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Research Article

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Synthesis and characterization of some new pyrazolines derivatives and their biological activity

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ABSTRACT

Six different hydrazine's (hydrazine hydrate 80%, phenyl hydrazine, thiosemicarbazide, semicarbazide,2,4-dinitrophenyl hydrazine and 4- phenyl thiosemicarbazide) were reacted with six different types of substituted chalcones α,β- unsaturated ketenes to yield pyrazole derivatives then characterized the products with infrared spectra, elemental analysis, mass spectra, ^{1}H and ^{13}C-NMR spectra. Finally their biological activity for some of the new pyrazoles were studied.

Keywords: pyrazolines derivatives, biological activity

INTRODUCTION

Benzalacetophenones(Chalcones) constitute an important class of naturally occurring flavanoid compounds that exhibit a wide spectrum of biological activities and are well known as intermediates for the synthesis of various heterocyclic products[1-4].

Chalcones(Benzalacetophenones) are useful synthons [5-7] in the synthesis of alarge number of bioactive molecules , such as pyrazolines [8] and isoxazoles that are well known nitrogen containing heterocyclic compounds [9-15]. The discovery of this class of compounds provides an outstanding case history of modern drug development and also emphasizes the unpredictability of biological activity from structure modification of a prototype drug [16, 17] molecule. The Benzalacetophenone were synthesized by the Claisen – Schmidt condensation reaction and the prazoles derivatives have been synthesized by the treatment of the appropriate chalcones (1-VI) with hydrazinehydrate, phenylhydrazine, semicarbazide, thiosemicarbazide, 2,4-dinitrophenylhydrazine and 4-phenylthiosemicarbazide in catalytic amount of sulpharic acid in ethanol.

EXPERIMENTAL SECTION

All the chemicals were supplied from Merck , Aldrich and Fulka chemicals company and used as received .

Uncorrected melting points were determined by using thermal scientific apparatus.

Fourier transform Infrared spectra were recorded using potassium bromide discs on 84005,Shimadzu, Japan . spectrophotometer and the measurements were made at chemistry department , education college for pure science / Basrah University .

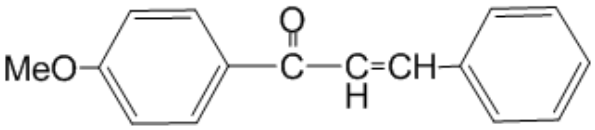
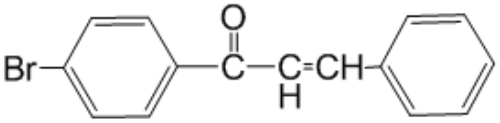
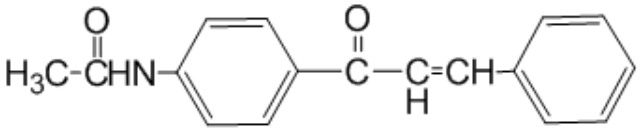
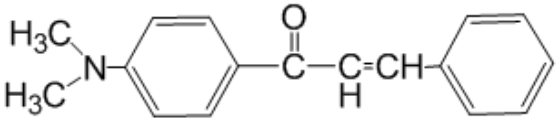
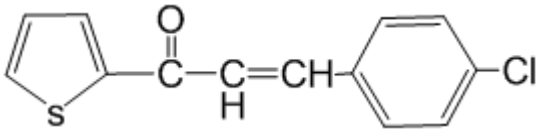
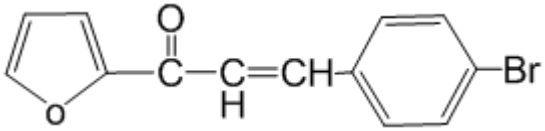
^{1}HNMR and ^{13}C NMR spectra were carried in Iran by using Burker (400MHZ)

And the samples were reported in ppm (s) , DMSO and CDCl3 used as solvents with TMS as an internal standard while the mass spectra were done in Iran, by using Agilent technology (HP) model 5973 in Tehran university .

The elemental analysis were done in India using Carlo-Erbaanalyzer .

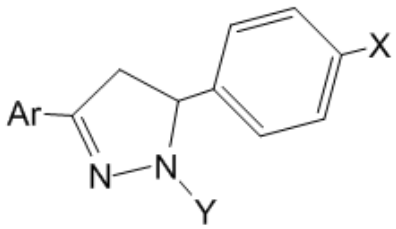
Procedure :
Table (1) shows the six different α,β- unsaturated ketones (Chalcones) which were prepared by using the claisen – Schmidt method once in basic media and twice by acidic media.

Table (1)The Prepared Chalcones , Physical data and its structure

Structure	M.p^{0}C	Yield %	Color / State	Rec.Solvent
	105	80	Yellow/Crystal	aq.Methanol
	104	75	Powder/Faint Brown	aq.Ethanol
	109-111	67	Powder/Faint Red	*aq.Methanol
	163-165	59	Crystal/Orange	*aq.EtOH
	127-129	65	Powder/White	aq.EtOH
	80-82	60	Powder/Green	aq.EtOH

**Using column of silica gel using (cyclohexane : benzene) (3:7)*

The general procedure for the preparation of pyrazolinederivatives (Schiff bases) .



To 0.01 mole of chalconesdissolved in 30 ml of aqueous ethanol , adding 0.01 mole of the six hydrazine's derivatives (hydrazine hydrate ; phenylhydrazine ,2,4-dintrophenylhydrazine semicarbazide, thiosemicarbazide and 4-phenyl thiosemicarbazide) in room temperature , with 3-drops of sulphuric acid as a catalytic amount (except for the hydrazine hydrate we does not used the acid). The reaction was heated then reflux for 8-12 hours depend on the ending of reaction by using thin layer chromatography using (Ethyl acetate : hexane) (8:2) then cooling and poured

the reaction mixtures on ice/water, then filtered and recrystallized from aq. Ethanol. Table (2) shows all the physical data , chemical structure time reaction for the thirty six condensing products.

