ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Synthesis of Some New Pyrazoline Compounds Derived from α-β Unsaturated Compounds and Study their biological and Biochemical Effect

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Abstract

In this work Compounds Chalcones (w_{1-8}) have been prepared from the reaction of acetophenone derivatives with appropriable aromatic aldehyde in presence of Noah (10%). The reaction of thiosemicarbazide with chalcones (w_{1-8}) yielded compounds (W_{9-16}) 5- (Aryl) -3- (4-sub. Phenyl) 4-5-dihro -1H-Pyrazole -1- Carbathiamide). All the new compounds have been characterized by using spectral (IR, H^1-NMR, TLC) data. And physical methods. The antibacterial activity have been tested in vitro by the disk diffusion assay method against two kinds of bacteria gram positive and gram negative .The minimum inhibitory concentration [MIC] have been determined with the reference of standard drugs the results showed that the pyrazoline derivatives are better than growth of both types of bacteria (gram-positive and germ–negative compared to drug .The effect of hydrogen peroxide (0.1%) H_2O_2 drinking water through the mouth for about (15) days on white male rats shows significant increase ($P<0.01$) at the level glucose and cholesterol and Triglyceride in the serum comparison with the control group, where as it shows a significant decrease($P<0.01$) in the level of ,glutathione, glutathione Peroxidase (GSH-Px), Superoxide dismutase (SOD), in serum. Whereas the prepared (W_{11}) compound was injected, results show after (5) days of treatment that the organic compound of (7.14) mg/kg of body weight through the mouth for white male rats exposed to oxidation, with an associated significant decrease ($P<0.05$) in serum (Glucose-Cholesterol-Triglyceride), with an associated a signification serum (glutathione, glutathione Peroxidase (GSH-Px), Superoxide dismutase (SOD) in comparison with the control group exposed to Oxidation of hydrogen peroxide. It concludes that (W_{11}) compound has an anti-oxidation effect on healthy male rats exposed to oxidation effect.

Introduction

Oxidative stress is characterized by an increased concentration of intracellular oxidizing species such as reactive oxygen species (ROS) and is often accompanied by the loss of antioxidant defense capacity it is well known that excess ROS attack many organs, and induce oxidative damage directly to critical biological molecules, such as lipoproteins, proteins and nucleotides, causing lipid peroxidation and protein oxidation.

Metabolic oxidative stress has been implicated, directly or indirectly, in the development of diseases and degenerative processes including inflammation, cancer, dementia and physiological aging moreover, oxidative stress also plays a central role in liver pathologies Antioxidant ,pharmacotherapy in various forms has emerged as a mean to minimize the bimolecular damage caused by the attack of ROS on vital constituents of living organisms ,Oxidative stress which may contribute to the pathogenesis of different complications .Furthermore, with diabetes several features appear including an increasing in lipid peroxidation, a decrease in the antioxidant enzyme activities .Furthermore, with diabetes several features appear including an increasing in lipid peroxidation, a decrease in the antioxidant enzyme activities.

These changes indicate an oxidative stress caused by hyperglycemia [1]. Anti-oxidants protect human body against oxidation through reducing free radicals and that prevailing such as glutathione Peroxidase and enzyme super oxide or through preventing serial reactions. That the notion of the paper is formed [2]. To study the effect of Glucose-Cholesterol and triglyceride in

serum, in addition to the level of glutathione, glutathione Peroxidase (GSH-Px), Superoxide dismutase (SOD) in serum of male rats exposed to oxidation of hydrogen peroxide. Therefore, there is a great interest in looking for new heterocyclic containing pyrazole moiety, which could represent a good pharmacological alternative to counteract oxidative stress. chalcones derivatives have displayed wide range of biological and pharma ecological activities such as, antitumor [3], anti-oxidant, anti-parasites, anti-proliferative[4], anti-tubercular, antiviral [5], antiprotozoal, ant leishmanial [6], analgesic, anti-platelet, anticancer [7] anti-ulcerative [8] inhibitor of topoisomerase I [9] anti HIV [10] antiplasmodial [11].

Chalcones were used to afforded pharmacologically interesting hetrocyclic sustems like five member ring six member ring, seven member ring. Pyrazoline for example are known to have, anticancer [12] anticonvulsant [13] antibacterial [14] anti-diabetic, [15] antipyretic [16] antifungal, antiviral [17] anti-tumour [18] anti-androgenic [19] antioxidant [20] anticonvulsant [21] analgesic [22] anti-tubercular (23) anti-inflammatory [24] sedative [25] anti-viral [26] anti-Parastiary [27] anti-filarial [28] as a result various procedure have been worked out for their synthesis. Numerous derivatives have been published [29]

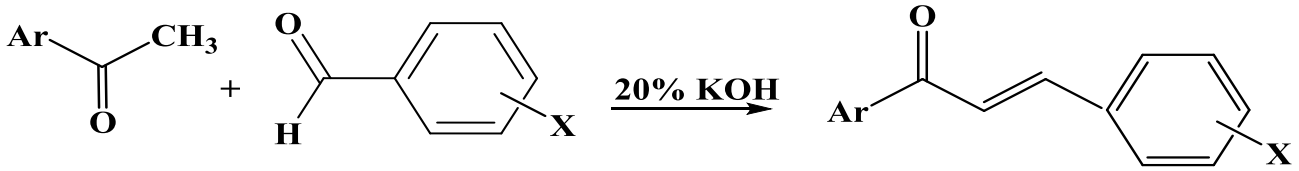
Materials & Methods

Experimental

Melting points are uncorrected and were recorded in an open capillary tube on Stuart melting point apparatus. Infared spectra have been recorded on a schemadzo FTIR-8100 spectrophotometer using KBr discs–and H^1-NMR Spectra have been measured on a MHZ spectrometer by using(DMSO) . All solvents and chemical reagents have been purchased form Aldrich, alfa aesar, sigma. Reaction monitoring and verification of the purity of the compounds were done by TLC on silica gel-percolated alumni sheets (type 60 F254 Merck, Darmstadt, Germany) using appropriate elaent.

