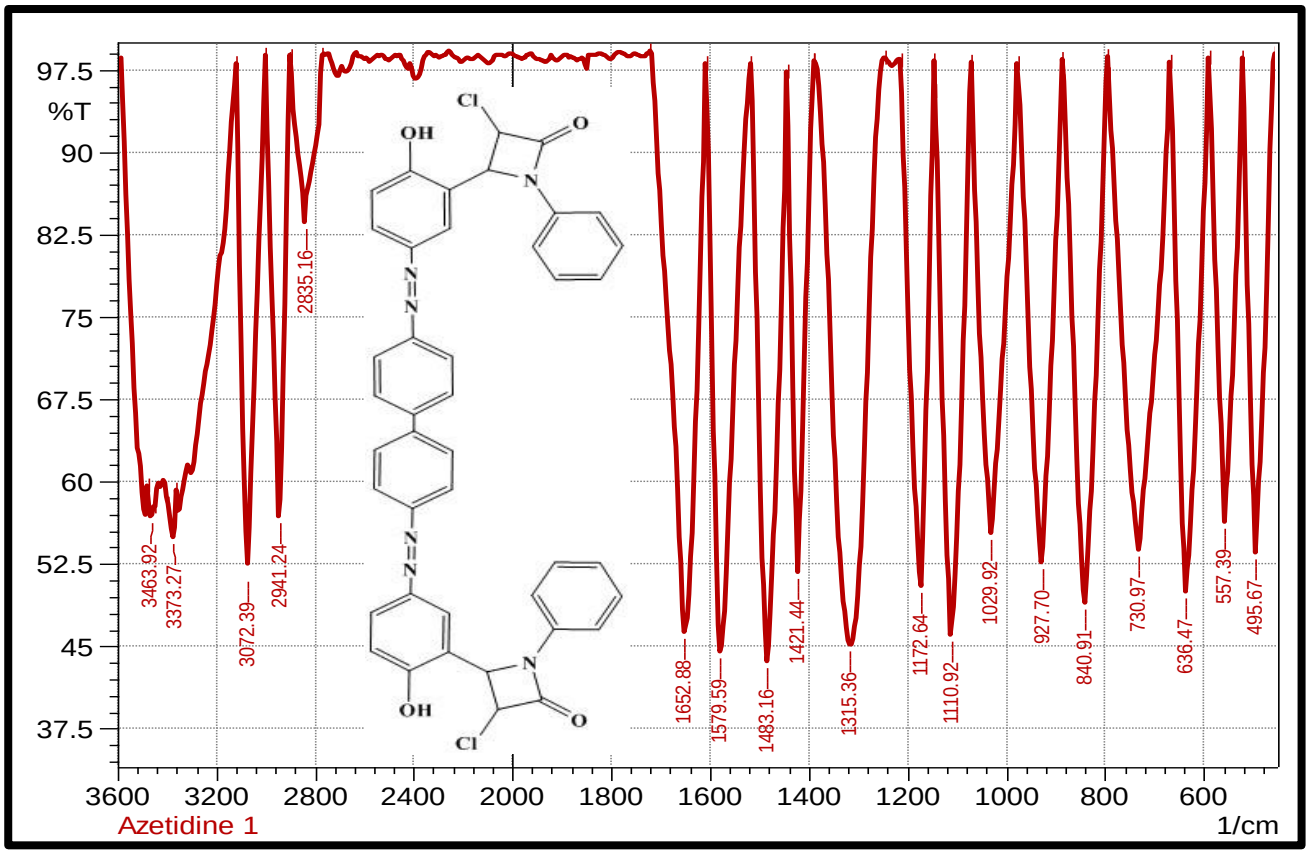


Fig. (1): FT-IR spectrum of compound [A_1]