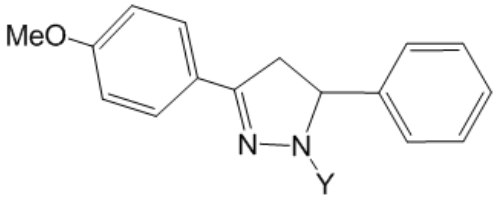
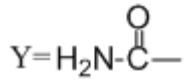
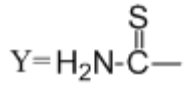
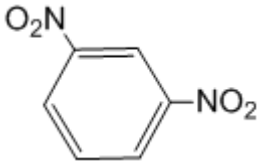
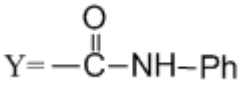
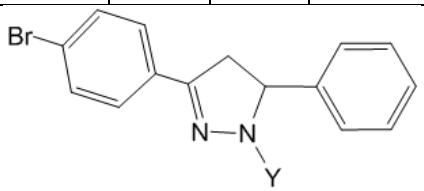
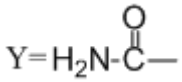
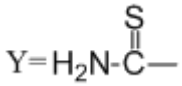
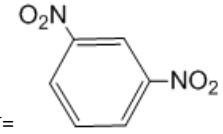
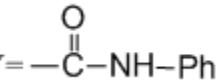
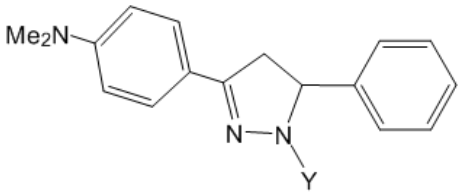


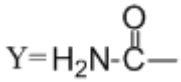
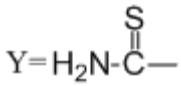
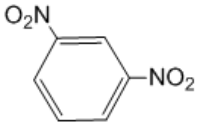
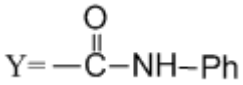
Table (2)The Pyrazoline derivatives data

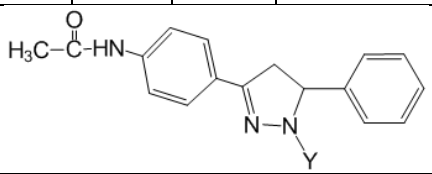
التركيب	M.p^{0}C	Yield %	Color /state	Time/Reaction hr
P1 	198-200	65	Yellow / powder	12
P2 	242-244	67	Orange / crystal	12
P3 y=H	190-191	68	Creamy/crystal	8
P4 y=ph	110-112	70	Dark blue crystal	12
P5 y= 	188-190	69	Red powder	12
P6 	203-205	68	Green crystal	12

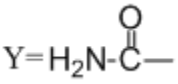
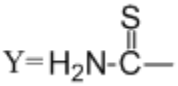
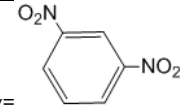
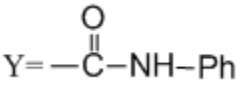


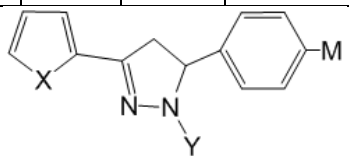
Subnet	M.p^{0}C	Yield %	State/color	Time of reaction (hrs)
P7 	124-126	66	Crystal/white	12
P8 	160-162	60	Yellow powder	12
P9 Y=H	193-195	70	Crystal/powder	8
P10 Y=ph	204-206	71	Powder/creamy	12
P11 Y= 	195-198	70	Yellow powder	12
P12 	105-106	72	Orange powder	12



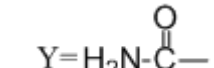
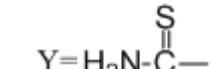
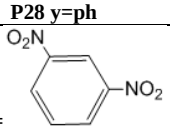
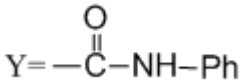
Subnet	M.p^{0}C	Yield %	State/color	Time of reaction (hrs)
P13 	146-148	76	Powder red	12
P14 	122-124	69	Grey crystal	12
P15 y=H	133-135	68	Brown crystal	8
P16y=ph	138-140	71	Brown powder	12
P17 y= 	192-194	61	Faint red crystal	12
P18 	220-222	60	Dark blue crystal	12

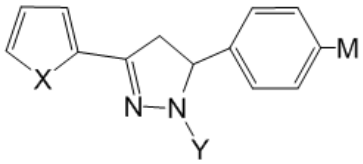


Subnet	M.p^{0}C	Yield %	State/color	Time of reaction (hrs)
P19 	133-135	61	Creamy crystal	12
P20 	190-192	60	Brown powder	12
P21 y=H	259-261	62	White crystal	8
P22 y=ph	135-137	59	Brown crystal	12
P23 y= 	313-314	61	Red crystal	12
P24 	196-198	53	Faint green powder	12

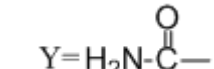
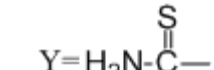
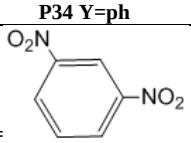
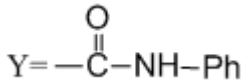


M=Cl, X=S

Subnet	M.p^{0}C	Yield %	State/color	Time of reaction (hrs)
P25 	126-127	72	Creamy crystal	12
P26 	89-91	77	Red powder	12
P27 Y=H	227-230	71	Creamy powder	8
P28 y=ph	128-130	69	Green powder	12
P29 y= 	203-205	65	Orange crystal	12
P30 	88-90	70	Brown powder	12



M=Br ,X=O

Subnet	M.p^{0}C	Yield %	State/color	Time of reaction (hrs)
P31 	71-73	71	Green powder	12
P32 	67-70	72	Yellow crystal	12
P33 Y=H	91-93	71	Green crystal	8
P34 Y=ph	122-124	72	Violet powder	12
P35 Y= 	180-182	70	Red powder	12
P36 	>300 dec.	68	Grey powder	12

RESULTS AND DISCUSSION

The chemical structure of the synthesized compounds were characterized with the help of TLC, FT.IR ,^{1}H , ^{13}C-NMR spectroscopy and mass spectroscopy. The I.R spectrum shows the characteristic band at 1500-1600 cm^{-1} due to the (C=N) imine group (Schiff base). There are disappearance for α,β- unsaturated carbonyl ketones (1600-1700) , also the presence of doublet of –CH2 near about (2-5) ppm in ^{1}H-NMR data confirmed the cyclisation of pyrazoline ring.

Treatment of Benzalacetophenones derivatives with six types of hydrazines [18]in boiling ethanol gave pyrazoline ring after purification and recrystallization from aqueous ethanol. With some exception for (P13 and P8), which does not give CH2 in pyrazoline ring due to the modification in experimental section (we were added the hydrazine derivatives with 10ml of glacial acetic acid to the Benzalacetophenone derivatives with catalytic amount of 4-5 drops of hydrochloric acid and then reflux for 4 hrs).

Pure pyrazoline derivatives (P1-P36) as shown in Table (2) in moderate yield. The elemental analysis (C H N) of the new products were fit and approximately identical with (± 0.5) error for the calculated and found data.