Synthesis of chalcones [30] (w_1-w_8)

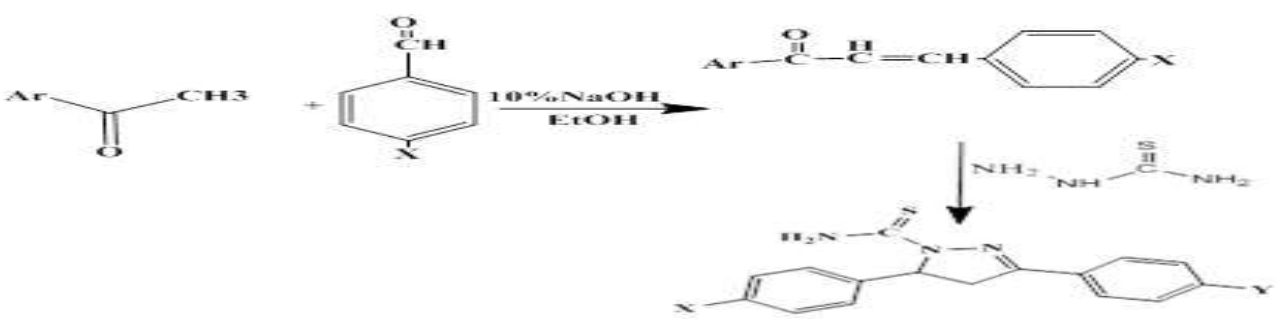
A mixture of appropriate acetophenone (0.01mol) and aromatic benzaldehyd (0.01mol) have been added to a solution of (10%) sodium hydroxide (5ml) and (3ml) of ethanol. The mixture was stirred for (2-3) hr at (20-40) C ° and kept in a refrigerator for (12) hr. Then it was diluted with ice-cold distilled water (30ml), filtered washed with cold water, dried in air and recrystallized from ethanol. The physical data are shows in Table (1).



Scheme 1: Synthesis of title compound (W_{1-8})

Synthesis of Pyrazoline [31] (W_{10-18})

Appropriate chalcones (W_{10-18}) (0.01) mole and thiosemicarbazide (0.01) mole in dioxan(10) ml have been refluxed in the presence of glacial acetic acid (1ml). The mass reactions have been concentrated to one- third volume under vacuum. The concentrated mass has been poured into ice-cold and filtered water. The separated product has been recrystallized from ethanol. The physical data are shown in Table (2).



Chemicals and Instruments

The biological activity has been estimated by using the propagation method whereas the biological activity has been estimated by the Kirby Bauer movement, where 0.1 ml of bacterial suspension has spread to the ager Muller Hinton dishes and left for 5 minutes to absorb the suspension. After that, holes were prepared for each dish using a Cork Porer and a diameter of 5 mm hole (0. 1) ml of the prepared solutions of the fourth hole as DMSO as control sample and incubated the dishes for (24) hours at a temperature of 37°C [32, 33] and then measuring the inhibition zone diameters around each hole in milometer. Of depending on the method of Prescott [32].

Selection of Anti-bacterial Activity of Some Prepared Compounds

In this study two species of pathogenic bacteria have been used *E. Coli* and *Proteus spp* .The two species are important in the medical aspect in resistance against antibiotics and took these types of bacteria ready and isolated ,this test has been done in the following ways:-

Cultivation Media

- Nutrient broth have been prepared according to the methods of the manufacturing and sterilized in the auto clave in121 ° C for 15 minutes under pressure (15) and then poured into the dishes or tubes and leaving cooled [34].
- Mullerhimnton, this medium is used to measure the biological activity of antibiotics and pharmaceuticals. This medium is used to measure, the diameter of inhibition zone [35].

Biochemistry Part

Experimental

Males albino rats from spragned awely that aged between (2-3) months and weighted ravaged between (175-200)gm were used in this study plastic cage and feuded with water and special feed ,the au rats were suffered to the same conditions like a normal light and temperature 2±25 C °.

Study of Experimental Preparing and Standard Compound Effect on Rats

Animals were divided randomly to the (3) groups and each group included (10) rates that have weight ranging between (175-200) gm. The groups divided as bellow

Groups 1: The healthy control group that feeding water and special feed without treatment for (15) days.

Group 2: In faceted groups with oxidative stress causing by at concentrated (0, 1%) with drinking water for (15) days and leaved without treatment by preparing compound.

Group 3: Susceptible to the oxidative stress that causing by hydrogen peroxide at concentrate (0.01 %) mole with drinking water for 15) days then daily treated with the preparing compound (W_{11}) by orally dosage (7.14mg/kg) [36] from body weight by using special dose tube for 5 days.

