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Synthesis and Evaluation of Biological Activity of Pyrazolone Compounds

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Abstract: Treatment of a mixture of 3-methyl-1-phenylpyrazol-5-one **1** with a diversity of aromatic aldehydes and piperidine in absolute ethanol (24 h at 0 ºC) gave products in good yields. Biological screening of the synthesized derivatives for their antimicrobial activity against various bacteria (gram positive and gram negative) as well as against fungi have been evaluated, where derivatives **3** and **11** were found to be the most potent.

Characterization of all the formed derivatives was emphasized by melting point determination, NMR data in addition to their mass spectra.

Keywords: pyrazolone, Benz aldehydes, Pathological microorganisms.

1 Introduction

1.1 Pyrazole and its Derivatives

Heterocyclic compounds constitute the largest and most varied family of organic compounds.(Al-Mulla, 2017) [1].

It is well known that heterocyclic compounds are widely distributed in nature and are essential for life activities of plants and animals. They play a vital role in the metabolism of all living cells, for example the vitamins and co-enzyme precursors thiamine, riboflavin etc. Some of these are natural products, for example antibiotics such as penicillin and cephalosporin etc, and they have attracted considerable attention in the design of biologically active molecules and advanced organic materials(Hote & Bhoyar, 2014).[2]

Today, heterocyclic compounds have a role in most fields of science such as medicinal chemistry and biochemistry as well as other areas of science.

Pyrazole and its derivatives constitute an important class of five member heterocyclic compounds. The presence of the pyrazole nucleus in different structures leads to diversified applications in different areas such as technology, medicine and agriculture. In particular, they are described as inhibitors of protein glycerin, antibacterial, antifungal, anticancer, antidepressant, anti-inflammatory, anti-tuberculosis, antioxidant as well as antiviral agents(Nirwan, Pareek, & Chohadia, 2015).[3]

Pyrazoles have illustrious history; in 1883, a German chemist Ludwig Knorr was the first to discover antipyretic action of pyrazole derivative in men, he named the compound antipyrine. When he attempted to synthesize quinoline derivatives with antipyretic activity, accidentally obtained antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) which has analgesic, antipyretic and antirheumatic activity; which stimulated interest in pyrazole chemistry(Rajpara, 2017). [4]

1.2 Pyrazolone and Their Derivatives

Pyrazolones are five membered nitrogen containing heterocyclic compounds, where pyrazolone ring system represents an important class of compounds not only for their theoretical interest but also for their bio-activity. The 3-pyrazolone and 5- pyrazolone are most dominant classes having importance in pharmaceutical industry.(Vijesh et al., 2011). [5].

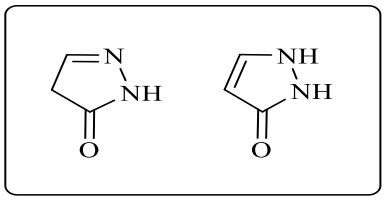

Fig.1 Chemical structures of 3- and 5-pyrazolone.

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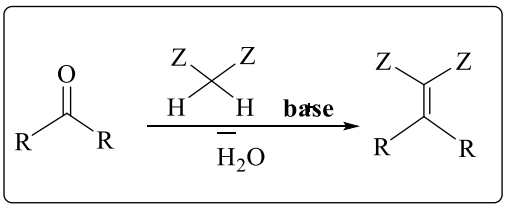
1.3.3-Methyl-1-Phenyl-Pyrazol-5-one

In our current research study, the 3-Methyl-1-Phenyl-Pyrazol-5-one derivatives have been the subject of many research studies due to varieties of potential biological activities such as antimicrobial antiviral biological activities such as antimicrobial antiviral such as antimicrobial antiviral, antitumor, antihistaminic, antidepressant, anti-inflammatory, anticancer, antioxidant, anticonvulsant, anti-diabetic activities, and cytotoxic activities.

The pyrazolone derivatives constitute an important moiety of numerous pharmaceuticals, agrochemicals, dyesand pigments, chelating and extracting agent. Pyrazolone can be considered as intermediate compound for the synthesis of various cyclic compounds of high biological activity (Wang, Jin, Cheng, & Li, 2010). [6]

In organic chemistry, the Knoevenagel reaction is widely used for C=C bond formation. The product obtain is α,β-unsaturated compound which is mostly used as intermediate in the formation of natural products, therapeutic agents, adequate chemicals, polymers having different functional groups, insecticides and pesticides. It is usually carried out in organic solvents and catalyzed by organic bases such as pyridine or piperidine..

The Knoevenagel condensation is a nucleophilic addition of an active α-hydrogen compound to a carbonyl group followed by a dehydration reaction in which a molecule of water is eliminated (hence *condensation*). The product is often an α,β- conjugated enone **(Scheme1)** (Denney, 1969; List & Turberg, 2019). [7].



Scheme 1: Knoevenagel condensation.

In this reaction, the carbonyl compound is an aldehyde or a ketone. The catalyst is usually a weakly basic amine. The active hydrogen component may have the following forms:

- Z–CH_2-Z or Z–CHR–Z , for instance diethyl malonate, Meldrum's acid, ethyl acetoacetate or malonic acid, or cyanoacetic acid.
- Z–CHR_1R_2 , for instance nitromethane.

where Z is an electron withdrawing functional group. Z must be powerful enough to facilitate de protonation to the enolate ion even with a mild base. Using a strong base, this reaction would induce self-condensation of the aldehyde or ketone.

2 Experimental Sections

All melting points of the synthesized compounds were measured by capillary tubes using Stuart scientific apparatus and are uncorrected.

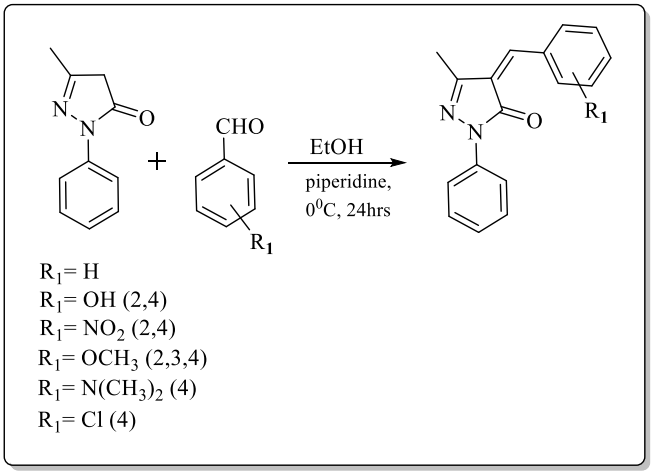
The accurate analyzes of NMR were conducted at Research Center of the Factuality of science, University of Cape Town.

The^{1}H-NMR spectra were recorded on a Varian Mercury NMR spectrometer (Varian, CA, USA; 400 MHz) and a Bruker Ultrashield-Plus spectrometer (Bruker, MA, USA; 400 MHz).

The ^{13}C-NMR spectra were recorded on the same instruments at 101 MHz. NMR samples were dissolved in deuterated dimethylsulfoxide (DMSO-d_6), or chloroform ($CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm) and rounded to two decimal places.