The mass spectra for two compounds P5 and P7 were done with (m/2-H+)418and 345 respectively

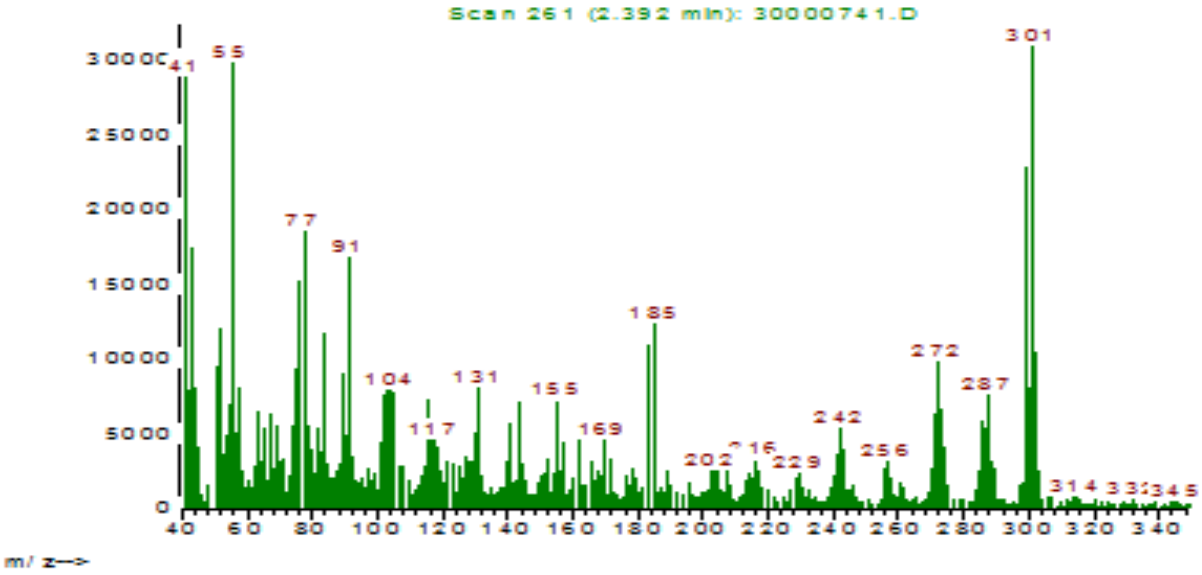


Figure.1 : Mass spectra for P5

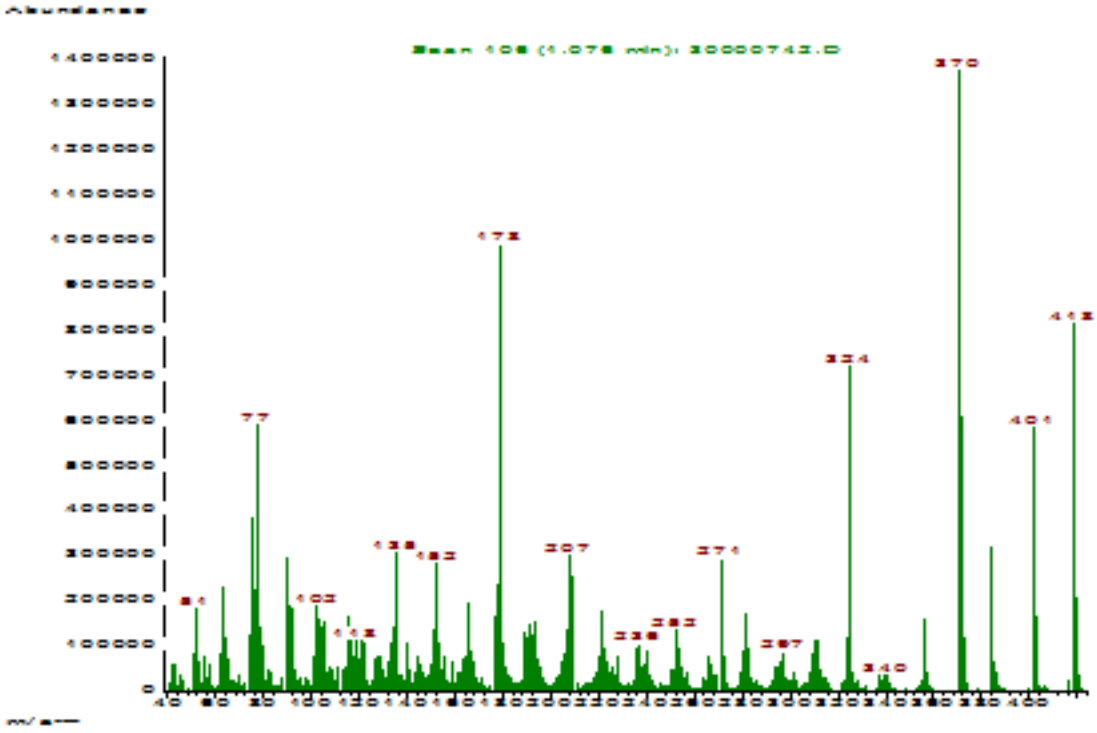


Figure.2 : Mass spectra for P7

Benzalacetophenones(Chalcones) were synthesized by Claisen –Schmidt condensation reaction. The correctness of the prepared condensed compound was fit from the literature and from TLC and melting point. The mechanism of the addition reaction of Benzalacetophenones with hydrazine (NH2NHY) were shown in Scheme (1) , it may be outlined as follows.

