Article

Cyclic imides , azo dyes: Synthesis, Identification and biological activity.

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Abstract

The research included the preparation of cyclic imide compounds (M1-M4) from the reaction of the amino compound 4,4-methylene diniline with various cyclic anhydrides (malic, succinic, Phthalic, and 1,8-naphthalic) in one step and by the microwave method. Azo dye compounds (M5-M9) were prepared in two steps. The first was preparing the diazonium salt of the amino compound with sodium nitrite and HCl. The second step was the reaction of the diazonium salt with the coupling materials (2-naphthol, vanillin, salicylic aldehyde, salicylic acid, 8-hydroxyquinoline) . The prepared compounds were characterized by several spectroscopic methods, including FT-IR, 1H, 13C-NMR, and mass spectrometry. The prepared compounds (M1-M9) were tested against two types of bacteria: Staphylococcus aureus, which is gram-positive and Escherichia Coli, which is gram-negative.

The results showed that some compounds have high effectiveness against E-Coli bacteria, such as compounds M3 and M6, and compounds M1, M2, M5, M8, and M9 showed high effectiveness against Staphylococcus aureus bacteria compared to the standard antibiotic, gentamicin sulfate, at a concentration of 6.12 mg/ml.

The compounds (M3, M4, M6 and M9) also showed excellent effectiveness against the fungus Candida albicans compared to the standard antibiotic Nystatin.Compound M5 has shown to be a good azo dye in the dyeing process (cotton, wool, brocade and wood.

Keywords: Cyclic imides, cyclic anhydrides, azo dyes, succinic.

1. Introduction

Cyclic imides are organic compounds prepared from the direct reaction of cyclic anhydrides with aliphatic or aromatic primary amines in one step. [1] Cyclic imides have multiple activities, including antibacterial, anti-inflammatory, and antifungal, which makes them of potential medicinal value [2]. The synthesis of Cyclic imides have been an interesting topic for researchers, as multiple methods have been developed to synthesize highly metabolites bearing functional functional groups [3]. In addition, cyclic imides are important compounds with diverse applications in various fields of chemistry and biology [4]. Cyclic imides are important Also in crop protection chemistry, where they are used as agricultural pesticides, fungicides and insecticides, they can act as receptors for crop protection agents, and are also used as intermediates in the manufacture of other agricultural chemicals [5,6,7]

Azo dyes: organic compounds resulting from the reaction of diazonium salt with various aromatic phenolic or amino compounds called coupling reagent in the dialysis reaction [8]. These dyes have been used in dyeing various types of fabrics, including wool and silk. Their stability on fabrics was evaluated, and the composite dyes showed good properties in terms of resistance to washing, light, and perspiration. These dyes showed good penetration and color stability on polyester fabrics. These dyes also have antibacterial activity against some bacteria [9,10] , and azo dyes are used. It is widely used in many industries such as textiles, leather, food, paper printing, pharmaceuticals, cosmetics and plastics [11,12}.

2.Practical part

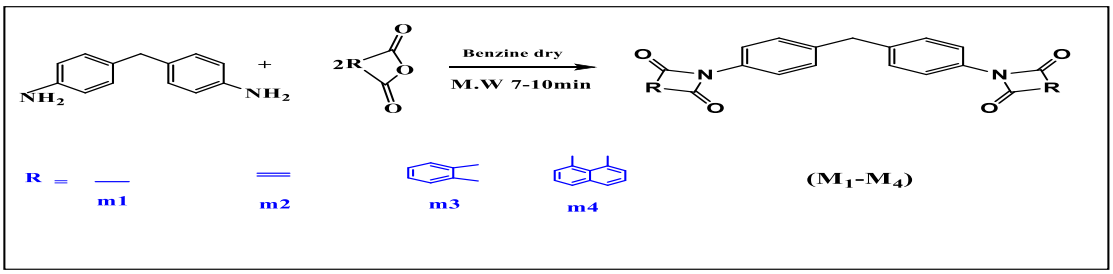
1.2Instrumentals used

The chemicals were obtained only from CDH and Aldrich. The melting point was determined using the Digital Advanced electrothermal SMP30 apparatus. The IR spectra were recorded with a Shimadzu Infrared spectrophotometer of the FTIR-8400S model, with the assistance of a KBr disc. The Proton-NMR spectra were acquired with a 600MHz nucleic magnetic resonance model, namely the Jeol ECA. The internal reference used in this study was TMS, whereas DMSO served as the solvent.

Preparation of cyclic imides (M1-M4)

(o.oo1mol, 0.5 grams) of the compound 4,4-methylene dianiline was mixed in 20 ml of absolute ethanol in a circular flask, and after completion of dissolution, (0.002)mole ml of different cyclic anhydrides (succinic, maleic, phthalic, 1,8- naphthalic anhydride) were added. with continuous stirring and the reaction mixture was heated in a rotating microwave device for (7-10 minutes) (425watt). After ensuring that the reaction had ended, the reaction mixture was cooled at room temperature, and the reaction mixture was poured over alittle cold water and a precipitate was obtained in the form of Crystals were filtered, washed with ethanol, and dried in an oven [13].