2.1 General Procedure for the Synthesis of 4-Substituted Pyrazolone Compounds:

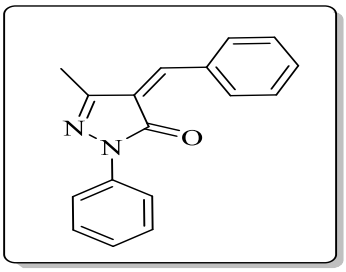
A mixture of different substituted aromatic benzaldehyde (2.8 mmol), 3-methyle-1-phenyl-5-pyrazolone (0.5 g, 2.8 mmol) and piperidine (1 ml) in 30 ml absolute ethanol was stirred at 0 C° for 24 hrs. The desired product was separated as solid, washed by cold water, then filtered, dried and recrystalized from Ethanol to give the pure compound. The progress of the reaction was checked by TLC using a mixture of EtOAc : Hexane (50:50) and completed in times of 24 hrs as given in **Table 1** (See **Scheme 2**).



Scheme 2: General Equation for Synthesis of 4-pyrazolone compounds.

2.2 Synthesis of 4-benzylidene-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (3).

0.7 g of **3** was obtained from 0.5g (2.8 mmol) of 3-methyl-1-phenyl-5-pyrazolone, and 0.29 g (2.8 mmol) of benzaldehyde and (1ml) of piperidine as mentioned above. White crystals of **3** from ethanol with melting point 195-197 °C and yield 93%.

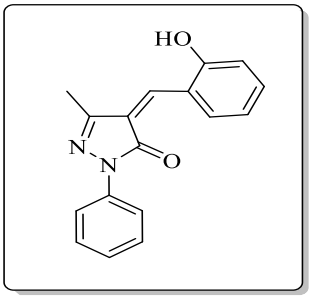


Chemical Formula: $C_{17}H_{14}N_2O$, Mol.Wt 262.31

The spectral data for **3** : ^{1}H-NMR (400 MHz, $CDCL_3$) δ (ppm) : δ7.2 (s, 1H; olefinic proton), 7.65 (d, 2H; H-ph, *J* = 7.4 Hz), 7.31 (t, 2H; H-ph, *J* = 7.4 Hz), 7.12 (t, 1H; H-ph, *J* = 7.4 Hz), 7.38-7.5 (m, 5H, H-Ar), 1.29 (s, 3H, $-CH_3$).
^{13}C-NMR (101 MHz, $CDCL_3$) δ 22.1, 122, 125.1, 128, 129.5, 139.8, 144.1, 147.9, 158.5.
Mass data: The molecular weight of compound **3** is 262 and the mass spectral data matching the same as 263 *m/e* showing the M^++1 peak.

2.3 Synthesis of 4-(2-hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (5).

Yellowish white crystals obtained from ethanol with m.p. 173-175 °C and yield 75%.

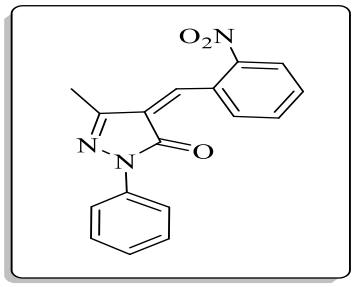


Chemical Formula: $C_{17}H_{14}N_2O_2$, M.W. 278.31.

The spectral data for **5** :^{1}H-NMR(400 MHz,DMSO-d_6) : δ2.1(s,3H;-CH_3), 7.35 (s,^{1}H; olefinic proton), 5.0(s,^{1}H; -OH), 6.6-6.8(m,4H;H-Ar),7.0(t,1H;H-ph, *J* = 7.5 Hz), 7.4(t,2H;H-Ph, *J* = 7.5 Hz), 7.6-7.8 (dd , 2H; H-Ph, *J* = 7.5 Hz).
^{13}C-NMR (101 MHz, DMSO-d_6): δ22.4, 115.1, 118, 119.8, 123, 126.1, 128.2, 130.1, 134.2, 140, 142.1, 146.5, 154.1, 159.2.

2.4 Synthesis of 5-Methyl-4-(2-nitrobenzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (7).

Yellow crystals from ethanol; m. p. 210-212 °C and yield 70 %.

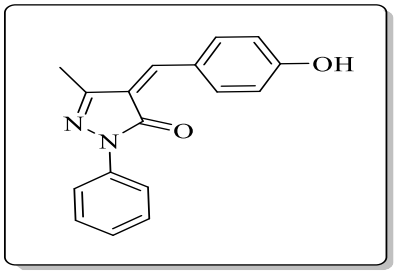


Chemical Formula: $C_{17}H_{13}N_3O_3$, Mol.Wt 307.30.

The spectral data for **7** : ^{1}H-NMR (400 MHz, DMSO-d_6): δ7.9 **(**s**,** 1H; olefinic proton), 2.0 (s, 3H; -CH_3), 8.1 (d, 1H; H-Ar, *J* =7.5Hz), 7.3-7.4 (m, 3H; H-Ar), 7.25 (t, 2H, H-ph, *J* =7.5Hz), 7.1 (t,1H;H-ph, *J* =7.5Hz), 7.7-7.8 (dd, 2H; H-ph, *J* =7.5,1.5 Hz).
^{13}C-NMR (101 MHz, DMSO-d_6): δ 22.5, 119.9, 123.4, 126.9, 128.8, 130.8, 132.1, 139.1, 142.5, 146.7, 150.1, 158.9.
Mass data: the molecular weight of compound is 307 and the mass spectral data matching the same as 308 *m/e* showing the M^++1 peak.

2.5 Synthesis of 4-(4-hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (9) .

Light yellow crystals from ethanol; m.p. 199-200°C and yield 92 %.

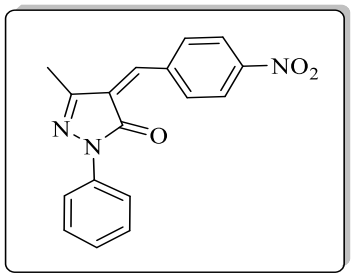


Chemical Formula: $C_{17}H_{14}N_2O_2$, M.W. 278.31.

The spectral data for **9** : ^{1}H-NMR (400 MHz,DMSO-d_6): δ 1.1 **(**s, 3H; -CH_3), 7.03 (s, 1H; olefinic proton), 6.6 (d, 2H; H-Ar, *J* =7.35 Hz), 7.2 (d, 2H; H-Ar, *J* =7.35 Hz), 4.5 (s, 1H;-OH), 7.7-7.9 (m, 5H; H-ph).
^{13}C-NMR (101 MHz, DMSO-d_6): δ22.5, 114.8, 119.4, 123.1, 124.6, 128.6, 129.4, 138.1, 141.4, 146.2, 155.1, 157.7.

2.6 Synthesis of-5-Methyl-4-(4-nitrobenzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (11) .

Glossy brown crystals from ethanol; m.p. 192-195°C and yield 72 %.

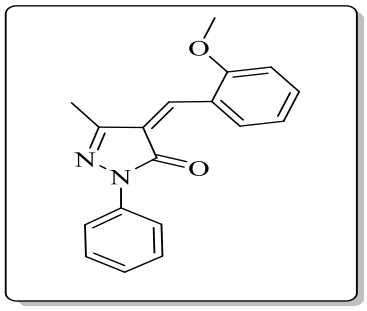


Chemical Formula: $C_{17}H_{13}N_3O_3$, M.W. 307.30.

The spectral data for **11** : ^{1}H-NMR(400 MHz,DMSO-d_6): δ8.15 (d, 2H; H-Ar, *J*= 7.5 Hz), 7.6 (d, 2H; H-Ar, *J*= 7.5 Hz), 7.3 (t, 1H; H-ph, *J*= 7.4 Hz), 7.8-7.9 (m, 4H; H-ph), 2.1(s, 3H; -CH_3), 7.15 (s, 1H;olefinic proton) .
^{13}C-NMR (101 MHz, DMSO-d_6): δ 22.1, 119.4, 123.0, 126.3, 128.1, 129.5, 130.9 139.6, 142.1, 145.1, 156.0, 159.9.
Mass data**:** the molecular weight of compound is 307 and the mass spectral data matching the same as 308 *m/e* showing the M^++1 peak.