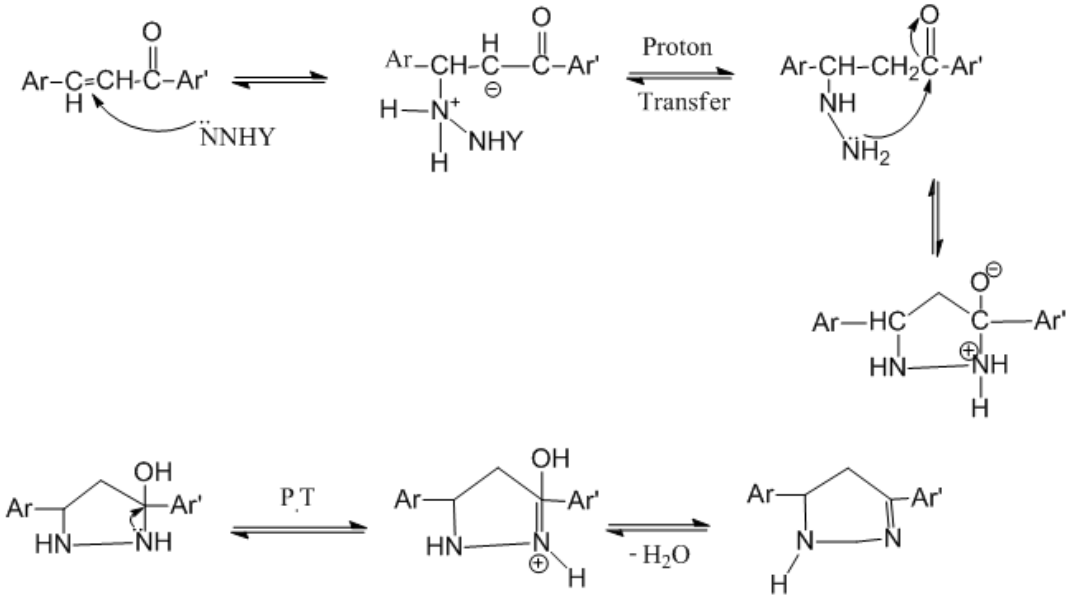


Table (3) shows the important selection in general important bands for IR data (cm^{-1})Which shows

C=N str. (w) 1614-1625 CH straliph.(m) 2880-2900
C-N str. (m) 1210-1219 NH (m) 3330-3338
C=C str. (w) 1590-1597
C-H str. (w) arom 3020-3024
w= weak
m = medium

Table (3) Some important selected stretching bands for some of the pyrazoline products

Compd. Symbol	Stretching											
	NH	NH2	Char.	Ch.alpt	C-O	C=N	C=C	C=S	ArO	C-O	C-S	C-N
P1	-	3437.15 3336.00	3250.00	3205.34	1068.00	1589.34	1456.26	-	-	1249.37	-	102801
P2	-	3375.43 3342.64	3250.05	3163.26	-	1602.85	1442.75	1471.69	-	1112.93	-	1089.78
P5	-	-	3483.44	3282.84	-	1616.35	1516.05	-	1257.59	1136.07	-	1085.92
P6	3336.85	-	3200.00	3055.24	-	1591.27	1450.47	1490.97	-	1176.58	-	1031.92
P8	-	3336.85 3319.00	3200.76	3059.10	-	1660.71	1514.12	1467.83	-	-	-	1070.49
P9	3047.53	-	2927.94	3054.92	-	1585.49	1521.84	-	-	-	-	1070.49
P10	-	-	3028.24	2910.58	-	1597.05	1570	-	-	-	-	1074.35
P15	3350.35	-	3150.00	3068.75	-	1645.28	1533.41	-	-	-	-	1186.22
P19	3228.84	3394.72 3334.92	3170.00	3050.00	1674.21	1541.27	1514.12	-	-	-	-	1016.49
P20	3267.41	3375.43 3298.28	3205.69	3186.40	1660.71	1589.39	1558.48	1494.83	-	-	-	1093.64
P22	3296.35	-	3296.34	3190.26	1670.35	1585.49	1494.83	-	-	-	-	1016.49
P27	3105.39	-	2971.94	-	-	1674.21	1517.98	-	-	-	1091.71	1058.92
P28	-	-	3028.24	2883.50	-	1597.06	1489.05	-	-	1134.14	1074.35	1008.71
P29	-	-	3261.63	3093.82	-	1616.35	1516.05	-	1255.66	1134.14	1103.28	1085.92
P33	3356.14	-	3197.98	3100	-	1587.42	1490.97	-	-	1149.57	-	1072.42
P34	-	-	3381.21	3273.20	-	1616.35	1500.62	-	1263.37	1138.00	-	1076.28
P35	3365.78	-	3309.89	3100.00	1697.36	1618.28	1510.26	-	-	-	-	1105.21

The ^{1}H-NMR spectra of pyrazoline compounds were shown in Fig (3) (P9) and Fig (4) (P13) while^{13}C shown in Fig (5) (P1) and Fig (6) (P10) for selected compounds respectively.