Estimating variables

The level of glucose, cholesterol and triglyceride was assessed using several analyzes (kit) Type of (Bio labo) French is an enzymatic method [37]. The concentration of glutathione in the serum was measured using the detection method of glutathione wane prepared in laboratory [38].The efficacy of glutathione Peroxidase and the effect of Superoxide dismutase was estimated using several analyzes company (SZaKits) [39].

Result &Discussion

Synthesis of Compounds (W_1-W_{16})

The reaction of acetophenon with aromatic aldehyde yielded the compounds ($W_{1\text{-}8}$), the IR spectra (data) of these compounds showed a band at (1593-1614 cm^{-1}) due to stretching (C=C) group. a band at (1668cm^{-1}) for (C=O) group band at (3078cm^{-1}) for (Ar-H) group, band at (1508 cm^{-1}) for ʋ (C≕C)group. The IR data showed in the figure (1) table (3). The reaction of chalcones with thiosemicarbazide yielded the compounds ($W_{9\text{-}16}$).

The IR spectral data of these Compounds showed band at (128-1268 cm^{-1}) due to stretching (C=S) group. a band at (1644-1679 cm^{-1}) for (C=N) group. a band at (3007-3057) for (Ar-H) group. a and at (3147-3344 cm^{-1}) for (NH_2) group. The IR DATA showed in the Figure (2) Table (4). The H^1-NMR Spectrum ($CDCl_3$) of compounds (W_6) Show signal at (2.50ppm) for (DMSO), signal at (6.99ppm) for (-CH=), signal at (7.49to7.81ppm) for phenyl, signal at (7.22ppm) for (CH=O=C), Figure (3) The ^{1}HNMR spectrum ($CDCl_3$) of compounds (W_{11}) Showed signal at (2.53 ppm) for (2H) pyrazolinering, Signal at (9.68 ppm)

for (H_2, NH_2), Signal at (7.33to7.54ppm) for phenol, signal at (6.59 to7.64 ppm) for (H=CH), the IR data showed in the Figure (2) Table (4).

Evaluation of Biological Activity

The antimicrobial activities of the synthesized compounds were determined in vitro against several pathogenic representative microorganism (*Escherichia coli* and *Proteus spp),* using Agar well-diffusion method [40]. Ciprofloxacin were used as standard drugs for studying the potential activities of these compounds, all the compounds were tested at different concentration level (0.01, 0.001, 0.0001 mg/ ml), DMSO was used as solvent and as control.

The inhibition zone diameter in mm (IZD) was used as a criterion for the antimicrobial activity. The lowest concentration required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC, µg/mL), was determined for all the compounds and compared with the control. The investigation of antibacterial screening data revealed that pyrazoline derivatives (w_{11}-w_{13}-w_{15}-w_{16}-w_{17}-w_{18}). Compounds (w_{11}-w_{13}-w_{15}-w_{16}-w_{17}-w_{18}) exhibited good antibacterial activity towards the both gram negative bacteria (*Escherichia coli*).

Compounds (w_{11}-w_{13}-w_{15}-w_{16}-w_{17}-w_{18}) have also exhibited good antibacterial activity towards gram positive bacteria (*Proteus spp)*; showed high activity against all the microorganisms employed in contrast with the ciprofloxacin derivatives. The maximum activity (MIC = 12.5 µg/mL) was indicated for compounds. He results are summarized in Table 5 [41].

Bio Chemical Study

Effect of Oxidative Stress with Hydrogen Peroxide on Some of the Biochemical Variables in the Serum of Rats

Treatment with hydrogen peroxide (0.1%) by mouth with drinking water and for (15) days in male white rats as shown in Table (6) to a significant increase ($P<0.01$) in serum glucose level when compared with the control group, this may be due to an increase in oxygen pressure from hydrogen peroxide and thus to an increase in the active oxygen species that attack beta cells in the pancreas, disrupting insulin synthesis [42].

The oxidative stress also resulted in a significant increase ($P<0.01$) in the total cholesterol level; it can be caused by a decrease in the effectiveness of the 7-hydroxyl enzyme responsible for total cholesterol to yellow acids [43]. Addition, oxidative stress and oxidative stress led to a significant increase ($P<0.01$) in the level of triglycerides when compared with the control group. This is consistent with studies [44].

This may be due to the low efficacy of riboprotein lupine [45]. The oxidative stress resulted in a significant decrease ($P<0.01$) in the level of glutathione; this may be due to the reduced efficacy of glutathione synthase, which is responsible for building glutathione. He oxidative stress induced a significant decrease ($P<0.01$) in the efficacy of both the glutathione peroxide and the superoxide dismutase, this may be due to an increase in the active oxygen groups that act to oxidize the effective sites, thereby reducing the effectiveness of the enzyme.