2.7 Synthesis of 4-(2-methoxybenzylidene)-5-methyl-2-phenyl-2,4- dihydro-3H-pyrazol-3-one (13) .

Glossy White crystals from ethanol; m.p. 202-203°C and yield 97 %.

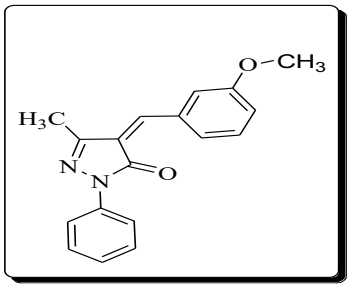


Chemical Formula: $C_{18}H_{16}N_2O_2$, Mol. Wt292.12.

The spectral data for **13** : ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 6.72-7.21 (m, 9H; H-Ar, H-ph), 3.75 (s, 3H; -OCH_3), 2.15 (s, 3H; -CH_3), 7.3 (s, 1H; olefinic proton) .
^{13}C-NMR (101 MHz, DMSO-d_6): δ 22, 55.6, 112, 119,120.1, 120.2, 123, 126.3, 128, 129.8, 135.3, 141.4, 146.5, 148.4, 156.8, 159.7.
Mass data**:** the molecular weight of compound is 292 and the mass spectral data matching the same as 293 *m/e* showing the M^++1 peak.

2.8 Synthesis of 4-(3-methoxybenzylidene)-5-methyl-2-phenyl-2,4- dihydro-3H-pyrazol-3-one (15).

Yellowish white crystals from ethanol; m.p. 227-228°C and yield 93 %.

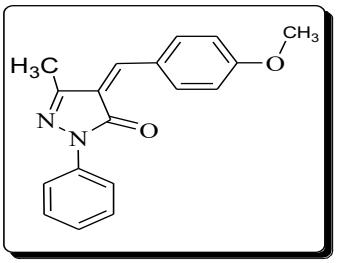


Chemical Formula: $C_{18}H_{16}N_2O_2$, Mol. Wt292.12.

The spectral data for **15** : ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 3.7 (s, 3H; -OCH_3), 2.1 (s, 3H; -CH_3), 7.15 (s, 1H; olefinic proton), 6.63 (d, 1H; H-Ar, *J*= 7.5 Hz), 6.80 (d, 2H; H-Ar, *J*= 7.5 Hz), 7.0 (t, 1H; H-Ar, *J*= 7.5 Hz), 7.24 (t, 2H; H-ph, *J*= 7.5 Hz), 7.75 (d, 2H; H-ph, *J*= 7.5 Hz), 7.1 (t, 1H; H-ph, *J*= 7.5 Hz) .
^{13}C-NMR (101 MHz, DMSO-d_6): δ 22.3, 55.5, 111.7, 115.8, 119.5, 121.8, 123,9, 125.8, 127, 134.7 141.4, 146.1, 149.1, 158.1, 160.1.
Mass data**:** the molecular weight of compound is 292 and the mass spectral data matching the same as 293 *m/e* showing the M^++1 peak.

2.9 Synthesis of 4-(4methoxybenzylidene)-5-methyl-2-phenyl-2,4- dihydro-3H-pyrazol-3-one 17 .

Shiny white crystals from ethanol; m.p. 207-206°C and yield 87.5 %.

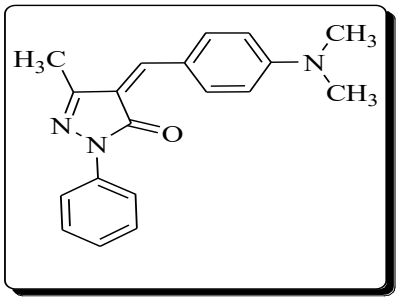


Chemical Formula: $C_{18}H_{16}N_2O_2$, Mol.Wt 292.12.

The spectral data for **17** : ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 3.71 (s, 3H; -OCH_3), 2.2 (s, 3H; -CH_3), 7.1 (s, 1H; olefinic proton), 6.7 (d, 2H; H-Ar, *J* = 7.5 Hz), 7.9 (d, 2H; H-Ar, *J* = 7.5 Hz), 7.19 (t, 1H; H-ph, *J* = 7.5 Hz), 7.25-7.49 (m, 4H; H-ph).
^{13}C-NMR (101 MHz, DMSO-d_6): δ 22.5, 55.4, 113.6, 119.5, 123.2, 125.2, 128.6, 129.4, 139.7, 142.9, 146.2, 157.3, 159.7 .
Mass data**:** the molecular weight of compound is 292 and the mass spectral data matching the same as 293 *m/e* showing the M^++1 peak.

2.10 Synthesis of 4-(4 dimethylamino)benzylidene)-5-methyl-2-phenyl-2,4- dihydro-3H-pyrazol-3-one (19) .

Bright red crystals from ethanol; m.p. 204-205°C and yield 88.8 %.

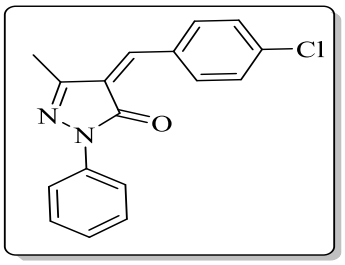


Chemical Formula: $C_{19}H_{19}N_3O$, Mol. Wt.305.38.

The spectral data for **19** : ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 2.3 (s, 3H; -CH_3), 7.25 (s, 1H; olefinic proton), 6.7 (d, 2H; H-ph, *J* =7.35 Hz), 7.2 (t, 1H; H-ph, *J* =7.35 Hz), 7.4 (t, 2H; H-ph, *J* =7.35 Hz), 3.2 (s, 6H; $N(CH_3)_2$), 8.0 (d, 2H; H-Ar, *J* =7.4 Hz), 8.5 (d, 2H; H-Ar, *J* =7.4 Hz).
^{13}C-NMR (101 MHz, DMSO-d_6): δ13.5, 40.2, 111.6, 119.3, 121.5, 122.3, 124.4, 128.8, 137.4, 139.2, 146.2,148.7, 151.2, 163. Mass data**:** the molecular weight of compound 19 is 305.3 and the mass spectral matching the same as 306.1*m/e* showing the M^++1 peak.

2.11 Synthesis of4-(4-chlorobenzylidene)-5-methyl-2-phenyl-2,4- dihydro-3H-pyrazol-3-one (21).

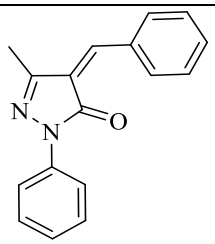
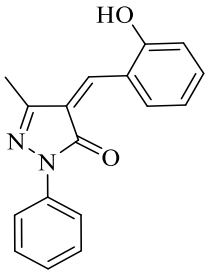
Pink crystals from ethanol; m.p. 201-203°C and yield 77.7 %.