Scheme (1): The general equation for the preparation of cyclic imides (M1-M4), and Table (1) shows some physical properties of the prepared compounds (M1-M4)



Scheme (1) General equation for the preparation of cyclic imides (M1-M4)

Table (1): Some physical properties of the prepared cyclic imide compounds (M1-M4)

Comp.No	Molecular Formula	Molecular Weight	Color	M.P ^{0}C	Yield %
M_1	$C_{29}H_{18}N2O_4$	458.47	Brown	302-304	**95**
M_2	$C_{21}H_{14}N_2O4$	358.35	Brown	192-194	**82**
M_3	$C_{21}H_{18}N2O4$	362.39	Yellow	221-223	**93**
M_4	$C_{37}H_{22}N2O4$	558.59	Yellow	241-244	**96**

Preparation of azo dye compounds (M5-M9)

The azo dye was prepared in two main steps and by forming diazonium salts:

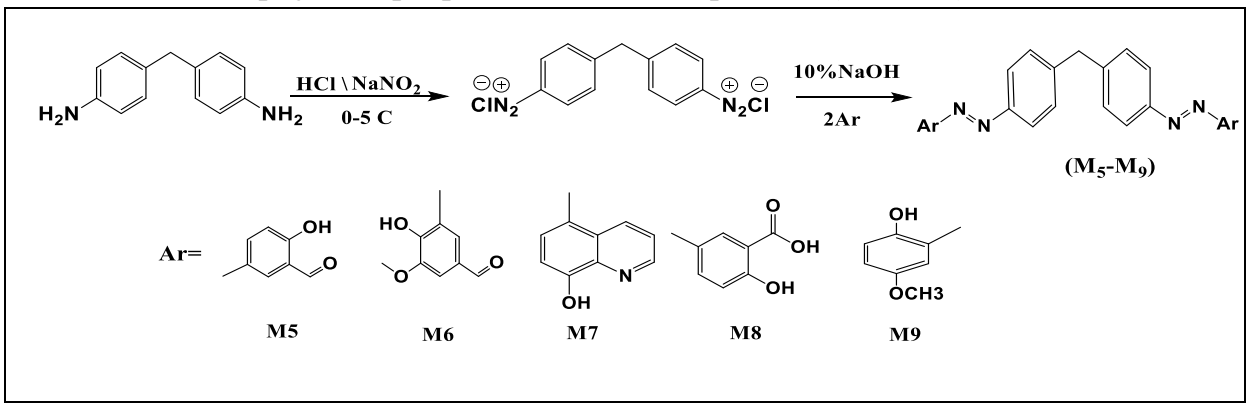
The first step: Preparation of diazonium salts:

(2.36g, 0.01 mol) of 4,4-methylene dianiline was mixed in (٪37 ، 2.5ml) acid HCl, concentration 37% (20 mL distilled water + 10 mL HCl), and dissolved in a second flask (0.7 g, 0.01 mol) of sodium nitrite. NaNO2 in the smallest amount of distilled water (4 ml), then added to the solution of the first beaker. The addition is done slowly and in the form of drops and at a temperature of -5 ^{o}C (0). With stirring, a colour change was observed, evidence of the formation of diazonium salt, which is stored at a temperature of 0-5 ^{o}C

Step Two: Prepare the coupling solution:

(1.22,0.01mol) of one of the substituted phenols (2-naphthol, salicylic acid, 4-hydroxyacetophenone, vanillin and 8-hydroxyquinoline) was dissolved in 10% dilute sodium hydroxide, and after completing the dissolution by continuous stirring, it was

added to it. Diazonium salt prepared in the first step in an ice bath with continuous stirring until the precipitate appears, then we leave the precipitate for a quarter of an hour, then filter it, wash it with ion-free water, and dry it in the oven [14] . Scheme (2) shows the general equation for preparing azo dye compounds (M_5-M_9) and Table (2) shows some of the physical properties of the compounds (M5-M9).



Scheme (2) General equation for preparing azo dye (M5-M9)

Table (2) Some physical properties of prepared azo dye compounds (M5-M9)

Comp. No	Molecular Formula	Molecular Weight	Color	M.P(°C)	Yield %
M_5	$C_{27}H_{20}N_4O_4$	464.48	Orange	255-257	**91**
M_6	$C_{29}H_{24}N_4O_6$	524.53	Dark yellow	224-226	**83**
M_7	$C_{31}H_{22}N_6O_2$	510.56	Dark yellow	245-248	**96**
M_8	$C_{27}H_{20}N_4O_6$	496.48	Orange	235-237	**78**
M_9	$C_{27}H_{24}N_4O_4$	448.51	Dark yellow	220-224	**86**

3. Results and Discussion

The infrared spectrum of cyclic imides showed absorption bands in the range (3072-3043 cm^{-1}) dating back to the aromatic (C-H) bond in the compounds (M1-M4). and it showed absorption bands in the range (2924-2922 cm^{-1}) dating back to The symmetric and asymmetric of the aliphatic (C-H) group. showed absorption bands at (1697-1600 cm^{-1}) returning to the imide carbonyl bond (C=O), and showed absorption bands at

(1597-1512 cm^{-1}) returning to the (C=C) bond Aromatic [15--16]. As shown in Table (3). Figure (1) shows the infrared spectrum of the compound (M3).

The infrared spectrum of some azo dye compounds (M5-M9) prepared from 4,4-Methlene aniline was measured, and showed absorption bands in the range (3414-3444 cm-1) dating back to the (O-H) bond. It showed absorption bands at (3209 cm^{-1}) belongs to the (0-H) carboxylate of the compound (M8), and showed absorption bands in the range (3022-3032 cm^{-1}). It goes back to the aromatic (C-H) bond in the compound, and showed absorption bands in the range (2885-2899 cm^{-1}) It belongs to the symmetric and asymmetric of the aliphatic (C-H) group, and it showed absorption bands in the range (1606-1627 cm^{-1}) that go back to the carbonyl bond (C=O) in the and showed absorption bands at (1444-1402 cm^{-1}) that go back to the bond (N=N) for all azo compounds [17-20,].