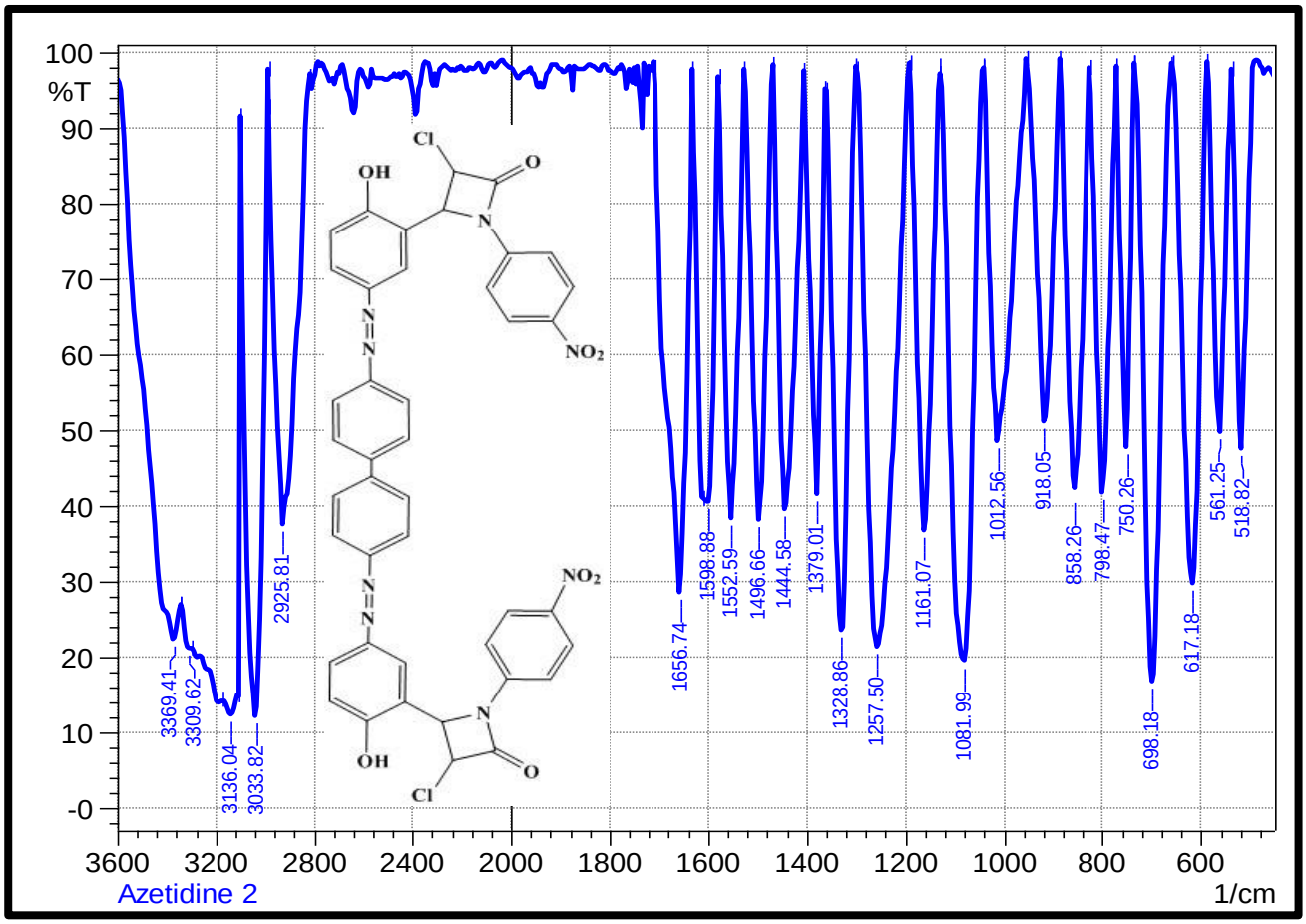


Fig. (2): FT-IR spectrum of compound [A_2]