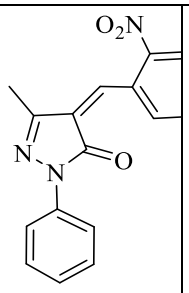
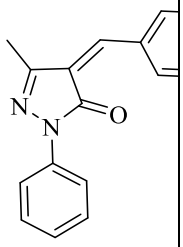
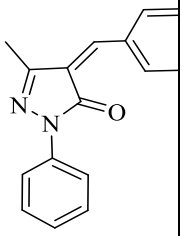
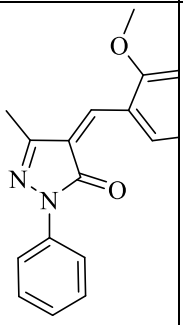
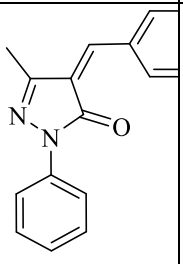
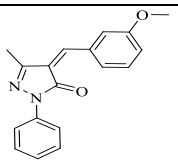


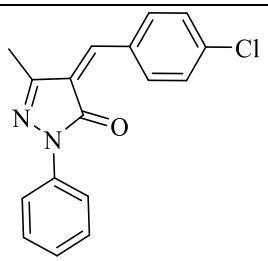
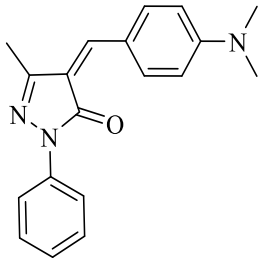
Chemical Formula: $C_{17}H_{13}CLN_2O$, Mol. Wt296.5.

The spectral data for **21** : ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 2.1(s, 3H; -CH_3), 7.1 (s, 1H; olefinic proton), 7.8 (d, 2H; H-Ar, *J* = 7.5 Hz), 7.0 (t, 1H; H-ph, *J* = 7.5 Hz), 7.2-7.4 (m, 6H; H-Ar, H-ph).
^{13}C-NMR (101 MHz, DMSO-d_6): δ13.5, 113.9, 120, 123.8, 128, 128.7, 130.3, 133, 141.3, 143.7, 146.8, 158 .
Mass data**:** the molecular weight of compound is 296.5 and the mass spectral data matching the same as 297.0 *m/e* showing the M^++1 peak.

Table 1. The physical characteristics of compounds.

S.No.	Compound	Structure	Solvent	Yield	m.p
1	**3**		ethanol	93%	195-197 °C
2	**5**		ethanol	75%	173-175 °C

3	**7**		ethanol	70%	210-212 °C
4	**9**		ethanol	92%	199-200 °C
5	**11**		ethanol	72%	192-195 °C
6	**13**		ethanol	97%	202-203 °C
7	**15**		ethanol	%87.5	206-207°C
8	**17**		ethanol	%93	227-228°C

9	**19**		ethanol	77.7%	201-203
10	**21**		ethanol	88.8%	204-205°C

3 Results and Discussion

The conventional knoevenagel condensation methodology was adopted to synthesize the titled compounds, which are characterized by TLC; melting points, and they have been assigned structures on the basis of their spectral data.

The starting material was 3-methyl-1-phenyl-5-pyrazolone **1**, which is commercially available. It is considered as an acidic hydrogen compound in knoevenagal condensation and so was converted into a nucleophile at 4-position by treatment with piperidine. Then, it was added to a carbonyl group of some benzaldehydes substituted with electron-donating and with electron-withdrawing groups to synthesize the target products in good yields in a period of time (24 hrs) determined by TLC using EtoAc : hexane mixture (50:50). The products were obtained as a result of **knovenagel condensation** according to the following mechanism **(Scheme 3).**

The 3-methyl-1-phenyl-5-pyrazolone **1** was added to benzaldehyde **2** in absolute ethanol at 0 °C for a period of time (24 hrs) affording 4-benzylidene-5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **3** as shown below (**Scheme 4**).

The formed product **3** was characterized by TLC and its spectroscopic data.

The ^{1}H-NMR spectrum ($CDCl_3$) showed a signal at δ7.25 ppm for a vinylic proton of the α,β-unsaturated unit in addition to the disappearance of the signal of methylene protons of pyrazolone ring which resonates at δ3.2 ppm In addition, the ^{13}C-NMR spectrum revealed the absence of a signal at δ28.5 ppm for methylene carbon of pyrazolone ring, and showed resonance signal of olefinic carbons at δ139.8 ppm. Furthermore, its mass spectrum showed M^{+}+1 ion peak at 263 *m/e*, corresponding to a molecular mass 262 of **3**. Based on the above data, the structure was assigned for compound **3**.

Condensation of 3-methyl-1-phenyl-5-pyrazolone **1** with *o*-hydroxybenzaldehyde **4** for the preparation of 4-(2-pyrazol-3-one **5** is briefly illustrated bellow **(Scheme 5)**.

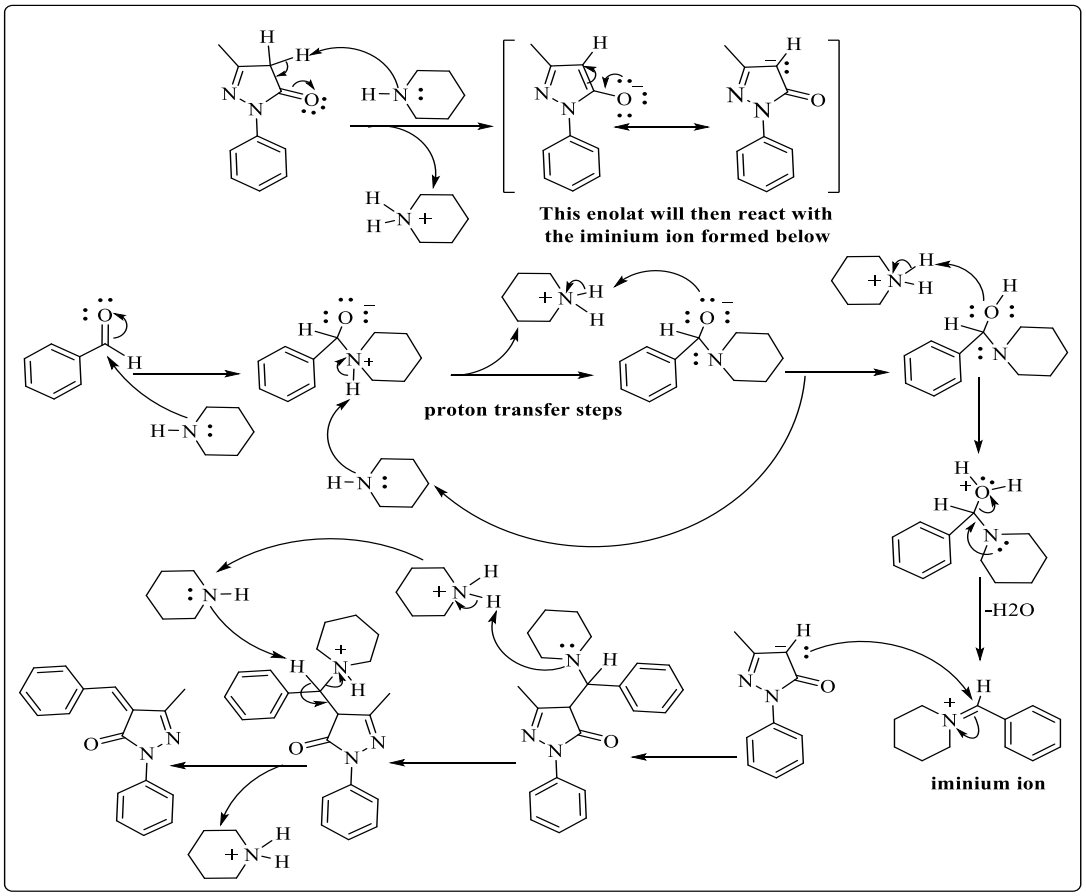
The ^{1}H-NMR spectrum (DMSO-d_6) showed a resonance signal of β-H (olefinic proton) at δ7.35 ppm indicates a shifting of this poroton to the lower field due to inductive effect (driving force) of hydroxyl group in ortho- position compared to that of olelifinic proton in case of un substituted benzaldehyde (compound **3**), and asinglet signal at δ 5.0 ppm for the OH-group.