Figures (2) and (3) show the infrared spectrum of the compound (M5, M8), as shown in Table No. (4).

NMR spectrum (1H.NMR), (13C.NMR) of the prepared imide compound*:*

The 1H.NMR spectrum of compounds (M1) showed signals between (δ7.55 -7.87 ppm) dating back to the protons of the aromatic rings, a single signal at (δ3.99 ppm) dating back to the protons of the CH2groupattached to the nitrogen atom, and a single signal at (δ2.51 ppm is due to the protons of the (CH_3) group attached to the pentagonal ring, and the appearance of a signal CH_2, while the signal at the location (δ2.51 ppm) is due to the protons of the solvent used (DMSO**)** [21-19)]. As shown in Figure (4) and it was measured 1H.NMR spectrum of compounds(M2) showed signals between (δ7.56 -7.17 ppm) dating back to the protons of the aromatic rings, a single signal at (δ3.87 ppm) dating back to the protons of the (CH_3) group attached to the nitrogen atom, and a single signal at (δ2.61 ppm is due to the protons of the CH_2 group attached to the pentagonal ring, and the appearance of a signal (4.00 ppm) is due to the protons of CH_2, while the signal at the location (δ2.51 ppm) is due to the protons of the solvent used (DMSO**)** [21-23)]. As shown in Figure (5)

^{13} C.NMR spectrum measurements were carried out for the compound (M1), and the measurements were identical to the prepared compounds, as a signal appeared (δ 158.38 ppm) belonging to the carbonyl groups in the compounds. Signals appeared between (δ 121.76-130.02 ppm) belonging to the carbon atoms inside the benzene

rings. A signal (δ. 44.72 ppm) appeared, belonging to the carbon atoms (CH_2) [19-21], as shown in Figure (6)and it was measured ^{13} C.NMR spectrum measurements were carried out for the compound (M2), and the measurements were identical to the prepared compounds, as a signal appeared (δ 137.49 ppm) belonging to the carbonyl groups in the compounds. Signals appeared between (δ 129.46-131.16 ppm) belonging to the carbon atoms inside the benzene rings. A signal (δ. 47.63 ppm) appeared, belonging to the carbon atoms (CH_2)[24-26], as shown in Figure (7)

To confirm the validity of the proposed structures of the prepared compounds, mass spectrometric analyzes were conducted to determine the molecular weight of the prepared compounds, by determining the molecular ion peak of the prepared compounds and the base peak, in addition to some peaks of the fragments generated from the molecule after ionization.

The integration of the results of the infrared spectrum, nuclear resonance spectrum, and mass spectrum gives clear evidence of the validity of the compositions assigned to the prepared compounds, as the mass spectrum of the compound (M3) was recorded, showing a main signal at (362 m/z) and in relative abundance (15%) It belongs to the molecular ion of the compound with the molecular formula ($C_{21}H_{18}N_2O_4$) [22- 25]. as shown in Figure (8).

Table (3) FT-IR data absorption results for cyclic imides compound (M1-M4)

Comp No.	ν(C-H) Arom	ν(C-H) Aliph.	ν(C=O) Amide	ν(C=C) Arom.	ν(C-N)
M_1	3039 3072	2922 2924	1664 1697	1527 1597	1228
M_2	3039 3072	------- 	1633 1697	1558 1581	1261
M_3	3043 3072	2933 2942	1600 1697	1512 1598	1219
M_4	3066 3074	2925 2955	1636 1697	1512 1579	1230

Table (4) FT-IR data results for azo dye compounds (M5-M9)

Comp No.	(O-H)	(C-H) Arom.	(C-H) Aliph.	C=O	N=N
M_5	3414 3444	3032	2970 2930	1606	1444
M_6	3416	3055	2984 2932	1627	1433
M_7	3412	3022	2980 2947	1612	1425
M_8	3433	3044	2984 2923	1666	1400
M_9	3458	3036	2930 2902	1673	1455

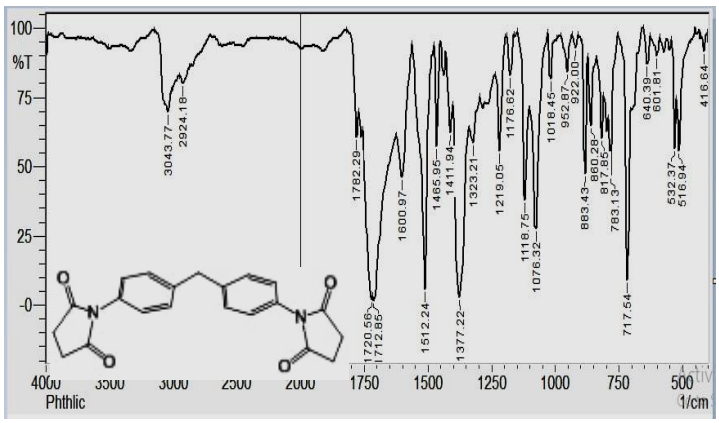


Figure (1) FT-IR spectrum of compound (M3)