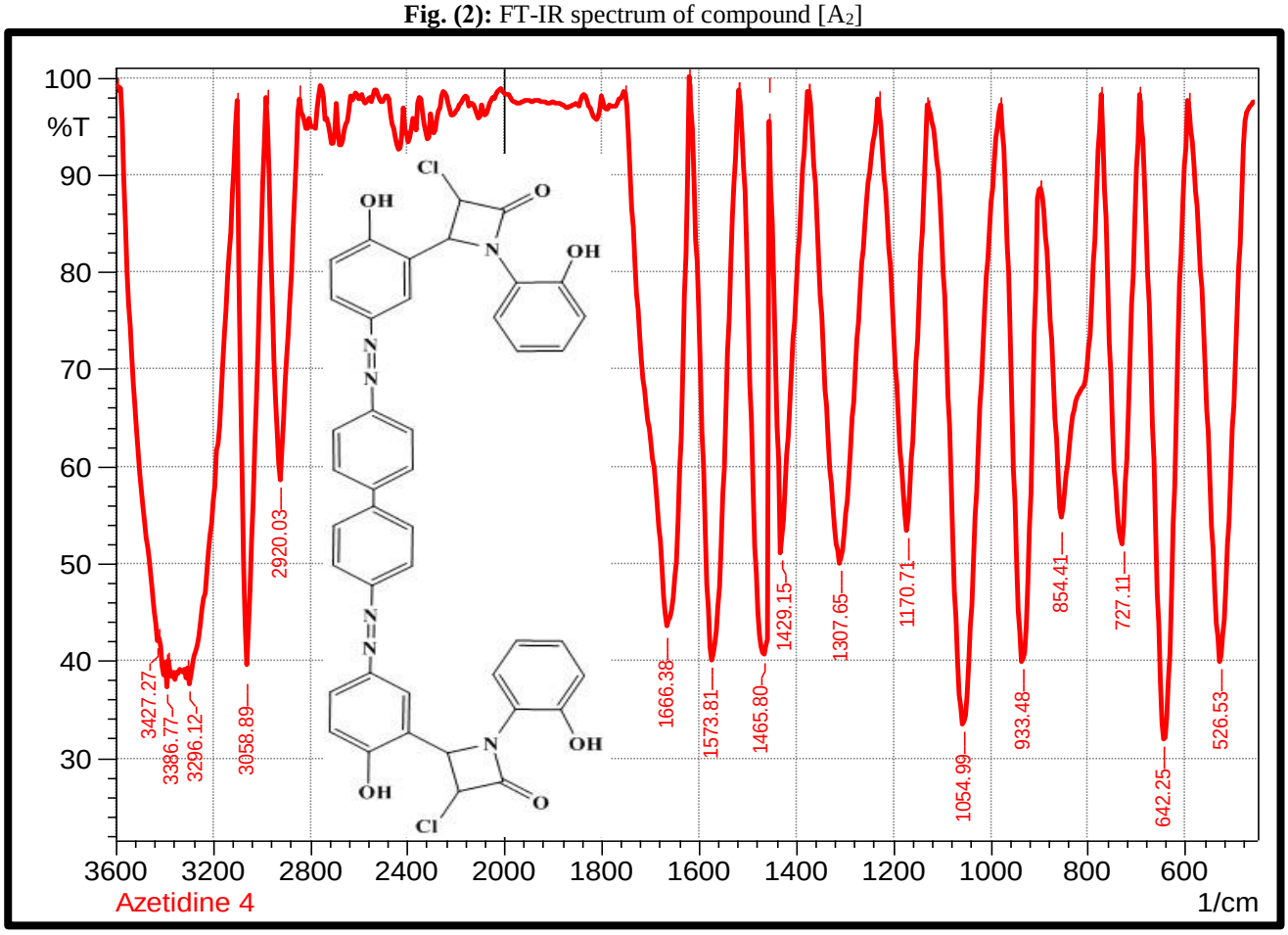


Fig. (3): FT-IR spectrum of compound [A_4]