The Effect of Pyrazoline Compounds on Some biochemical Variables in the Serum of Rats Exposed to Oxidative Stress

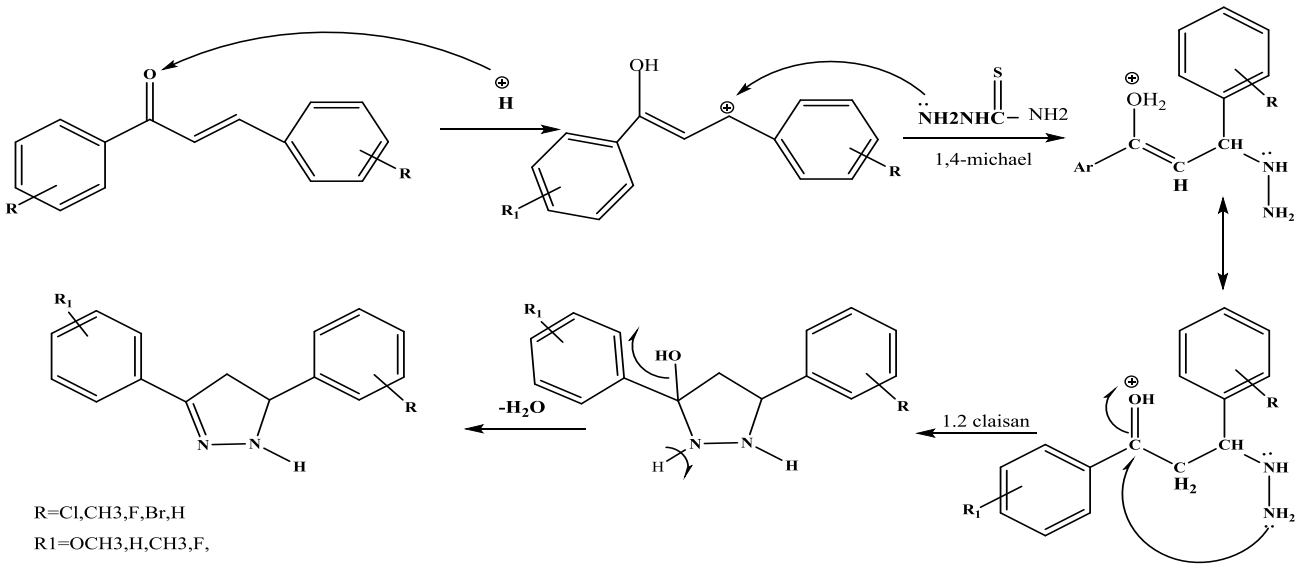
The treatment with pyrazoline and (7.14)mg\kg of oral weight with drinking water for male rats has showed a significant decrease ($P<0.01$) in blood glucose level as compared with controlled group exposed to hydrogen peroxide oxidation-based control [46], as shown in tables(7) this is due to their(rats) ability to inhibit the enzyme glucose-6-phosphatse [47]. GU additions the do sage of the same compound and the same dose, have significantly reduced the level of total cholesterol ,triglyceride [48].

The reasons have, may be due to the high efficacy of -7-alpha hydroxylase, which is responsible for the conversion of low-density lipoprotein cholesterol and very low density lipoproteins [49]. The case of triglycerides, the cans of the decrease many are due to the increased effectiveness of the enzyme Lippo protein lipase, which increases the intake of low-fat lipoprotein [50].

The increase in the level of glutathione is due to the increase of building (GSH) by stimulating the glutathione syntheses enzyme. The increase in the efficacy of both glutathione peroxidase and superoxide dismutase [51], which is consistent with. This many due to the ability of these oral

compound with drinking water to protect the effective location of these enzymes by inhibiting the glycation process through the preventing of non-enzymatic association of glucose with acids minis located in the active sit [52].

Scheme



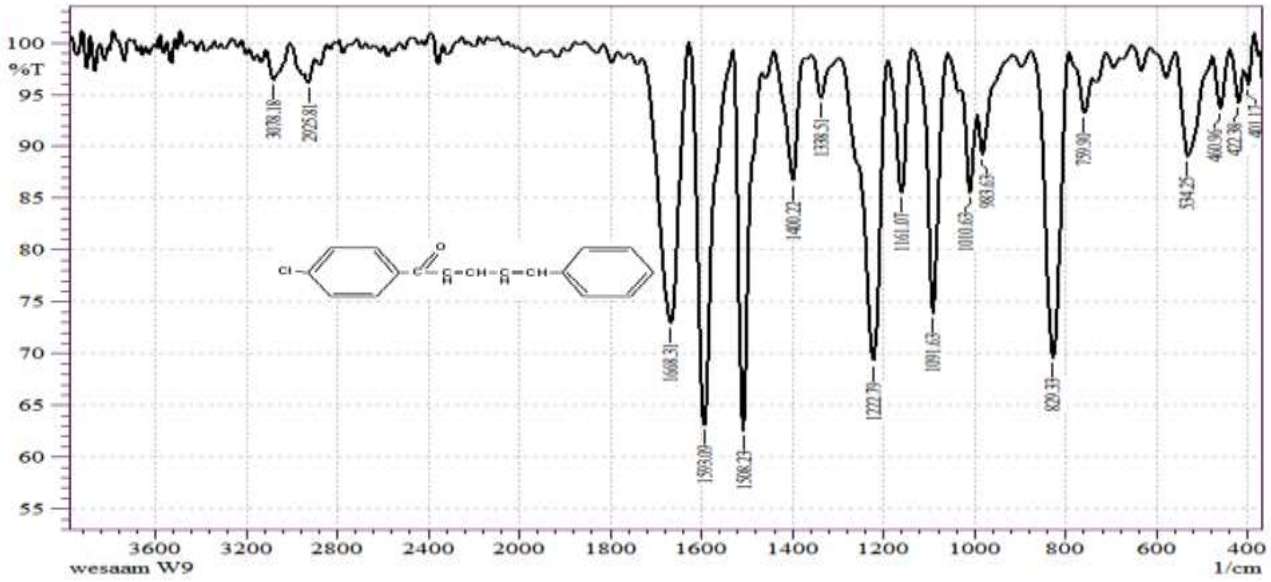


Figure 1: FTIR spectrum of compound (W_6)