The rest of signals are presented in the experimental part. On the other hand, its ^{13}C-NMR spectrum of this molecule consists as expected of one line in the sp^3-region and 12-lines in the sp^2-region in addition to one line in the carbonyl carbons region confirm the structure of the molecule.

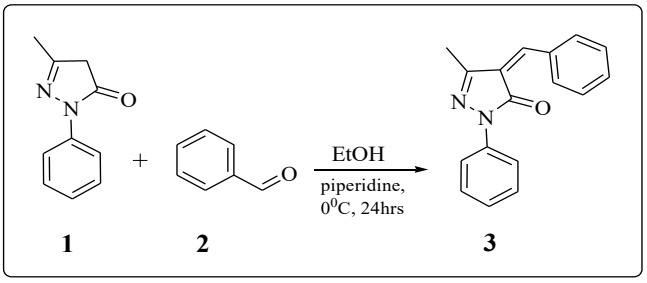
Reaction of 3-methyl-1-phenyl-5-pyrazolone **1** with *o*-nitrobenzaldehyde **6** in ethanol in presence of catalytic amount of piperidine gave 5-methyl-4-(2-nitro benzylidene)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **7** as shown below **(Scheme 6).**

Its structure assignment was based on the spectral data. The ^{1}H-NMR spectrum (in D_6-DMOS) revealed the clear signal at δ7.9 ppm for the olefinic proton indicating and proving that the nucleophilic addition reaction and formation of condensed product **7** have taken place. Due to the withdrawal effect of -NO_2 group in the ortho position, it caused the shift of olefinic proton signal to lower field, i.e. from δ7.15 ppm in the case of product **11** into δ7.9 ppm. On the other hand, its ^{13}C-NMR spectrum (in D_6-DMSO) showed 13 resonance lines as expected and a signal at δ126.9 and δ146.7 ppm for β and α-olefinic carbons, respectively, as well as the absence of a resonance signal for methylenic carbon of the pyrazolone ring at δ28.5 ppm, revealing the formation of the product **7**. Furthermore, its mass data showed M^{+}+1 peak at 308.0 *m/z*, matching the molecular mass of **7**.

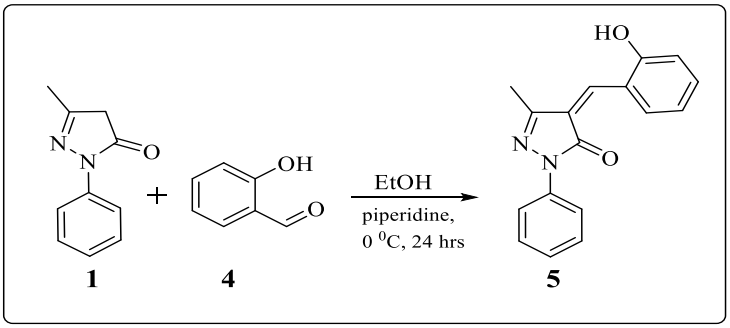
Treatment of 3-methyl-1-phenyl-5-pyrazolone **1** with *p*-hydroxybenzaldehyde **8** in the same manner as mentioned above (**Scheme 6**) afforded product **9**.



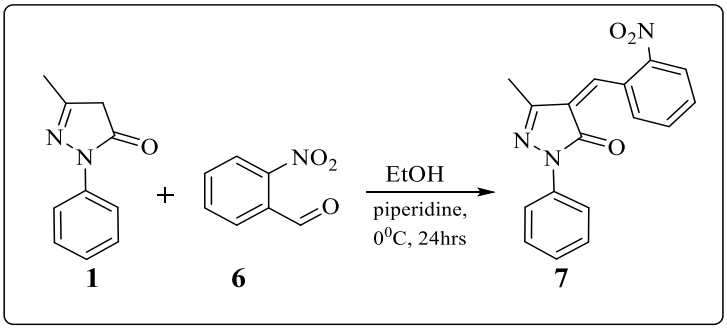
Scheme 3. General reasonable mechanism for the formation of products.



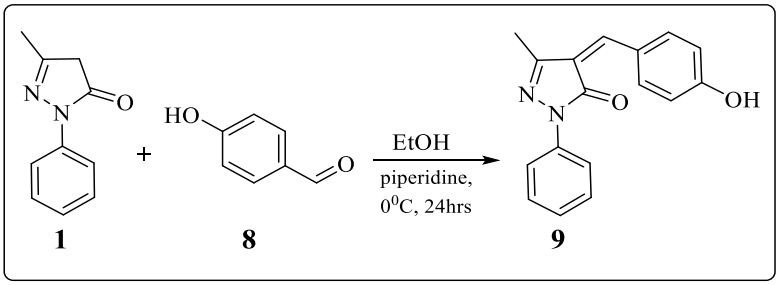
Scheme 4



Scheme 5



Scheme 6



Scheme 7

The correct structure for **9** was established based on its spectral data. In its ^{1}H-NMR spectrum (DMSO-d_6) the olefinic proton (β-H) resonated at high field δ7.03 ppm, compared with that in product **5** which means that the inductive effect of **-**OH group (P-postion) has no effect on the resonance of olefinic proton. On the other hand, the ^{13}C-NMR spectrum showed 12 lines at 22.5, 114.8, 119.4, 123.1, 124.6, 128.6, 129.4, 138.1, 141.4, 146.2, 155.1, 157.7.

Condensation of 3-methyl-1-phenyl-5-pyrazolone **1** with *p*-nitrobenzaldehyde **10** in the presence of piperidine, furnished the 5-methyl-4-(4-nitrobenzylidene)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **11** in accordance with the same manner in (**Scheme8)**.

The structure of **11** was characterized by TLC and NMR, mass spectra. Its ^{1}H-NMR spectrum showed beside the predictable aromatic signals, i.e. a doublet at δ8.15 ppm, a doublet at δ7.6 for protons of aromatic ring, and a triplet at δ7.3 ppm for one proton of phenyl ring attached to pyrazolone ring in

addition to multiplet at δ 7.8-7.9 ppm for the rest of protons attached to phenyl ring respectively, The singlet at δ7.15 ppm for proton attached to olefinic carbon. On the other hand, its ^{13}C-NMR spectrum. Displayed along with the aromatic signal, there are four signals at δ22.1, 142.1, 145.1, 156.0 ppm in addition to a signal at 159.9 ppm for the carbonyl carbon. The mass spectrum of **11** observed the molecular weight of the compound **11** is 307 and the mass spectral data matched M^{+}+1 peak at *m/z* 308.0.

methoxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3*H*pyrazol-3-one **13 (Scheme9**).