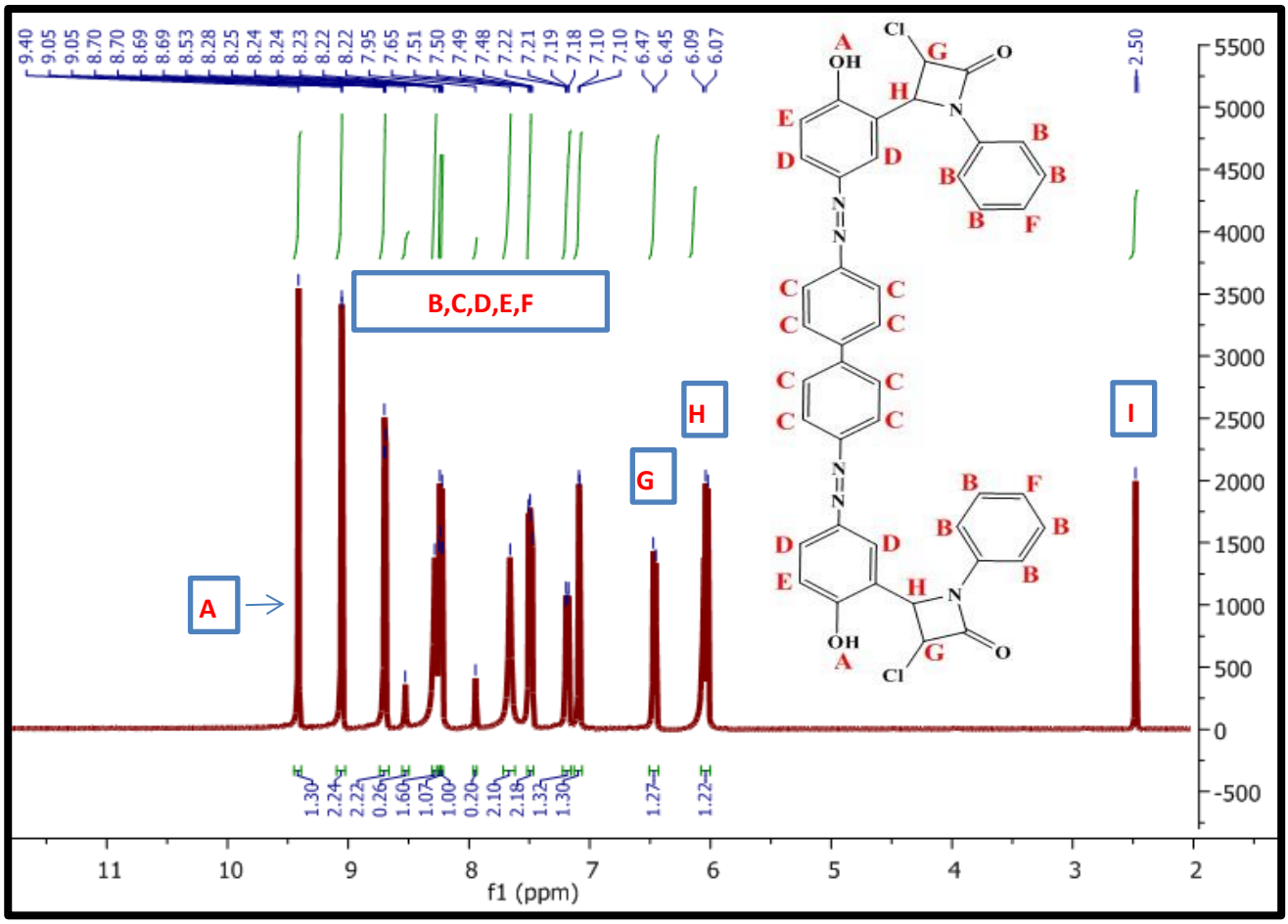


Fig. (4): ^{1}H-NMR spectrum of compound [A_1]