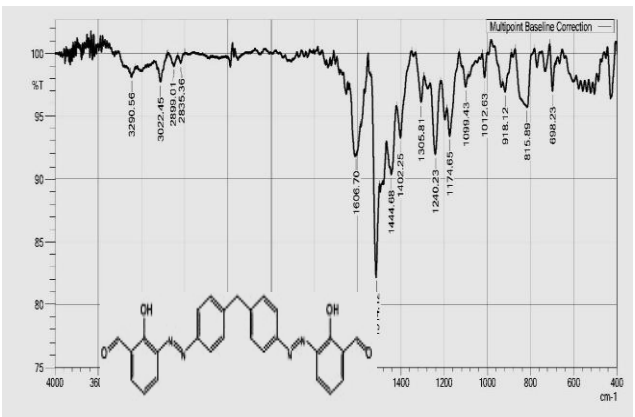


Figure (2) FT-IR spectrum of compound (M5)

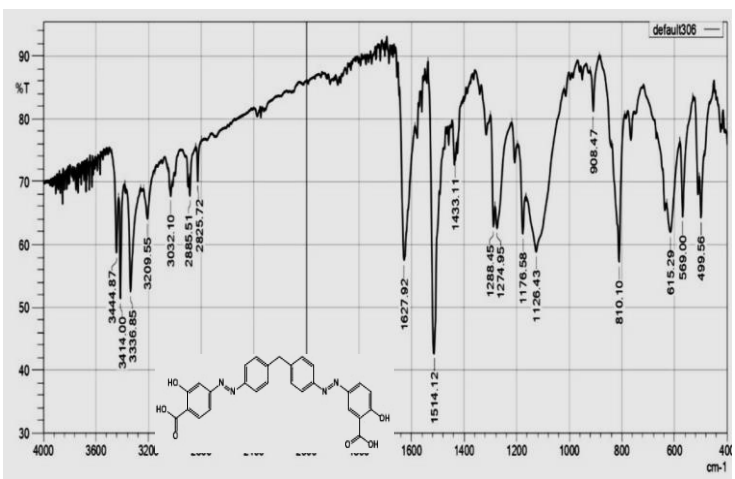


Figure (3) FT-IR spectrum of compound (M8)

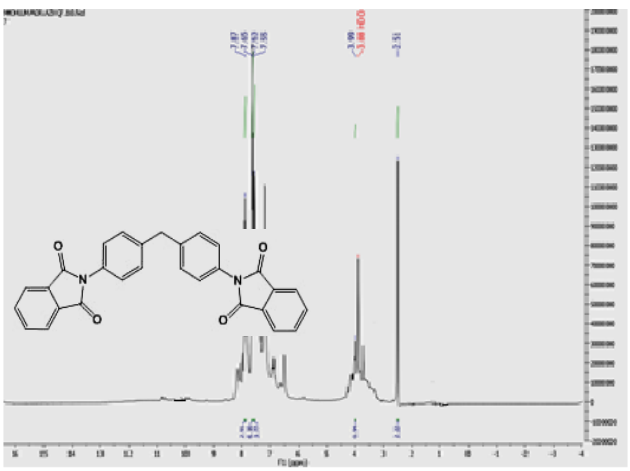


Figure (4) ^{1}H- NMR of compound (M1)

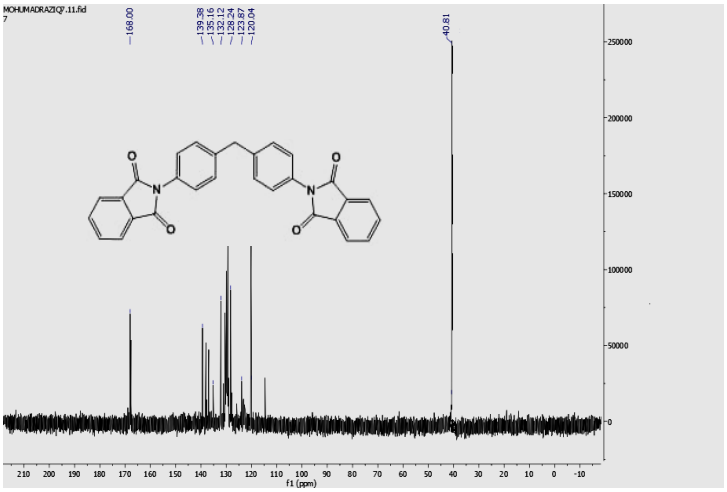


Figure (5) ^{13}C- NMR of compound (M1)

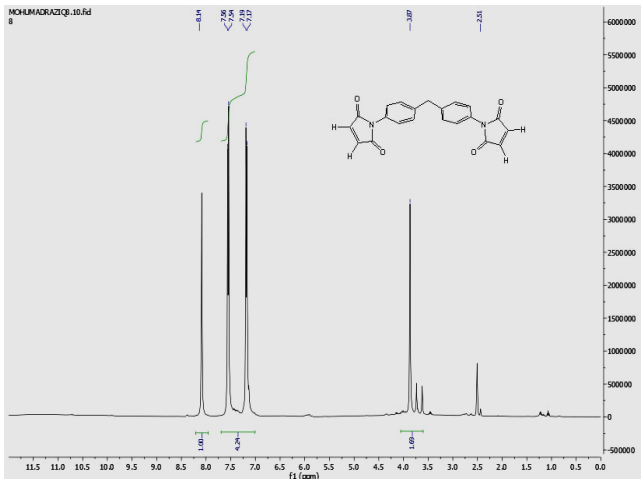


Figure (6) ^{1}H- NMR of compound (M2)