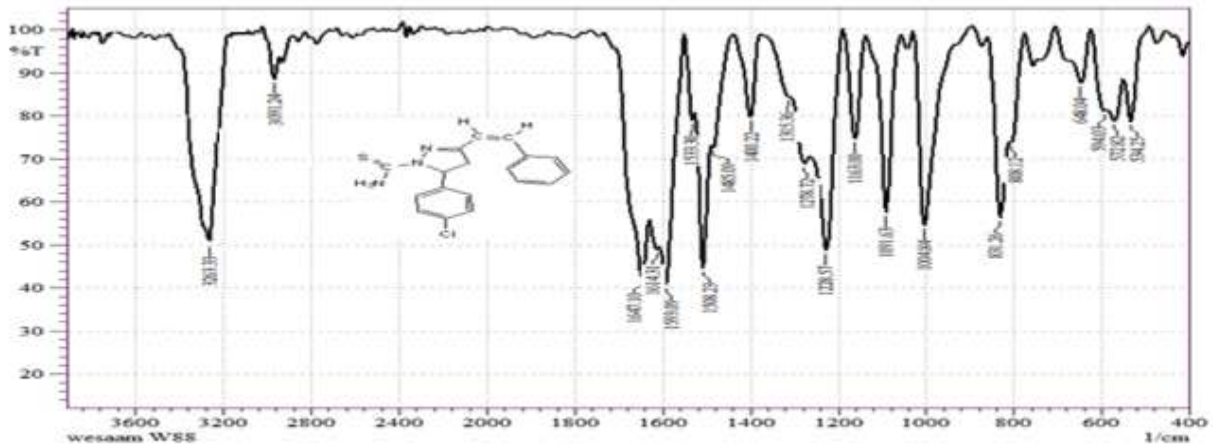


Figure 2: FTIR spectrum of compound (W_{11})

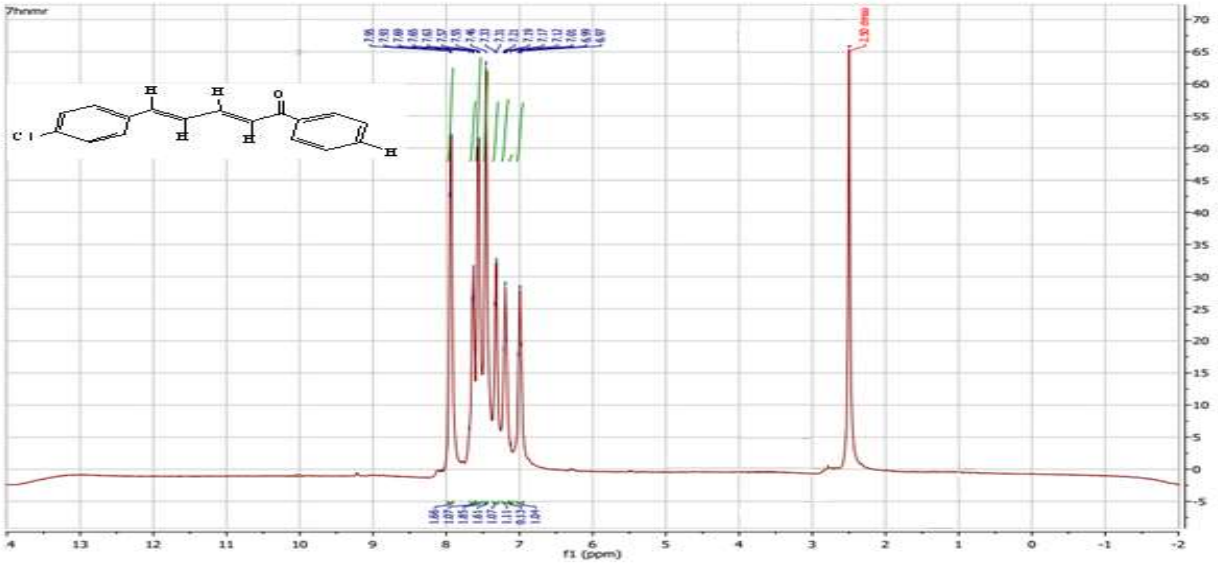


Figure 3: H[1]- NMR spectrum of compound (W_6)