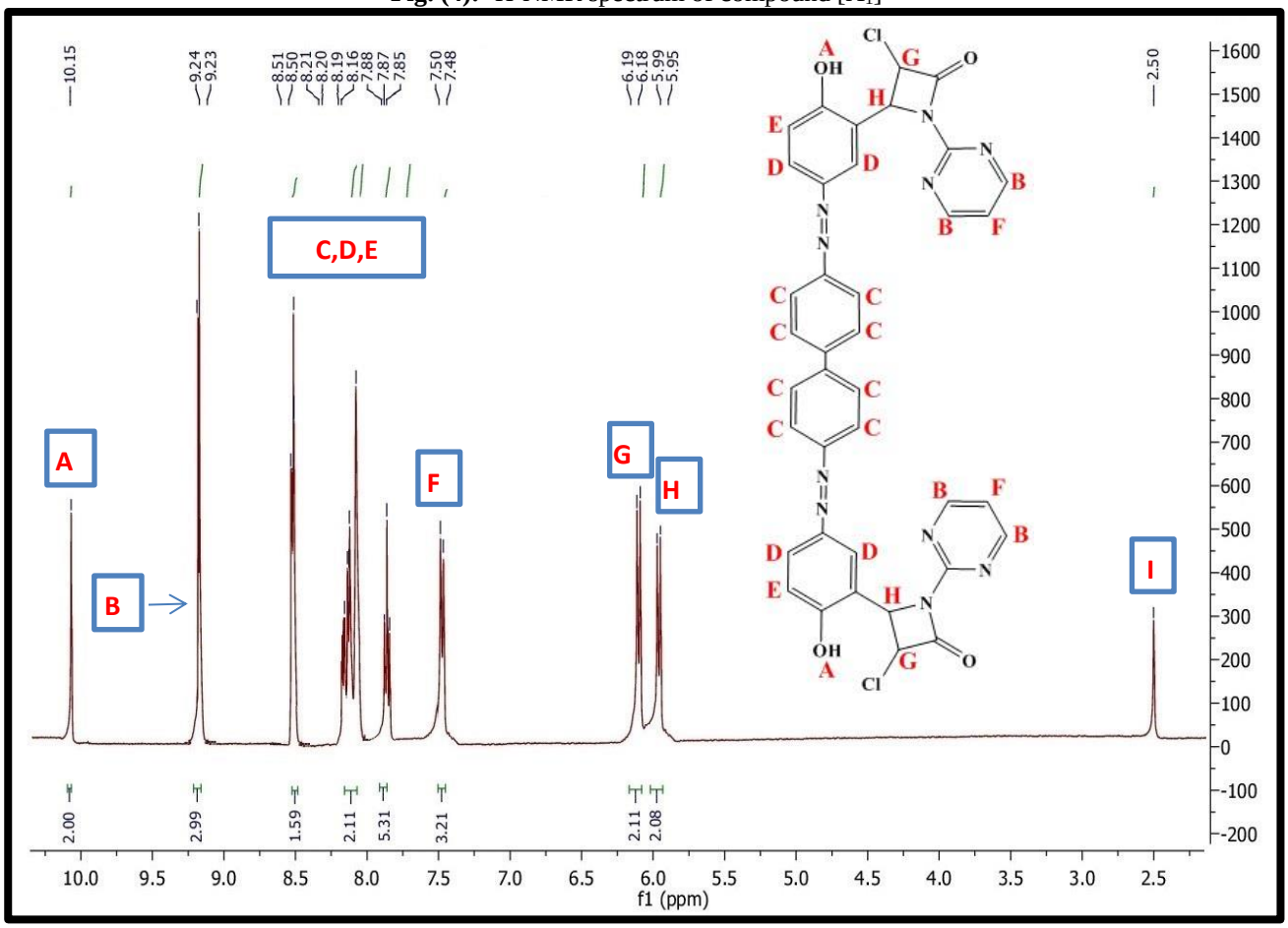


Fig. (5): ^{1}H-NMR spectrum of compound [A_3]

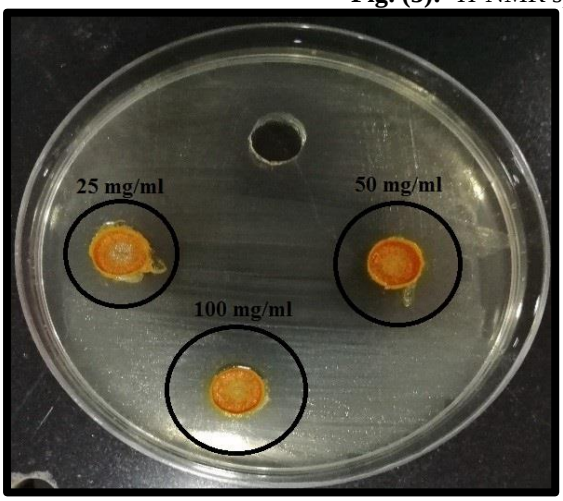


Fig. (6): Antibacterial activity of compound [A_1] against *E. coli*.