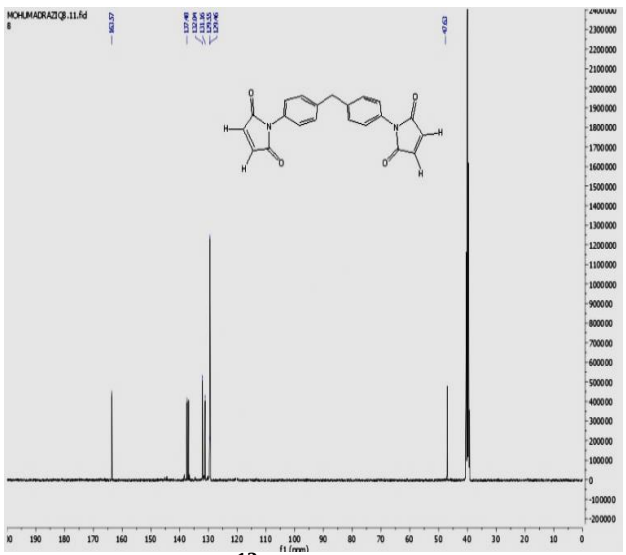


Figure (7) ^{13}C- NMR of compound (M2)

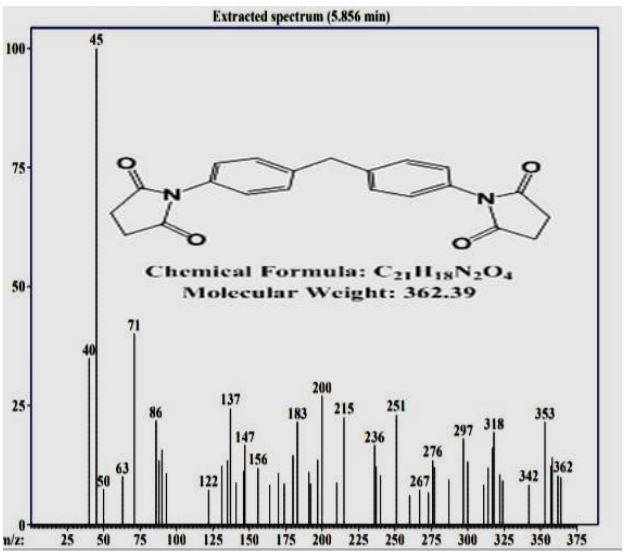


Figure (8) Mass spectrum of the compound (M3)

The biological activity of some prepared compounds*:*

The biological activity of some prepared compounds (M1-M9) was tested on a type of fungus, *Candida albicans*, and on two types of bacterial isolates, one of which is gram positive and the other is gram negative, namely*:*

-*Staphylococcus aureus*, which is gram positive.

-Gram-negative *Escherichia Coli* bacilli.

The results showed inhibitory activity of the selected prepared compounds against the *Candida* fungus compared to the standard antibiotic (gentamicin sulfate for bacteria - nystatin for fungi), and against two types of bacteria used. The results of the prepared

compounds were compared with the standard antibiotic (gentamicin sulfate) for bacteria and (nictatin) for fungi. Concentrations (mg\ml 6.25, 12.5, (25)

The results indicate that some of the prepared compounds have the ability to inhibit fungi and bacteria used using different concentrations of the compounds (25mg\ml), (12.5mg\ ml), and (6.25mg\ ml)

Candida albicans Fungus

At the concentration (12.5mg\ml) and (6.25 mg\ml), all compounds showed an inhibitory activity between higher and lower than the standard antibiotic. At the concentration (25mg\ml), most of the compounds showed a higher inhibitory activity than the standard antibiotic, and through The data obtained in the table shows that the compounds (M4, M6, M9) at a concentration of 6.25 are the best inhibitors of this bacteria compared to inhibition with the standard antibiotic at a concentration of (6.25 mg\ml), as in Figure (9).