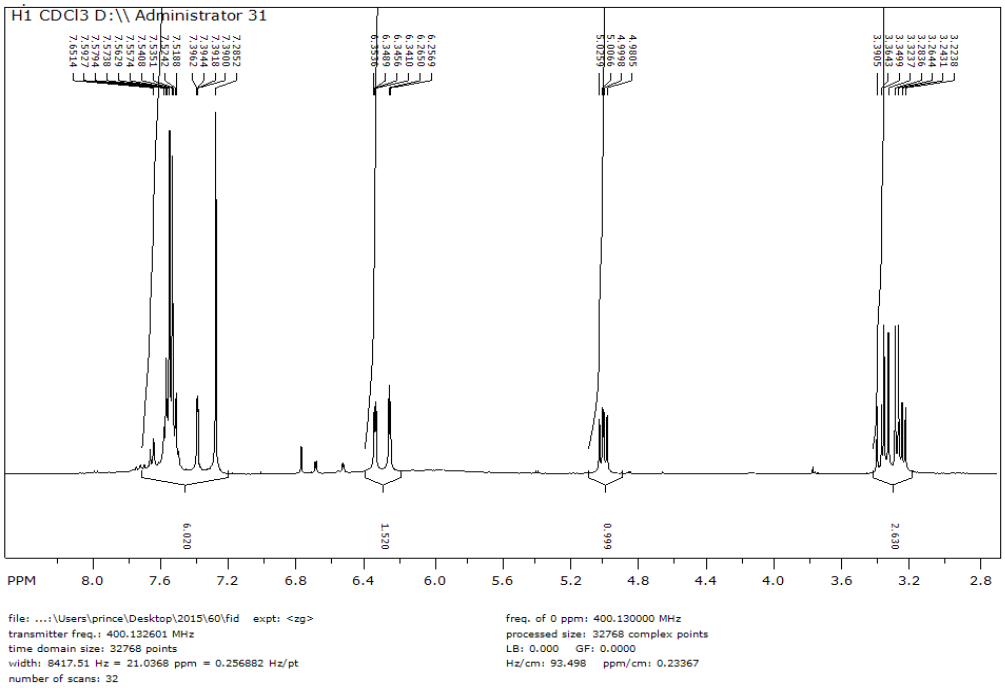


Figure : 3 Proton NMRfor P9

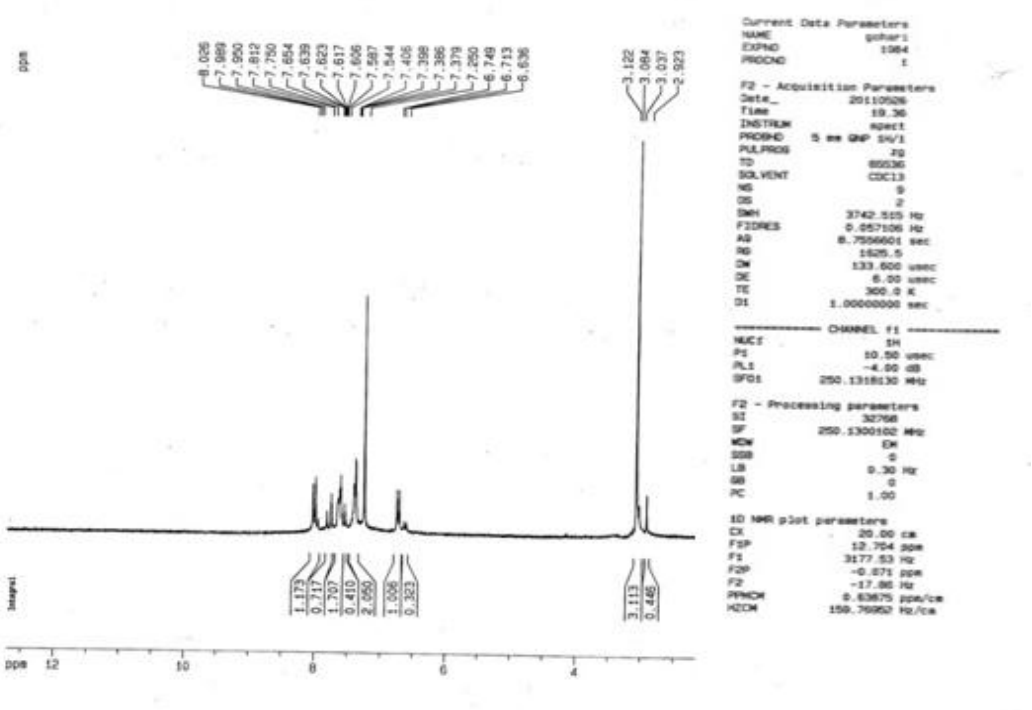


Figure : 4 Proton NMR for P13

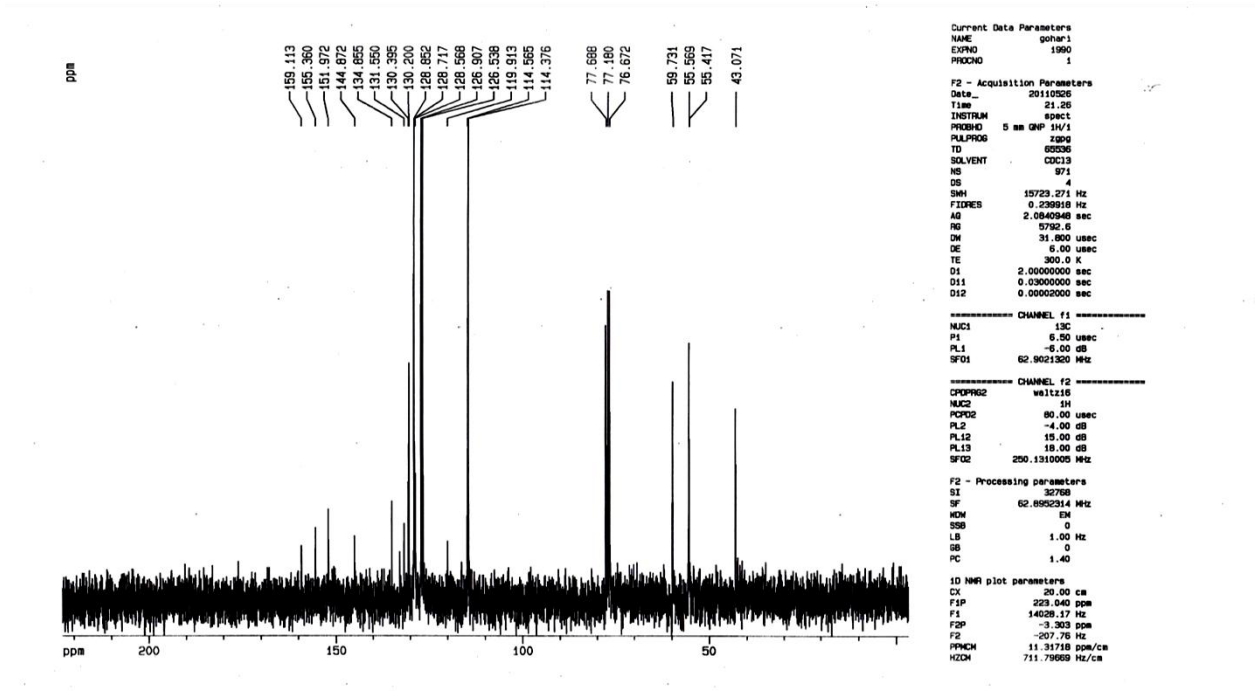


Figure : 5^{13}C NMR for P1

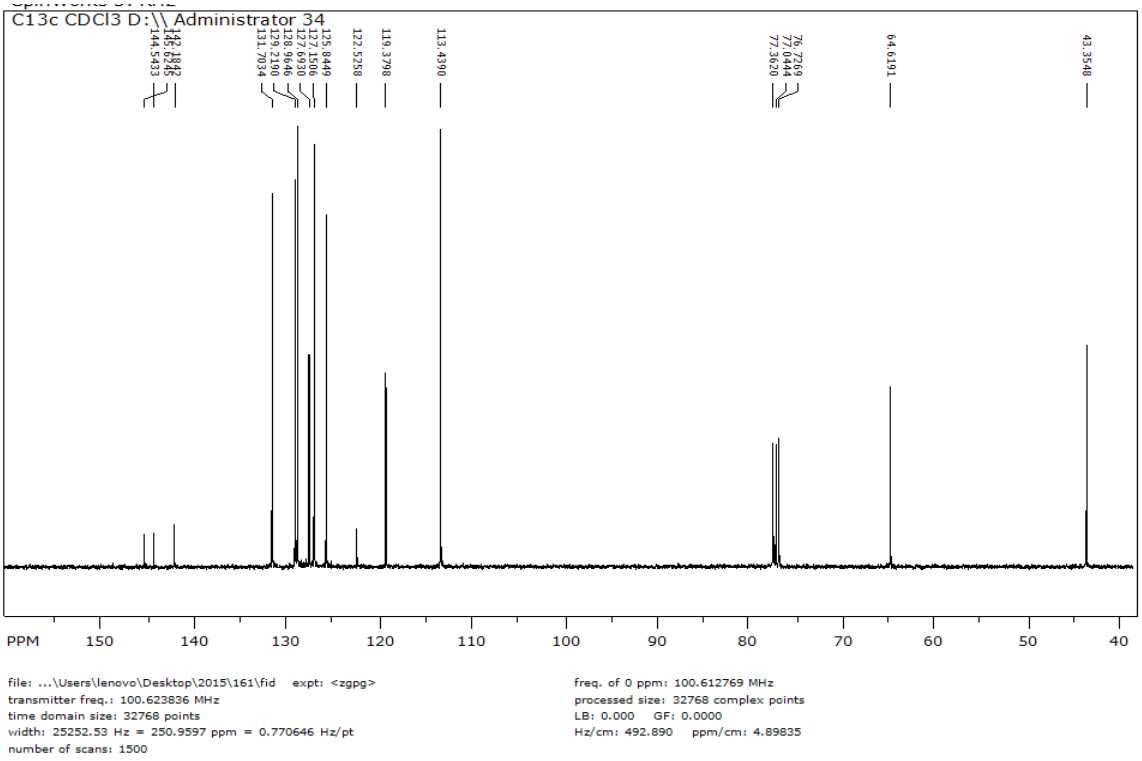
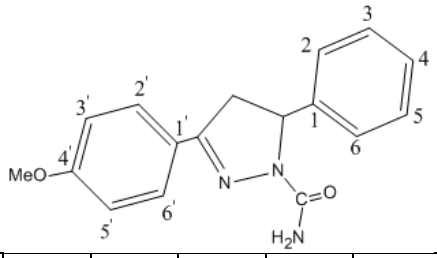


Figure : 6^{13}C NMR for P10

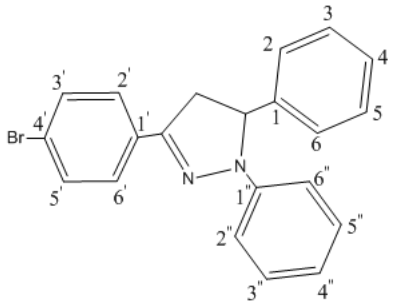
^{1}H-NMR spectra of pyrazoline were characterized by silverstienet.al [19], cooper [20] and shriner and Hermann [21], by the presence of 7.065 (NH), CH2 (pyrazoline) , d, 3.35-3.937 also the CH proton of pyrazoline , t, 4.62-4.70 ppm .

Compound (P8 and P13) their proton NMR proved that there is no CH_2-pyrazoline protons while the others compounds have the methylene in pyrazoline ring.

Table (4) 13C-NMR and 1H-NMR data some of for selected pyrazoline derivatives



Comp. No.	CH2	-C-	CH	Other OCH3	C=O	4	4’	I	I’	2,6	2’,6’	3	3’	5,5’
P1	43.07	151.97	5970	55.60	159.11	134.85	130.20	128.86	155.36	126.53 126.28	114.37 114.55	114.55	126.90	128.71 130.39



Com. No.	CH2	-C-	CH	Other OCH3	C=O	4	4’,4’’	I	I’,I’’	2,6	2’,6’	2’’,6’’	3,5	3’,5’	3’’,5’’