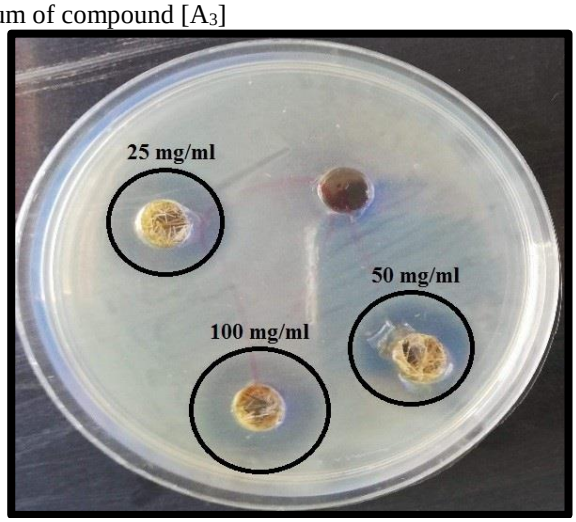
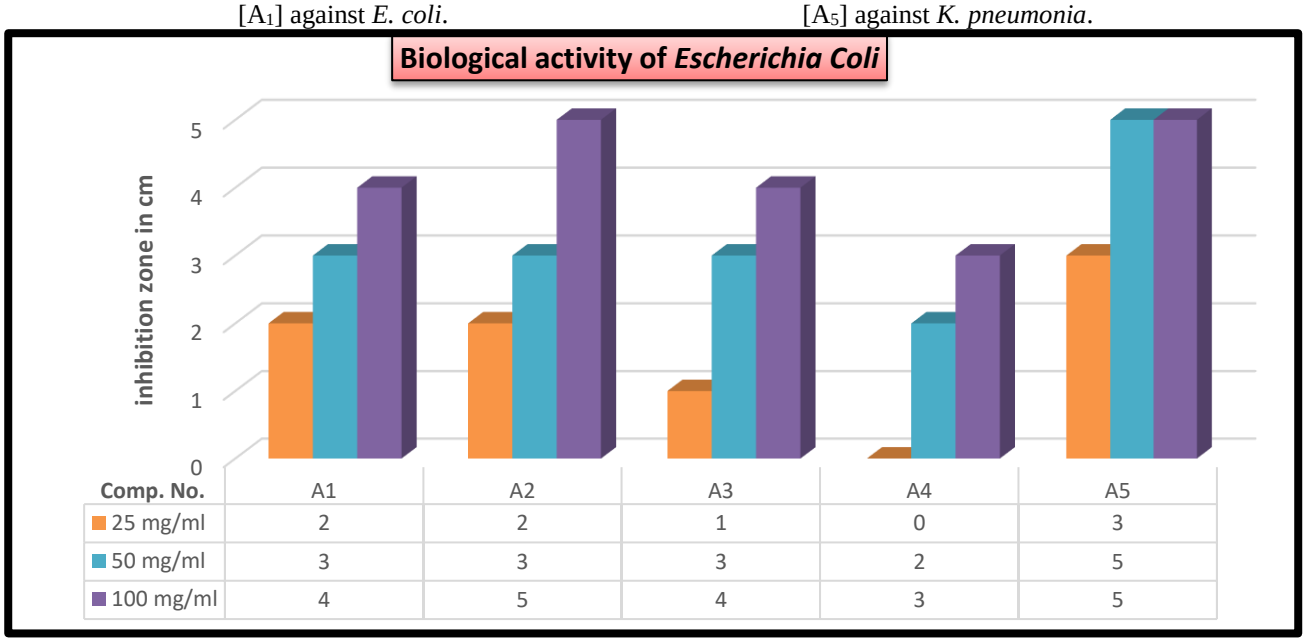
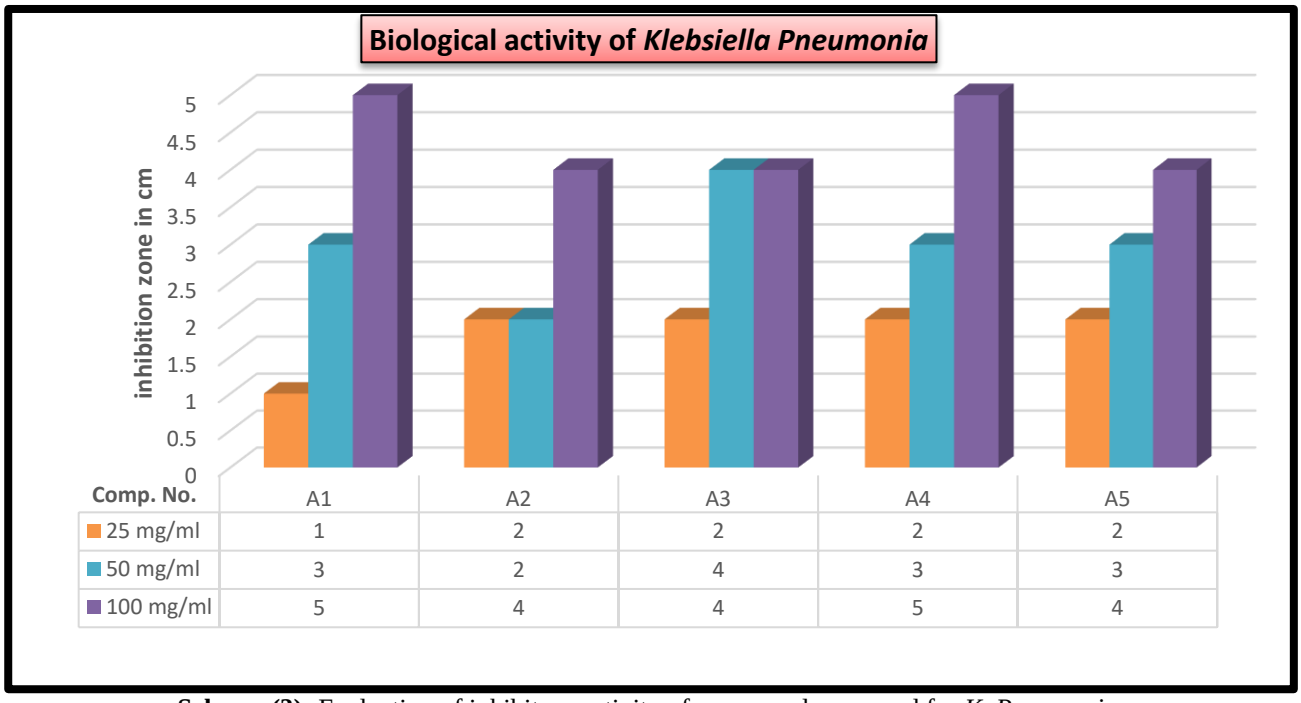