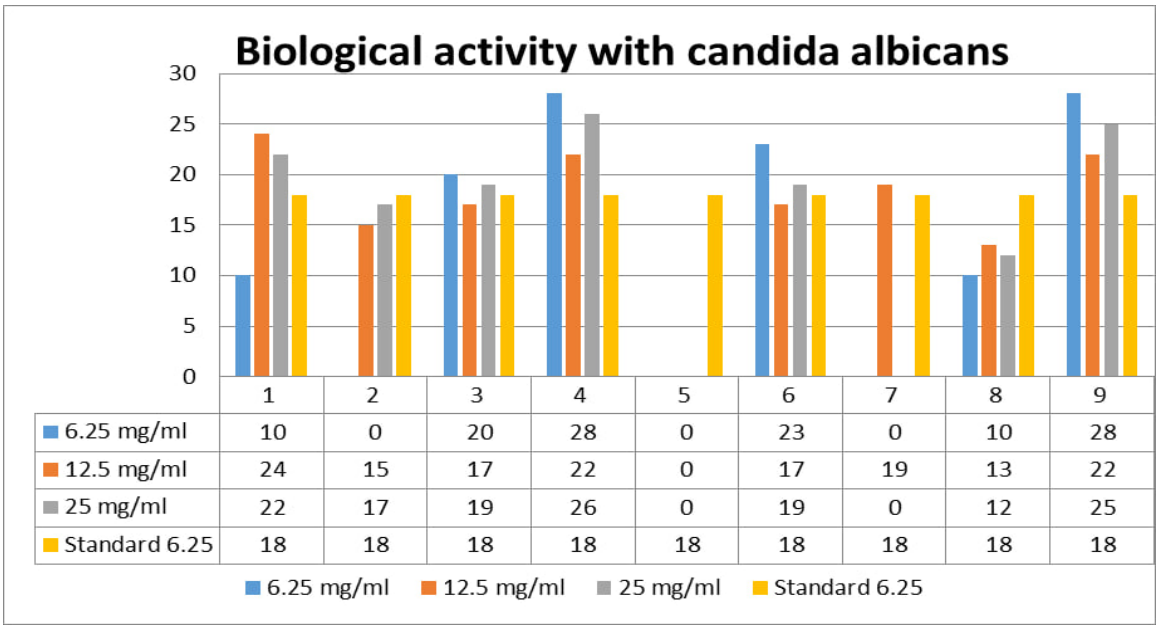
Escherichia coli bacilli

At the concentration (25mg/ml), some compounds showed higher to lower inhibitory effectiveness than the standard antibiotic, and most of the compounds at this concentration had a higher inhibitory effectiveness than the standard substance, while at the concentration (12.5mg/ml) and the concentration (6.25mg/ml). ml), so all compounds showed lower to higher effectiveness than the standard substance, and from the data obtained in the table it is clear that the compound (M9) at the concentration (25mg/ml) is the best inhibitor for this type of bacteria, as shown in Figure (10).

**Staphylococcus aureus*

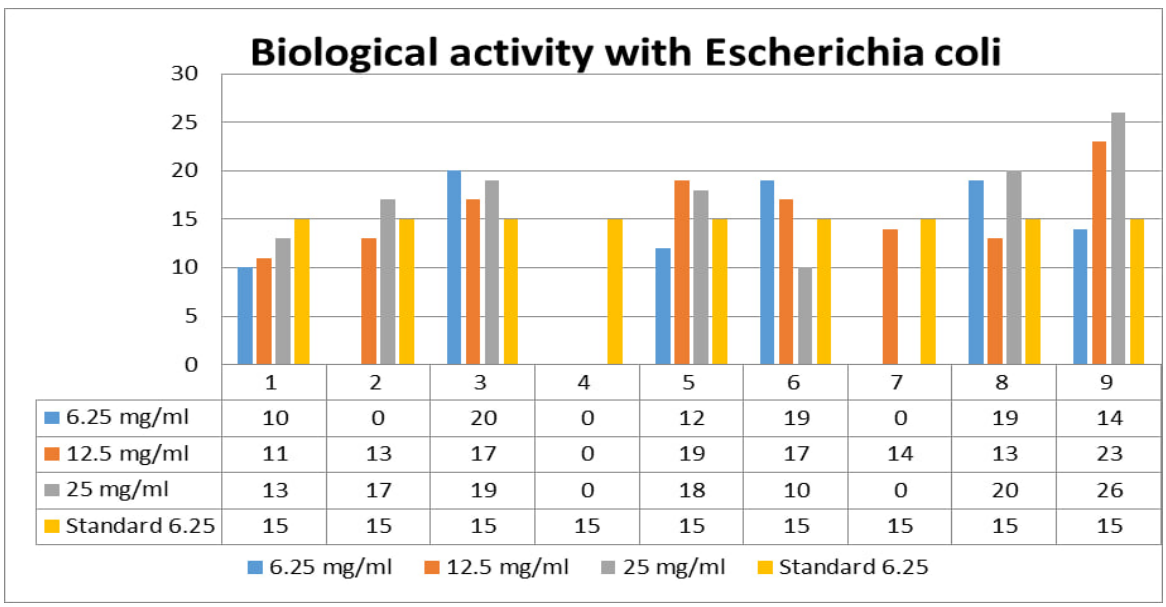
At the concentration (25mg/ml), all compounds showed a higher inhibitory activity than the standard substance

(12.5 mg./ml) (6,25mg/ml), some compounds showed high to moderate to low inhibitory activity compared to the standard antibiotic, as shown in Figure (11)



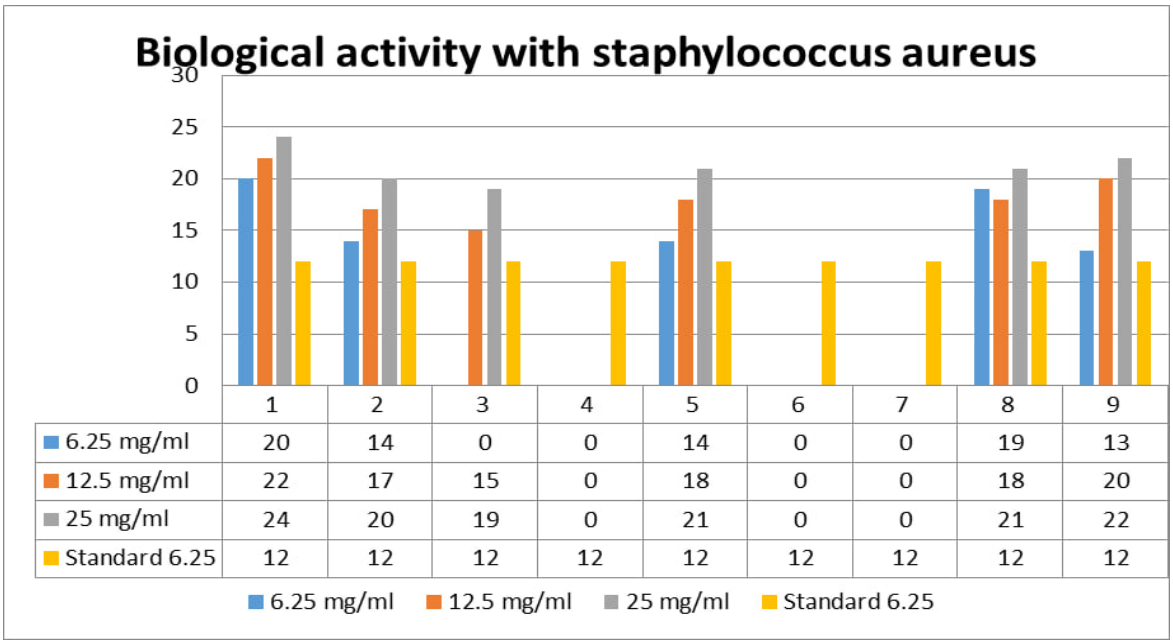
	1	2	3	4	5	6	7	8	9
6.25 mg/ml	10	0	20	28	0	23	0	10	28
12.5 mg/ml	24	15	17	22	0	17	19	13	22
25 mg/ml	22	17	19	26	0	19	0	12	25
Standard 6.25	18	18	18	18	18	18	18	18	18