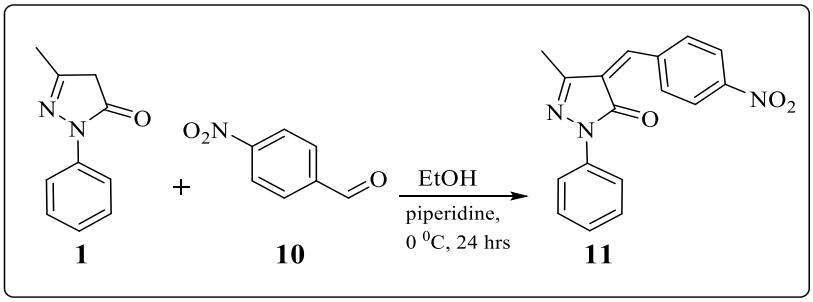
Its ^{1}H-NMR spectrum (DMSO-d_6) showed a singlet at δ7.3 ppm for olefinic proton, a singlet at δ2.15 ppm for methyl protons, and another signal at δ3.75 ppm for protons of the methoxy group. The rest of protons are written in the experimental part. The ^{13}C-NMR spectrum of **13** also confirmed the expected structure by giving two carbon signals in sp^3-region and the two signal at δ126.3 and 146.5 ppm of olefinic carbons. Finally, its mass spectrum was in agreement with its (M^{+}+1) at 293.1 *m/z*.

In addition to the above mentioned compounds **(3,5,7,9,11,13)** the physical characterization for compounds **(15**,**17,19,21)** including ^{1}H-NMR, ^{13}C-NMR, MS spectra have been measured, and are presented in the experimental part.

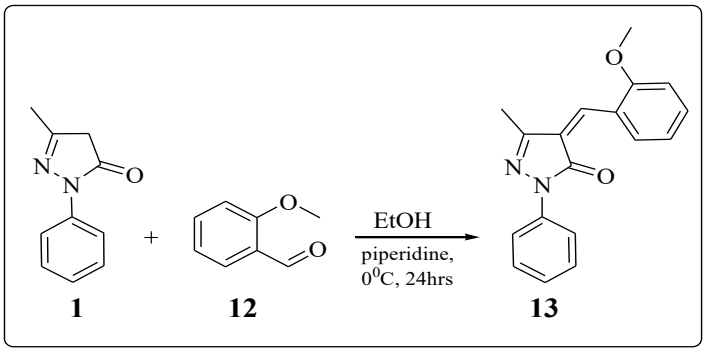
4 Antimicrobial Activities

4.1Microorganisms Used

The following microorganisms, Gram negative Bacteria: *Pseudomonas aeruginosa* (MTCC424) and *Escherichia Coli (*MTCC1302**)**; Gram positive Bacteria: *Staphylococcus aureus* (MTCC96), *Streptococcus pyogenes* (MTCC442), and fungal strains: *Aspergillus niger* (MTCC282), *Penicillium chrysogenum* (MTCC5108), growth The bacteria and fungi were cultured in nutrient agar and malt extract, respectively.