Fig. (7): Antibacterial activity of compound [A_5] against *K. pneumonia*.

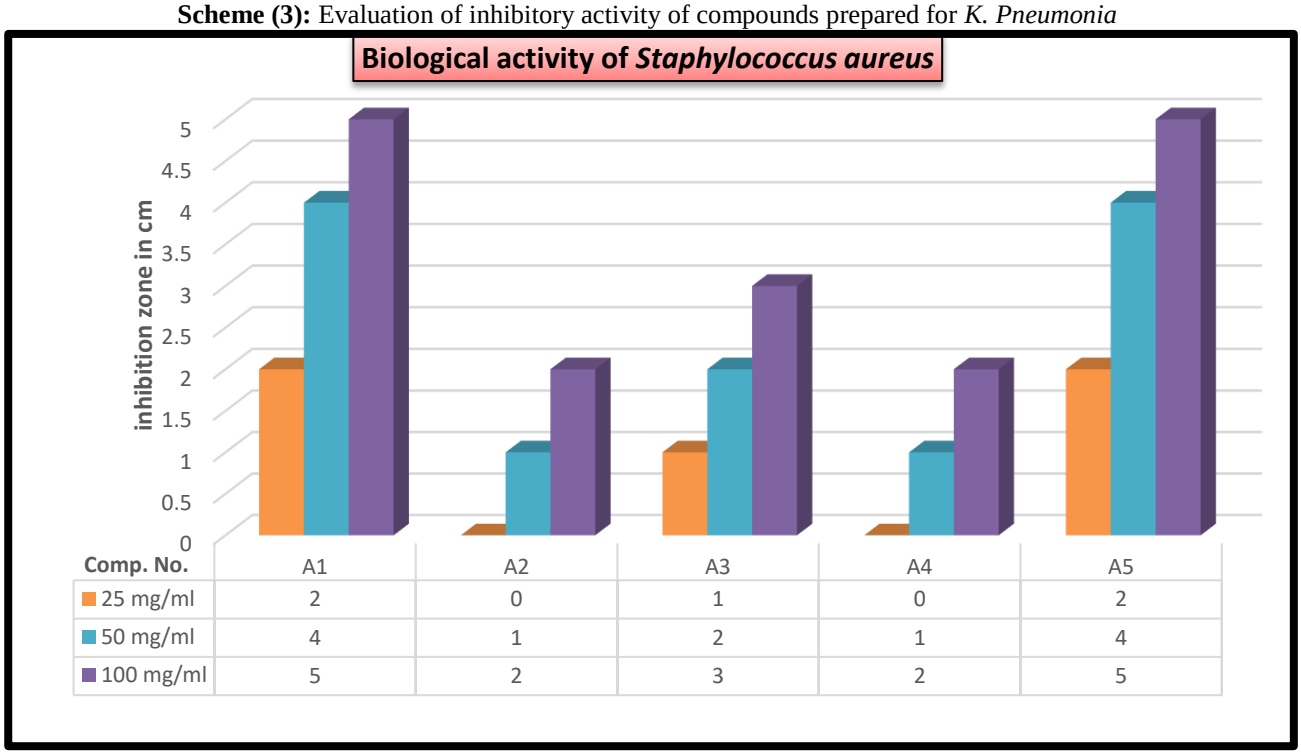


Scheme (2): Evaluation of inhibitory activity of compounds prepared for *Escherichia Coli*