P10	43.40	145.50	64.60	-	-	125.9	125.9, 129.0	127.1	142.3, 144.5	127.9	129.2	113.4	1.2, 7.1	131.7	113.4

Proton – NMR pyrazoline ring						
Compd. No.	CH2 (d)	CH (t)	NH2	NH	Aromatic rings M	Others (s)
P1	3.94-3.64	4.9	6.0	-	6.92-7.72 ppm (9 protons)	
P9	3.25. J=16.4Hz, 7.7Hz 3.35 J=16.4Hz, 10.4Hz	5.02	-	6.75	6.25-6.35 m 6.55-6.70 m 7.38 (m)	
P10	3.90-3.65	5.19	-	-	6.78 – 7.821 ppm (14 protons)	
P24	2.3-2.6	4.6	7.4	7.47	6.77 – 9.45 (m)	3.7 (s)
P8	-	7.12	8.56	-	7.51-8.05 (m)	-
P13	-	6.60	7.6	-	(2’,6’) J=9.1 (6.71-6.7) (3’,5’) T=9.25 (2,6) 7.61-7.62 (1) 7.58	3.1 (s) NMC
P28	3.85-3.95	3.9	-	-	6.81 – 7.99 ppm (12 protons)	
P 33	3.69-3.94	4.1(m)	-	7.0	6.41 – 7.74 ppm (7 protons)	

Finally a biological activity study was done for some of the prepared pyrazoline products and it shown

Antibacterial activity

Antibacterial [22, 23] activity of compounds were tested by agar – well diffusion method. Besides the antibacterial activity, Petri dishes with 20 ml of Mueller – Hinton agar were prepared, inoculated with 1x 10^6 cell/ml(0.1 optical density on 540 nm wave length), 100 µ of a 24 hours broth culture of test bacteria. Discs 6 mm diameter each were