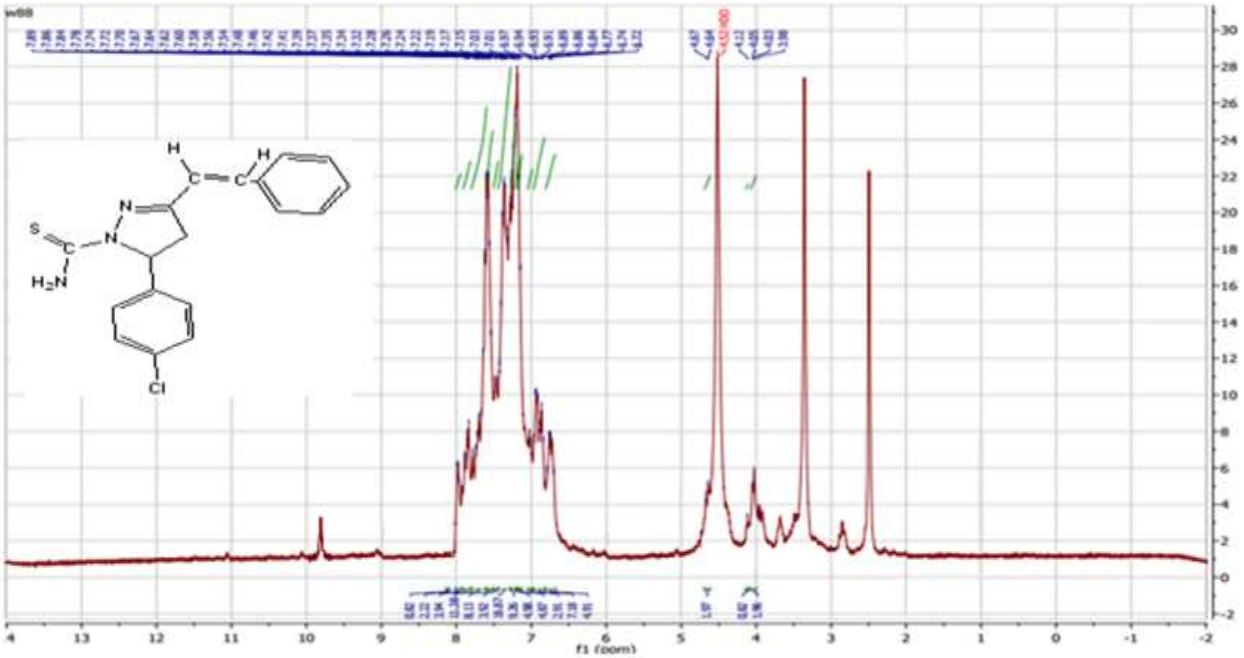


Figure 4: H[1]- NMR spectrum of compound (W_{11})