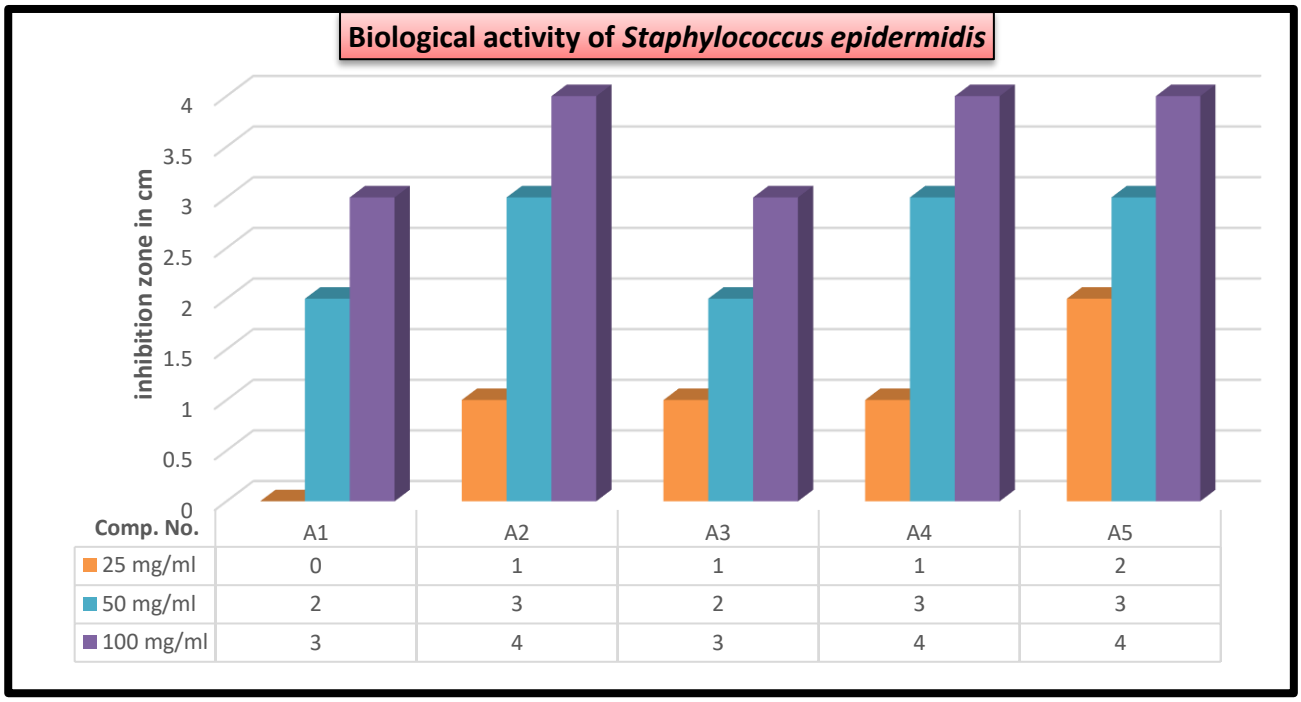
Comp. No.	A1	A2	A3	A4	A5
25 mg/ml	1	2	2	2	2
50 mg/ml	3	2	4	3	3
100 mg/ml	5	4	4	5	4

Scheme (3): Evaluation of inhibitory activity of compounds prepared for *K. Pneumonia*



Comp. No.	A1	A2	A3	A4	A5
25 mg/ml	2	0	1	0	2
50 mg/ml	4	1	2	1	4
100 mg/ml	5	2	3	2	5

Scheme (4): Evaluation of inhibitory activity of compounds prepared for *S. Aureus*



Scheme (5): Evaluation of inhibitory activity of compounds prepared for *S. Epidermidis*

4. Conclusions

The spectroscopic measurements showed the purity of the compounds prepared. In addition, some of the prepared compounds showed good antibacterial activity against the bacterial such as ***Eschershia coli, Klebislla Pneumonia*** **Gram (-ve),** ***Staphylococcus epidermidis*** **and** ***Staphylococcus aureus*** **Gram (+ve)**. Laser radiation of compounds [A_1-A_5] for (10, 20, 30) seconds was studied. It has been found the compounds have not been affected. When the melting point and color were determined, they neither disintegrated nor polymerized. This clarified that the laser beams used did not affect the stable compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Credit authorship contribution statement

Adil H. Dalaf: Conceptualization, Methodology, Investigation, Software, Validation, Data curation.

Fawzi H. Jumaa: Supervision, Investigation, Writing - review & editing.

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