Figure (9) Inhibitory effectiveness of the prepared compounds (M1 – M9) against Candida albicans fungus



	1	2	3	4	5	6	7	8	9
6.25 mg/ml	10	0	20	0	12	19	0	19	14
12.5 mg/ml	11	13	17	0	19	17	14	13	23
25 mg/ml	13	17	19	0	18	10	0	20	26
Standard 6.25	15	15	15	15	15	15	15	15	15

Figure (10) Inhibitory activity of the prepared compounds (M1-M9) against Escherichia Coli bactera



	1	2	3	4	5	6	7	8	9
6.25 mg/ml	20	14	0	0	14	0	0	19	13
12.5 mg/ml	22	17	15	0	18	0	0	18	20
25 mg/ml	24	20	19	0	21	0	0	21	22
Standard 6.25	12	12	12	12	12	12	12	12	12

Figure (11) Inhibitory activity of the prepared compounds (M1-M9) against Staphylococcus aureus bacteria.

The following pictures show zones of inhibition for some compounds prepared against the fungus *Candida albicans*, the bacteria *Escherichia-Coli*, and the bacteria *Staphylococcus aureus*.

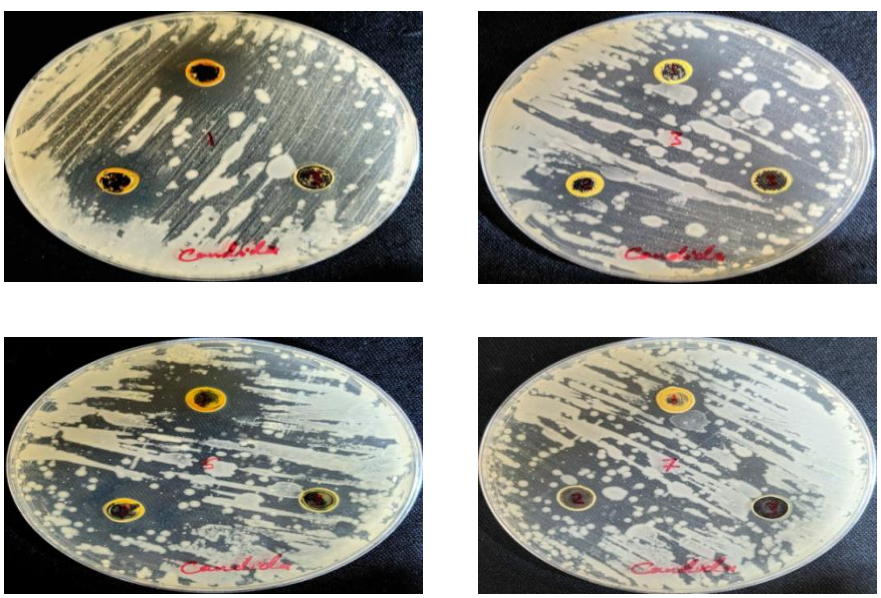


Figure (12) Inhibitory diameter of compounds (M1, M3, M5, M7) against the fungus Candid albican

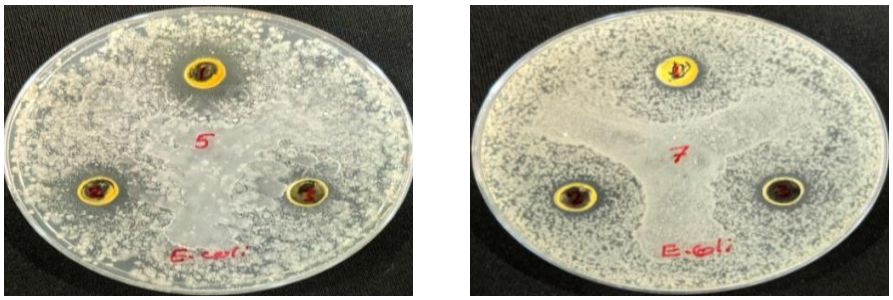


Figure (13) Inhibitory diameter of compounds (M1, M3, M5, M7)

against the bacteria *Escherichia-Coli*

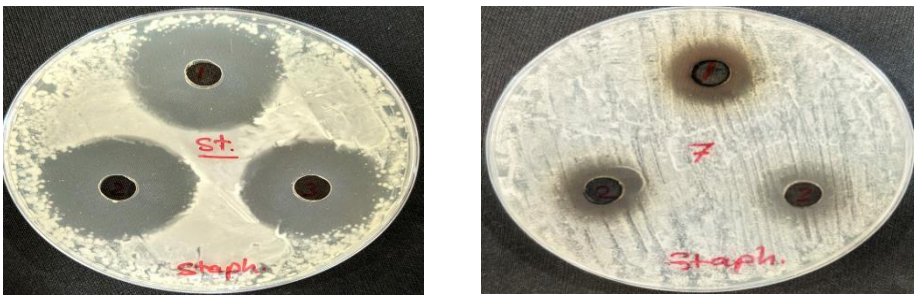


Figure (14) Inhibitory activity of compound M7

against the bacteria *Staphylococcus aureus*

The dyeing process:

Some prepared azo compounds (M5) were tested in the dyeing process (cotton, wool, brocade and wood), and according to the dyeing process in the practical part, the results showed clear stability after washing with water and weak to medium stability when washing with soap and liquid (bright) soap, and that most of the compounds Its stability is M5, and this is due to the high polarity of the compound, as well as the higher ability of wood to form hydrogen bonds with the test compounds. Dyeing is the colored material that can impart its color to a specific material, provided that the following conditions are met: that it has the ability to dye the material. To be dyed, it has an intense colour, and has stability against chemical and natural influences such as stability to light and washing[27-28]. as shown in Figure (15**)**

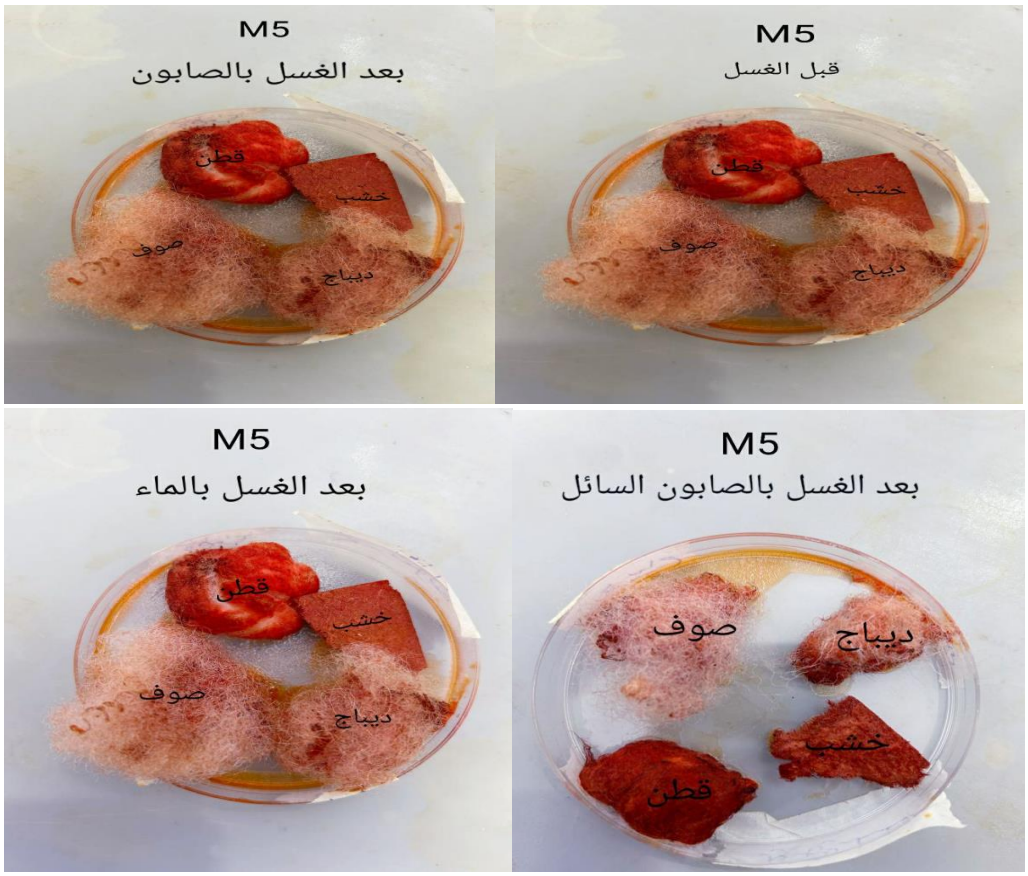


Figure (15): Tissues dyed with azo compound (M5) before and after washing

4.Conclusion

The research included the preparation of cyclic imide compounds (M1-M4) from the reaction of the amino compound 4,4-methylene dianiline with various cyclic anhydrides (malic, succinic, Phthalic, and 1,8-naphthalic) in one step and by the microwave method. Azo dye compounds (M9-M5) were prepared in two steps. The first was preparing the diazonium salt of the amino compound with sodium nitrite and HCl. The second step was the reaction of the diazonium salt with the coupling materials (2-naphthol, vanillin, salicylic aldehyde, salicylic acid, 8-hydroxyquinoline). . The prepared compounds were characterized by several spectroscopic methods, including FT-IR, 1H, 13C-NMR, and mass spectrometry. The prepared compounds (M1-M9) were tested against two types of bacteria: *Staphylococcus aureus*, which is gram-positive and *Escherichia Coli*, which is gram-negative.

The results showed that some compounds have high effectiveness against *E-Coli bacteria*, such as compounds M3 and M6, and compounds M1, M2, M5, M8, and M9 showed high effectiveness against *Staphylococcus aureus* bacteria compared to the standard antibiotic, gentamicin sulfate, at a concentration of 6.12 mg/ml.

The compounds (M3, M4, M6 and M9) also showed excellent effectiveness against the fungus *Candida albicans* compared to the standard antibiotic Nystatin.
Compound M5 has shown to be a good azo dye in the dyeing process (cotton, wool, brocade and wood)

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