Scheme 8



Scheme 9

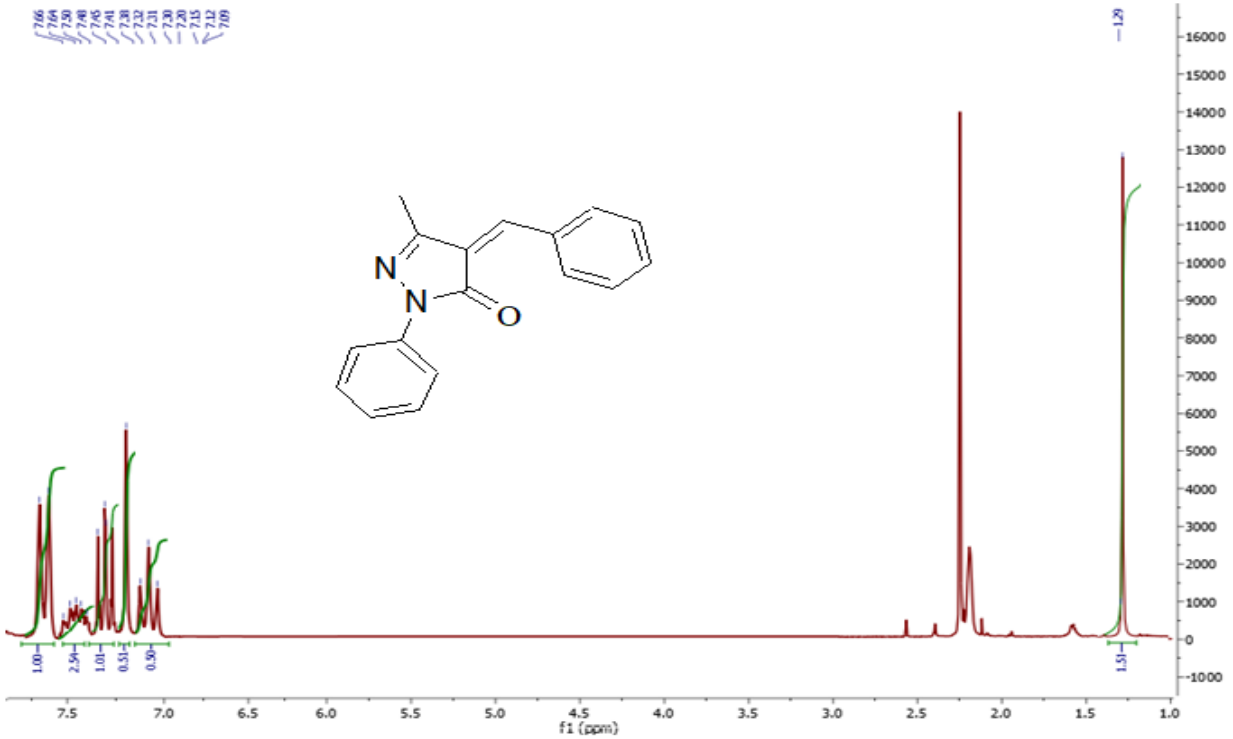


Fig. 2. ^{1}H-NMR($CDCl_3$, 400 MHz) for compound **3**

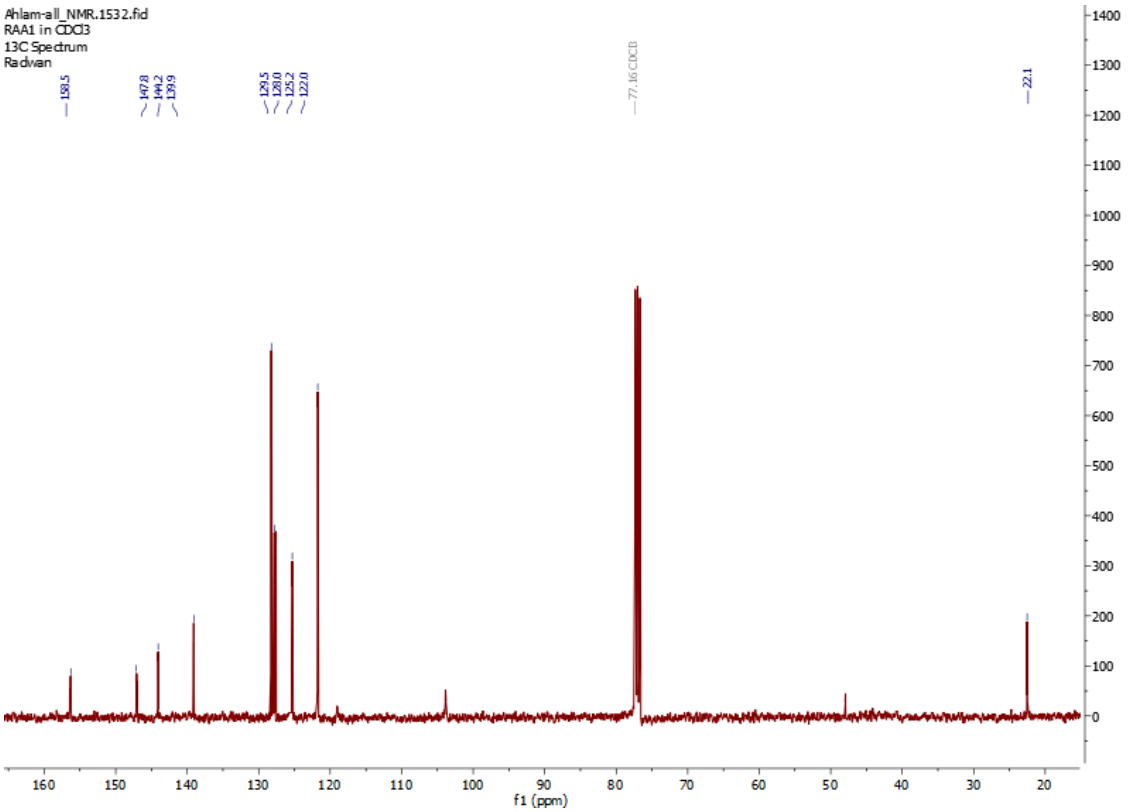


Fig. 3 ^{13}C-NMR ($CDCl_3$, 101 MHz) for compound **3**

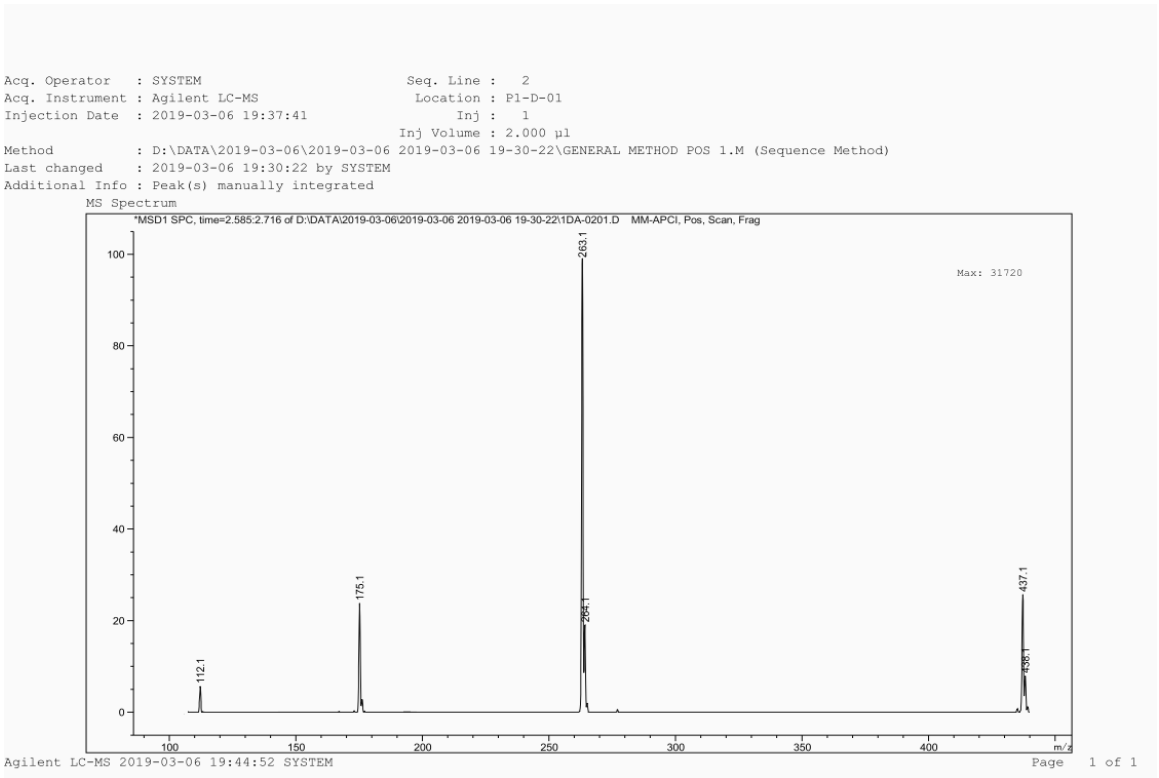


Fig.4Ms Spectrum Graph for compound **3**

4.2 Method Used for Screening

In the in-vitro evaluation of the antimicrobial activity, the 'agar holes well method was used for the antimicrobial susceptibility testing. Antimicrobial potentialities were expressed as the diameter of inhibition zones. Chemical extracts were examined as antimicrobial agent against all microbial isolates. Inoculum suspensions of all bacteria and fungi isolates were spread on the surface media. Equidistant (1 cm diameter) holes were made in the agar using sterile Cork borer, and dimethyl sulphoxide (DMSO) was used as a control, in media plates (10 x 10 cm), which had previously been seeded with bacteria and/or fungi tested, were filled by 100 µL concentration 10% with each extract. Plates were left in a cooled incubator at 4 (±2) °C for one hour and then incubated at 37 (±2) °C for 24 hour for bacterial growth and 28 ºC for 48 hours for fungal growth. Inhibition zones developed due to active extract ingredients were measured after 24-48 hours of incubation. The experiments were carried out in triplicates and the zone of inhibition was measured with the help of standard scale (Abd-El-Kader, 1995). [8]

Antimicrobial activity of derivatives: Susceptibilities to pathogenic bacterial isolation for the used DMSO extract of compounds were investigated by measuring their inhibitory, where both Gentamicin and Ampicillin were used as standard antibiotics for comparison (drug control), and dimethyl sulphoxide (DMSO) was used as a control (solvent control). The investigation results were shown in **(Table 2, Fig. 5, and plate 1, 2)**.

Table 2: Antimicrobial activity of tested compounds.

Samples		Inhibitory zones diameter(mm)					
		Gram (Ve^-)[a]		Gram (Ve^+)[a]		Fungi	
		E.coli	*P. aeruginosa*	*S. pyogenes*	*S. aurous*	*A.niger*	*P. chrysogenum*
Control: DMSO		-	-	-	-	-	-
Stander antibiotics	Gentamicin	-	-	21	13	-	14
	Ampicillin	-	-	2	15	-	16
3		12	-	-	18	-	-
5		-	-	-	-	-	-
7		3	3	-	1	-	3
9		5	5	5	5	-	2
11		12	-	-	18	-	-
13		-	-	-	-	-	-
15		3	5	-	3	-	2
17		3	5	-	3	-	-
19		3	3	-	1	-	3
21		-	-	-	-	-	-