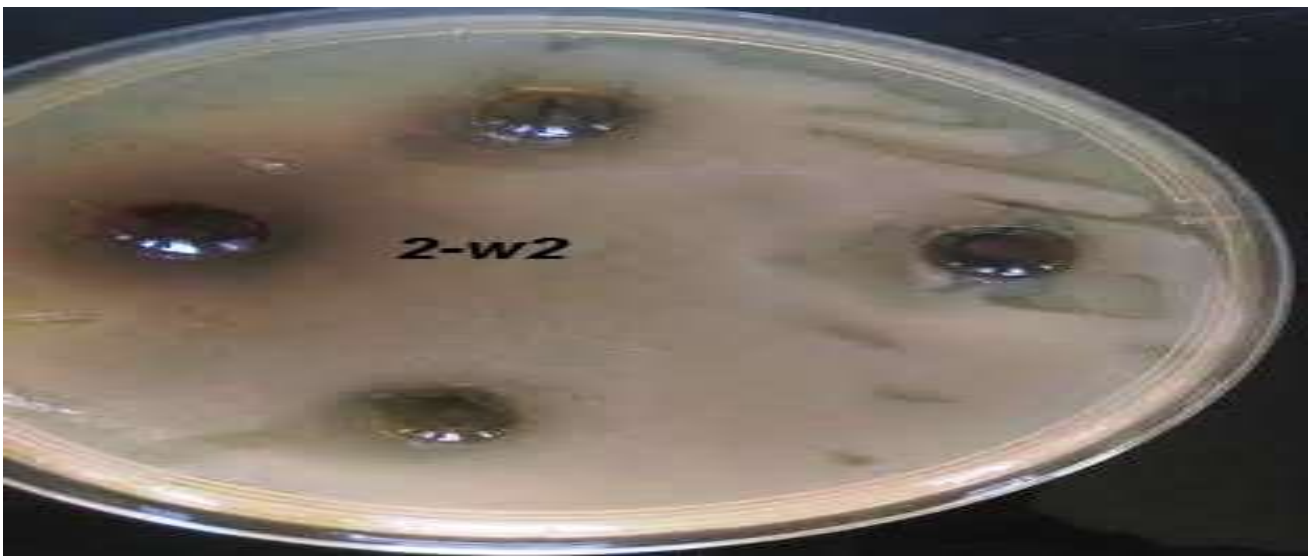


Figure 5: Compound (W_{15}) inhibits growth of bacteria E. coli

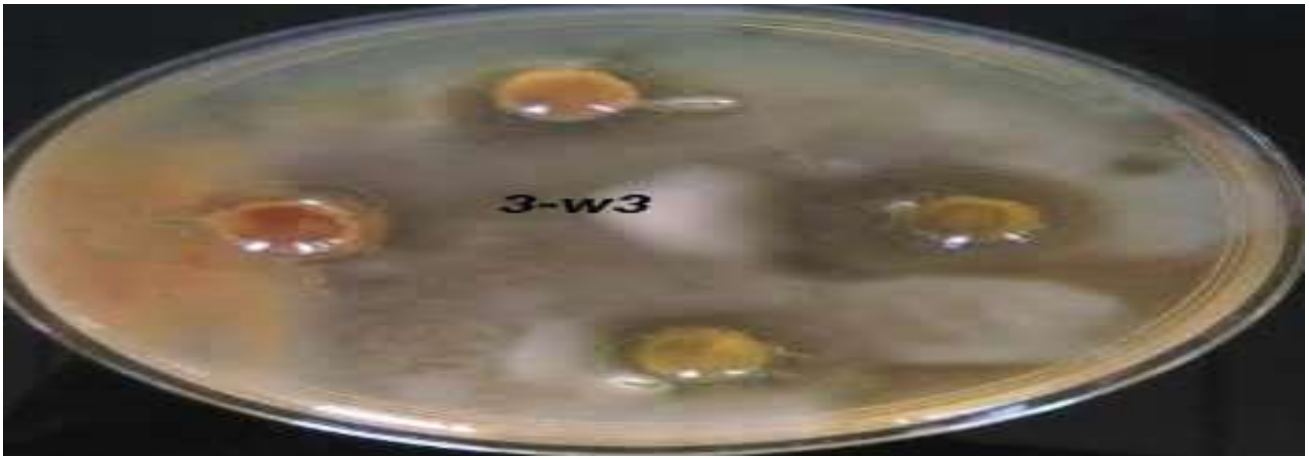


Figure 6: Compound (W_{17}) inhibits growth of bacteria E. coli

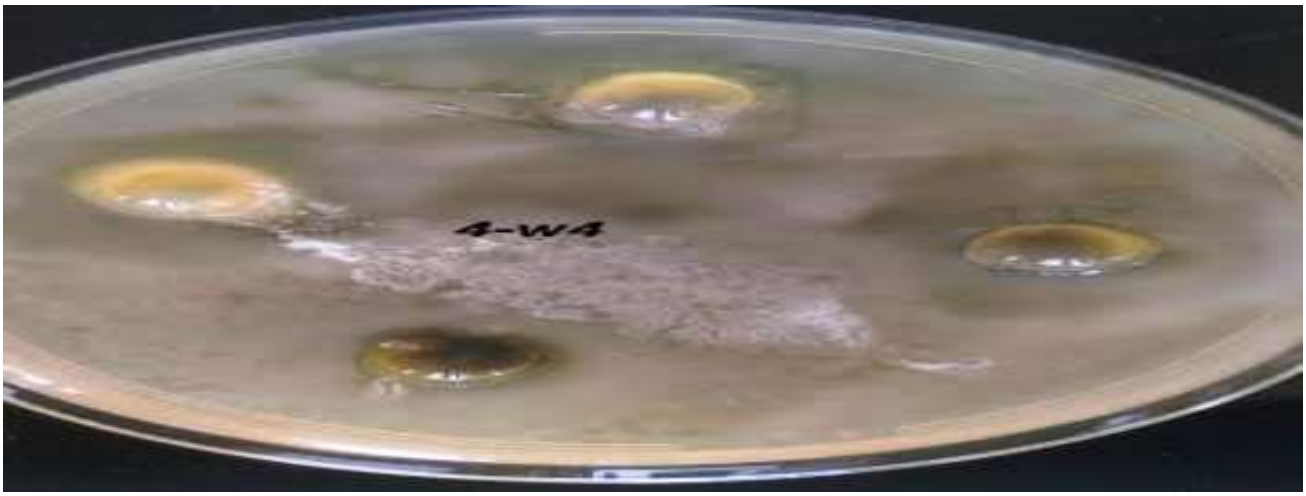


Figure 7: Compound (W_{11}) inhibits growth of bacteria E. coli

Figure 8: Compound (W_{16}) inhibits growth of bacteria Proteus Mirabilis

Table 1: the physical properties of compounds (w_1-w_8)

Comp. No.	X	Ar	Molecular formula	M.P (C)°	Yield (%)	Rf	Color
W_1	CH_3	$-C_6H_5$	$C_{16}H_{14}O$	45-48	85	0.78	White
W_2	CH_3	$4\text{-}OCH_3C_6H_5$	$C_{17}H_{16}O_2$	85-87	78	0.67	Yellow
W_3	CH_3	$-(CH{=}CH)\text{-}C_6H_5$	$C_{18}H_{16}O$	73-75	88	0.72	White
W_4	Cl	$4\text{-}F\text{-}C_6H_5$	$C_{15}H_{10}OFCl$	110-112	83	0.74	Yellow
W_5	Cl	$4\text{-}OCH_3\text{-}C_6H_5\text{-}$	$C_{16}H_{13}O\ Cl$	118-120	94	0.63	White
W_6	Cl	$-C_6H_5$	$C_{15}H_{11}O\ Cl$	102-105	79	0.71	Yellow
W_7	F	$-C_6H_5$	$C_{15}H_{11}OF$	73-75	84	0.69	Yellow
W_8	Br	$-C_6H_5$	$C_{15}H_{11}OBr$	117-119	77	0.47	White

Table 2: The physical properties of compounds (W_9-W_{16})