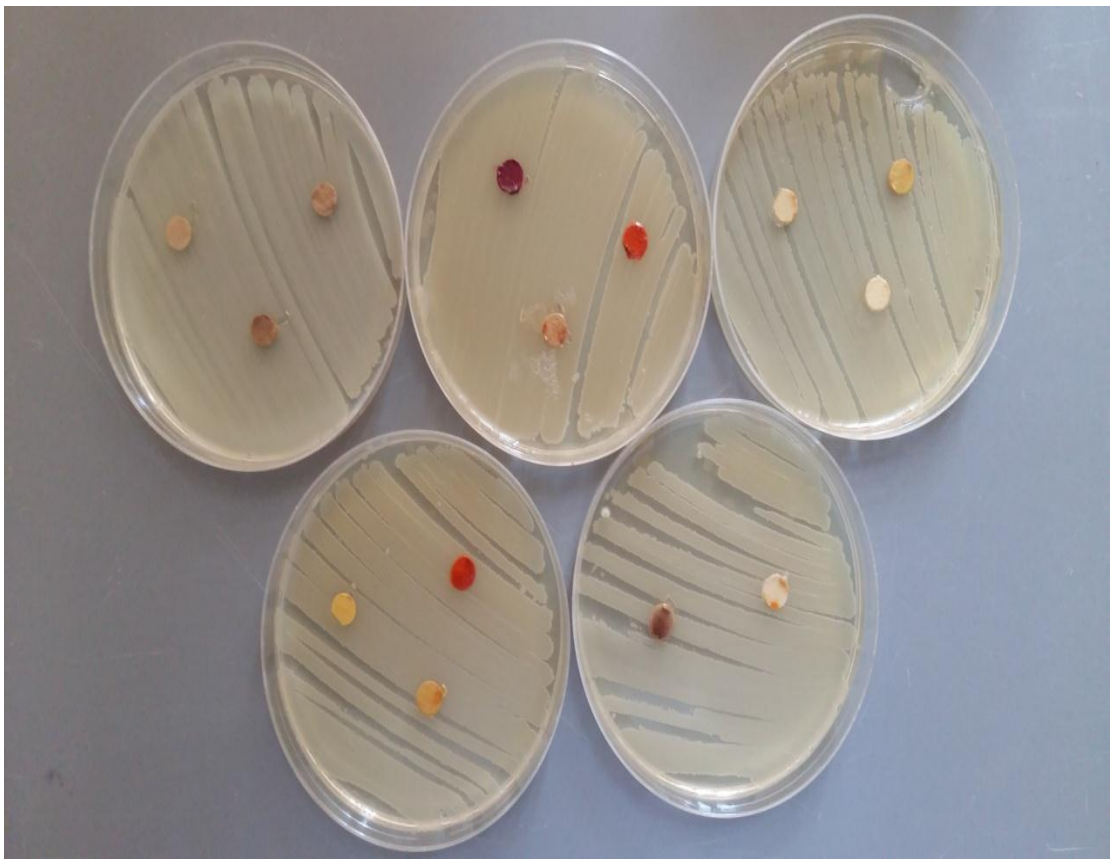
made and filled with 100 µl of (150 mg/ml) extracts. The inoculated plates were incubated for 24 hours at 37°C. After incubation, the diameters of inhibition zone diameter were measured in mm. (Perez *et al*.,1990).

Cytotoxicity

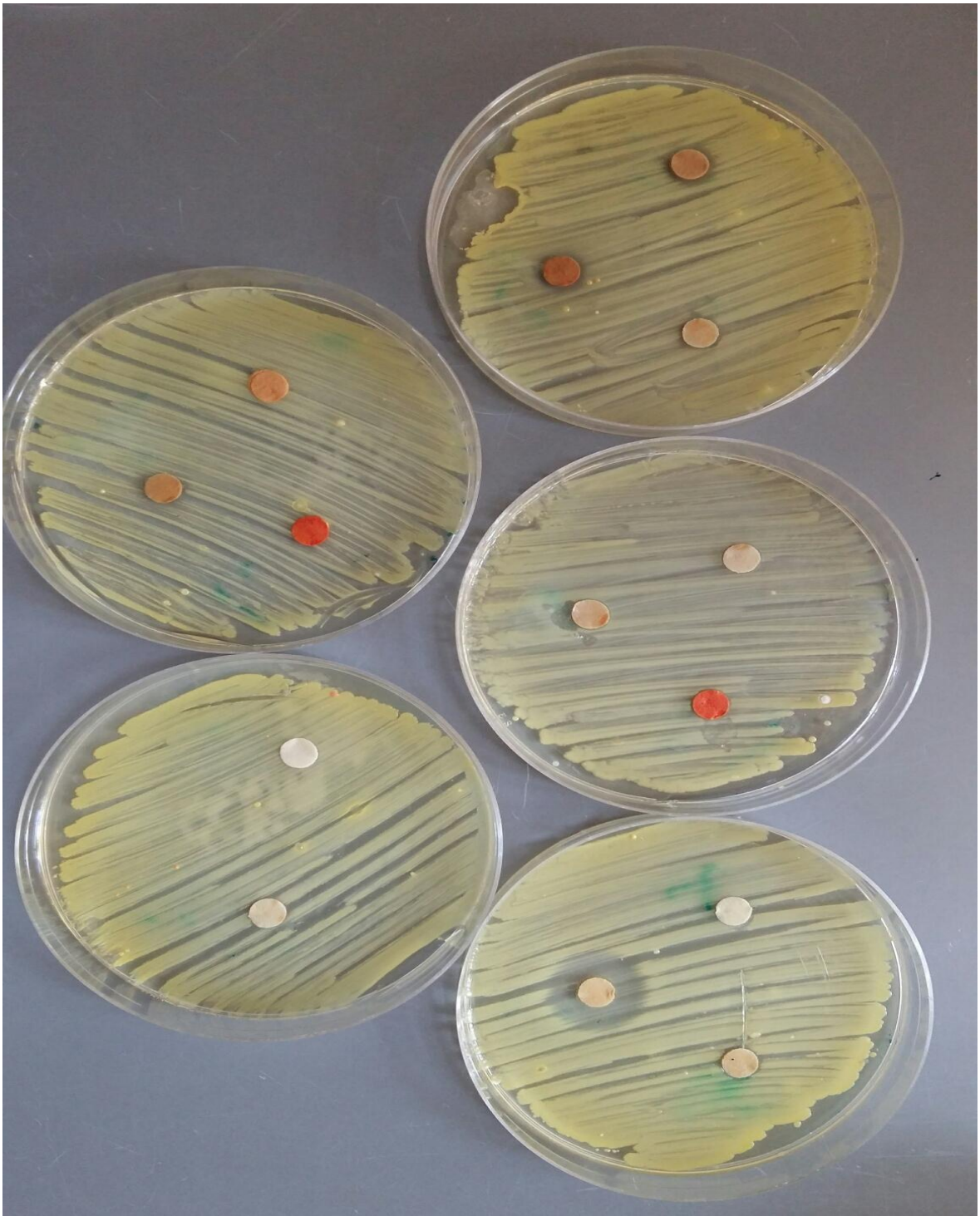
The methods described by Xian –guo and Ursula,(1994) was employed to study cellular toxicity .Briefly stock solution concentration (200 mg/ml)a serial of dilutions of each compound (1:1,1:10,1:100,1:1000) i.e. (100,20,2,0.2 mg/ml) were made in DMSO. A total volume of 0.8 ml for each concentration was placed in an eppendroff tube. A negative control tube (containing DMSO) and a positive control tube (containing tap water) were also included in the analysis . Fresh human red blood cells were added to each tube , to give final volume of 1 ml solution were incubated at 37 ^{o}C for 30 minutes and all tubes were centrifuged for 5 minutes and then observed for hemolysis.