Gram (Ve^-) = Gram negative Bacteria, Gram (Ve^+) = Gram positive Bacteria

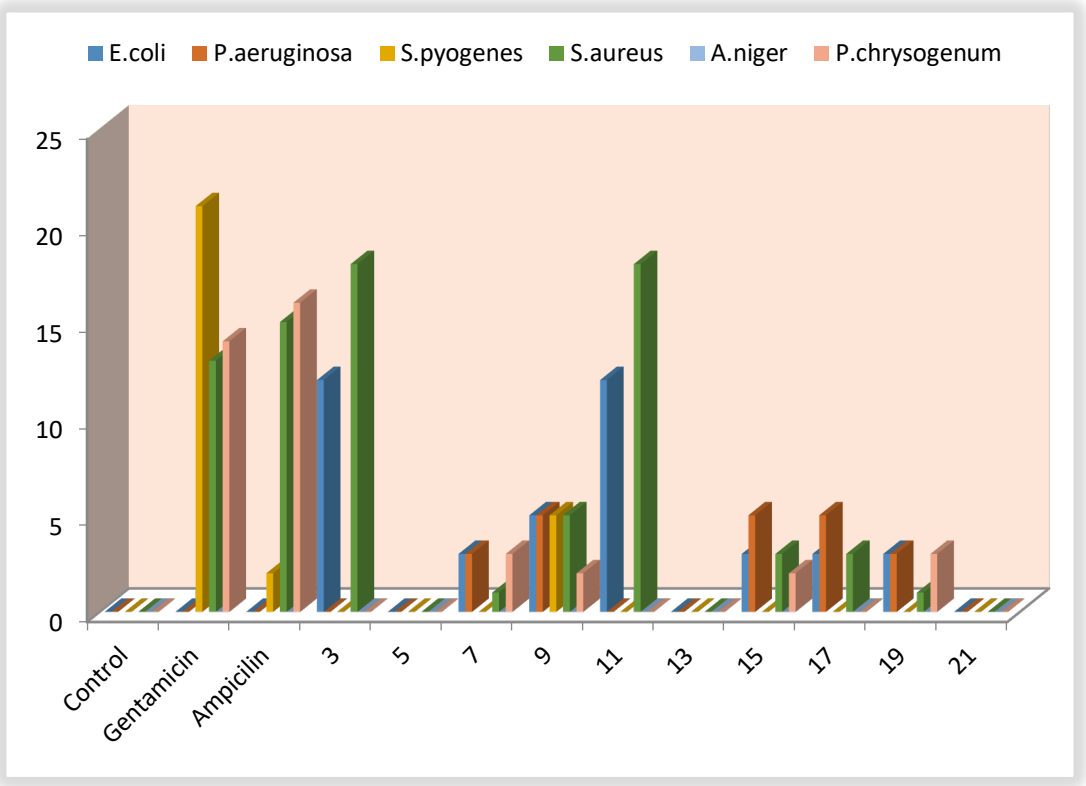
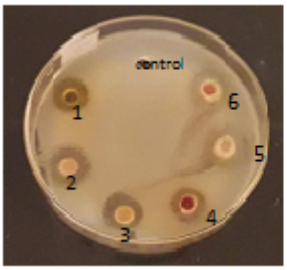
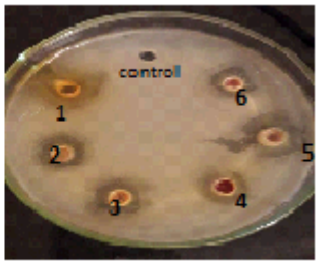


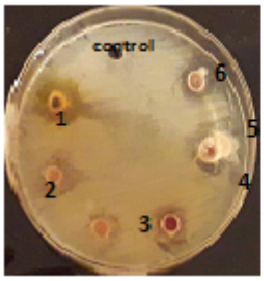
Fig. 5: Graphical representation of antimicrobial activity.



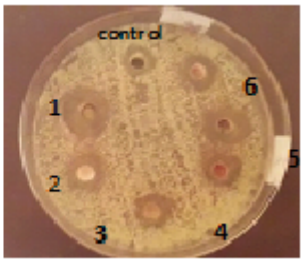
P.aeruginosa



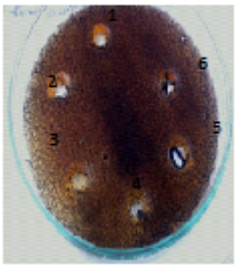
E.coli

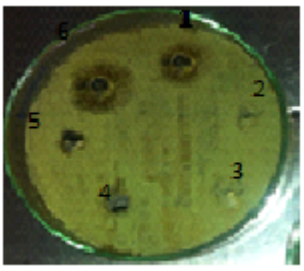


S. aureus



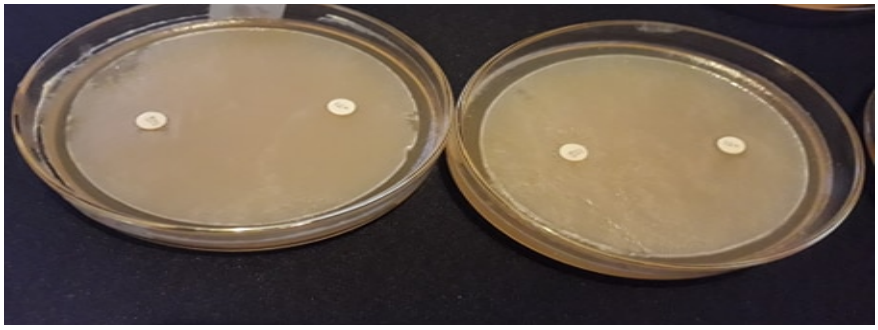
S. pyogenes



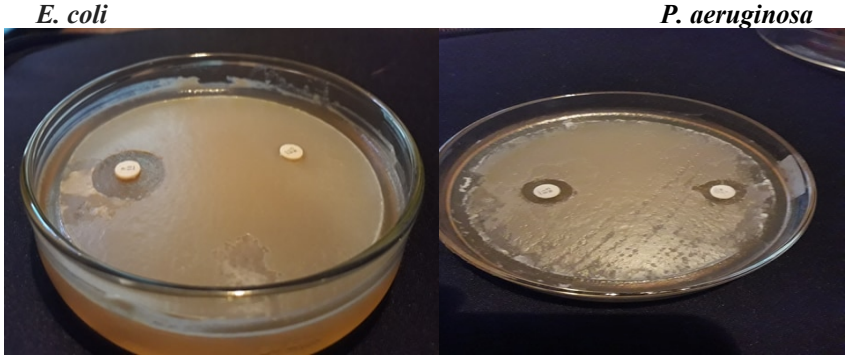


A.niger ***P.chrysogenum***

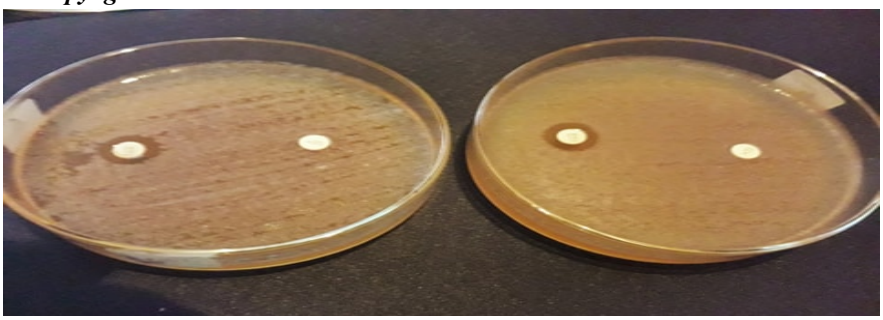
Plate 1. Inhibition zone produced by the synthetic DMSO extractagainst gram-positive, gram-negative bacteria and fungi.



E. coli ***P. aeruginosa***



S. pyogenes ***S. aureus***



A.niger ***P. chrysogenum***

Plate 2: Inhibition zone produced by the synthetic standard anti bioticsagainst gram- positive, gram-negative bacteria and fungi.

Compounds exhibited significant activity against some of the tested microorganisms.
In addition, compounds **9, 15 and 17** showed moderated activity against gram negative Bacteria *(E. coli* and *P. aeruginosa*) only. Whereas compounds **7** and **19** showed negligible activity against all microorganisms.

Acknowledgment

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