Comp. No.	X	Ar	Molecular formula	M.P. (C)	Yield (C)°	Rf	Physical State
W_9	CH_3	C_6H_5-	$C_{17}H_{17}N_3S$	160-163	93	0.74	Yellow Crystals
W_{10}	CH_3	4-$OCH_3C_6H_5$	$C_{18}H_{19}N_3SO$	168-170	81	0.79	Dark Yellow Crystals
W_{11}	CH_3	-(CH=CH)-C_6H_5	$C_{17}H_{17}N_3S$	137-139	93	0.67	Pale Yellow Crystals
W_{12}	Cl	4-F-C_6H_5	$C_{16}H_{13}N_3ClFS$	143-145	95	0.81	Yellow Crystals
W_{13}	Cl	4-OCH_3-C_6H_5	$C_{17}H_{16}N_3SOCl$	157-160	74	0.83	Brown Crystals
W_{14}	Cl	-C_6H_5	$C_{16}H_{14}N_3SCl$	122-125	72	0.71	Pale Yellow Crystals
W_{15}	F	-C_6H_5	$C_{16}H_{14}N_3SOF$	134-137	78	0.76	Dark Yellow Crystals
W_{16}	Br	4-F-C_6H_5	$C_{16}H_{13}N_3FBrS$	152-155	84	0.67	Pale White Crystals

Table 3: Characteristic FTIR absorption bands of synthesized compounds (w_1-w_8)

Comp .No	X	Y	FT.IR cm^{-1} (KBr)				
			-C=O	υ (Ar-H)	υ (C=C) olefin	υ(C=C)Ar	Others cm^{-1}
W_1	CH_3	H	1650	3057	1602	1446	2921asy,2878sy υ(CH_3)
W_2	CH_3	OCH_3	1662	3002	1606	1573	2925asy υ(CH_3)
W_3	CH_3	H	1644	3062	1595	1574	2937asy,2891sy υ(CH_3)
W_4	Cl	F	1666	3068	1592	1508	**υ** (C-F) 730
W_5	Cl	CH_3	1654	3031	1604	1509	**υ** (C-Cl)773
W_6	Cl	H	1668	3078	1593	1508	**υ** (C-Cl) 821
W_7	F	H	1658	3058	1602	1581	υ (C-F)829
W_8	Br	CH_3	1689	3073	1599	1572	υ (C-Br)861

Table 4: Characteristic FTIR absorption bands of synthesized compounds (w_9-w_{16})

Comp .No	X	Y	FT.IR cm^{-1} (KBr)					
			υ -C=N	υ C=S	υ (Ar-H)	NH2	υ C≡≡C	Others
W_9	Cl	OCH3	1677	1232	3057	3322	1588	**υ** (C-Cl) 819
W_{10}	F	H	1679	1236	3007	3344	1591	**υ** (C-F) 692
W_{11}	Cl	H	1674	1228	3018	3263	1593	813 **υ** (C-Cl)
W_{12}	Cl	CH3	1677	1243	3072	3222	1589	815 **υ** (C-Cl)
W_{13}	CH_3	OCH3	1664	1263	3056	3172	1512	**υ** (C-OCH_3) 828
W_{14}	CH_3	H	1663	1254	3042	3147	1566	**υ** (C-CH_3) 2916
W_{15}	Br	CH3	1667	1261	3014	3274	1578	(Br-OCH_3)714
W_{16}	Cl	F	1644	1268	3022	3243	1567	**υ** (Cl-F)823

Table 5: Antibacterial activity of the synthesized compounds (W_9-W_{14})

Compound God	Antibacterial activity (zone of inhibition in mm)		
	Conc. mg/m	*E. coil*	*Proteus spp*
W_9	0.01	15	25
	0.001	15	25
	0.0001	20	30
W_{10}	0.01	10	25
	0.001	25	30
	0.0001	27	25
W_{11}	0.01	24	30
	0.001	15	18
	0.0001	26	27
W_{12}	0.01	15	20
	0.001	20	15
	0.0001	25	27
W_{13}	0.01	15	30
	0.001	28	15
	0.0001	20	25
W_{14}	0.01	10	19
	0.001	15	17
	0.0001	25	28
Ciprofloxacin	**MIC**	12.5	12.5

Slight activity 15-18 mm, moderate activity 18-20 mm and high activity 21-25 mm; MIC: minimum inhibition concentration (μ g / mL)

Table 6: Shows the life variables in the two groups healthy control serum and hydrogen peroxide treatment

Parameter	Control	H_2O_2	Significant
glucose	8.28∓0.37	9.06∓0.57	0.009
Cholesterol mmol/L	1.92∓0.11	2.51∓0.29	0.00
Triglycerides mmol/L	0.57∓0.06	0.74∓0.04	0.00
glutathione U/L	1.69∓0.06	1.11∓0.01	0.00
Glutathione peroxides U/L	0.06∓0.01	0.05∓0.01	0.787
Superoxide dismutase U/L	15.20∓4.43	7.66∓1.88	0.00

The data in the table above refer to M∓ SD P<0.05 significant

Table 7: Shows the level of concentration (glu, Ch, TG, GSH, GPx, SOD), the normal and moral deviation and deviation of the peroxide group and the group treated by the registered compound

Parameter	H_2O_2	pyrazoline	Significant
glucose mmol/L	9.06∓0.57	7.77∓0.97	0.000
Cholesterol mmol/L	2.51∓0.29	2.26∓0.20	0.008
Triglycerides mmol/L	0.74∓0.04	1.09∓0.47	0.003
glutathione U/L	1.11∓0.01	1.19∓0.06	0.001
Glutathione peroxides U/L	0.05∓0.01	0.06∓0,01	0.632
Superoxide dismutase U/L	7.66∓1.88	10.75∓1.15	0.032

The data in the table above refer to M∓ SD P<0.05 significant

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