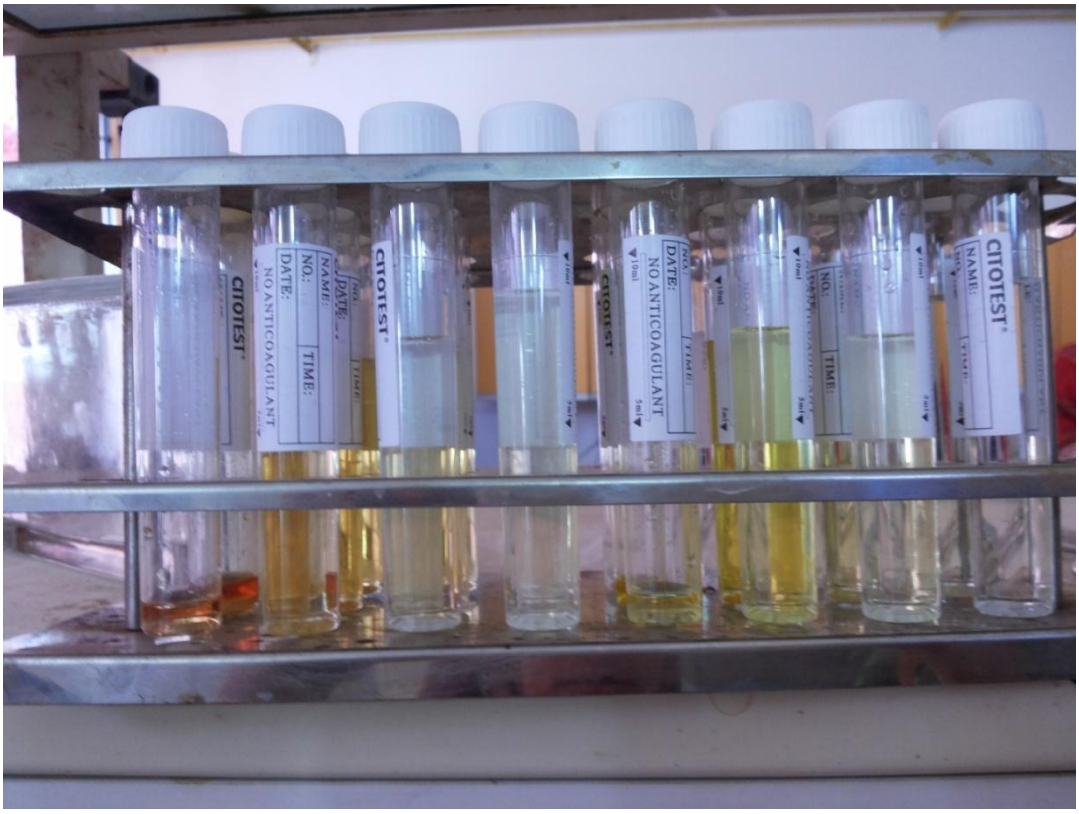
RESULTS

Results of the antibacterial activity against *Escherchiacoli*and *Staphylococcus aureus*. Bacteria showed that only the compound 33 had antibacterial activity against *Staphylococcus aureus*.

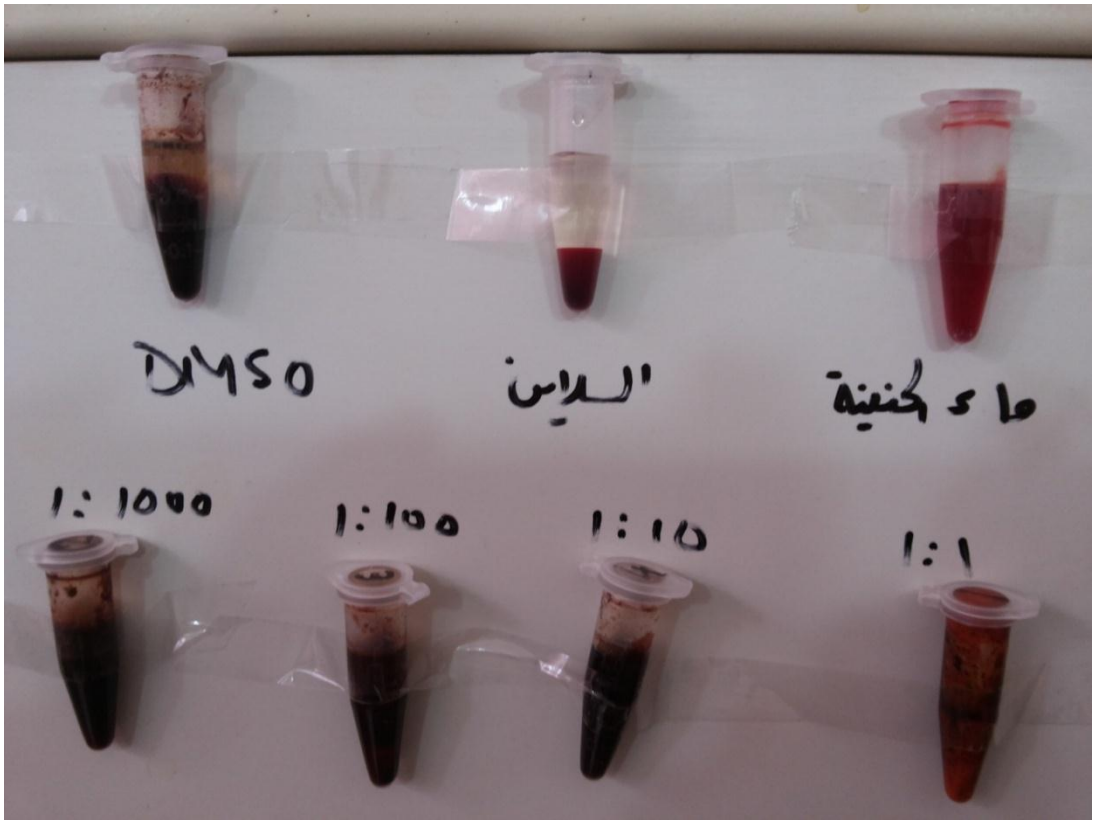


E.coli





Staphylococcus aureus



Cytotoxicity

The results of cytotoxicity of the compounds revealed that hemolysis of red blood cells occur at concentration (100 mg/ml) only(Table 1).

Table(1): The results of cytotoxicity of the compounds studied

compound	100 mg\l	20 mg\l	2 mg\l	0.2 mg\l	DMSO	Tap water	Normal salaine
P35	+	▬	▬	▬	▬	+	▬
P12	+	▬	▬	▬	▬	+	▬
P8	+	▬	▬	▬	▬	+	▬
P6	+	▬	▬	▬	▬	+	▬
P9	+	▬	▬	▬	▬	+	▬
P10	+	▬	▬	▬	▬	+	▬

+: hemolysisoccur

▬ : no hemolysis

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