



Synthesis, Characterization and Biological Evolution of Nitrogenous Heterocyclic Ring Containing Chalcones

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ABSTRACT

Chalcones were the key intermediates for the synthesis of various six and five membered heterocyclic compounds. In the present work Chalcones were synthesized by base catalysed Claisen–Schmidt condensation reaction of imidazolyl acetophenone with appropriate aromatic aldehydes followed by dehydration reaction. Ten Chalcones were synthesized and structures were confirmed by spectral analysis. The compounds were tested for their anti-microbial activity and antioxidant activity using diffusion method by measuring the zone of the inhibition and DPPH measuring by measuring the % of inhibition. The compound CH-08 showed maximum activity among all other chalcones, with *Bacillus subtilis* zone of inhibition 22,24,28 mm at 50 µg/ml, 100 µg/ml, 150 µg/ml, *Staphylococcus aureus* zone of inhibition 22,26,28 mm at 50 µg/ml, 100 µg/ml, 150 µg/ml, with *Pseudomonas vulgaris* zone of inhibition 22,24,30 mm at 50 µg/ml, 100 µg/ml, 150 µg/ml, with *Escherichia coli* the zone of inhibition 24,28,32 mm at 50 µg/ml, 100 µg/ml, 150 µg/ml compare with the standard streptomycin. In case of anti-oxidant activity the compound CH-02 shows inhibition at 56.24± 0.20, 125.24±.47, 185.24±0.25, 256.36±0.35, 380.36±0.36 at concentration of 100, 200, 300, 400, 500 µg/ml respectively compare with the standard ascorbic acid.

Keywords: Chalcones, Claisen–Schmidt condensation, anti-microbial, anti-oxidant, DPPH reagent.

INTRODUCTION

Heterocyclic systems are one of the most important classes of organic compounds present in nature or synthesized in laboratory. These compounds possess array of biological activities and are employed in treatment of a commonly occurring diseases. This has been the backbone for medicinal chemists to keep perpetuating interest to synthesize some novel derivatives of possible high biological activity¹. In recent years, Chalcones have found a wide range of applications in the pharmacological activities such as, potential cytotoxic agents, antiviral, anesthetics, mydriatics, antimicrobial, antimitotic, antitumor, cytotoxicity, and antipyretic properties^{2, 3}. They undergo a variety of chemical reactions and are found to be useful in the synthesis of variety of heterocyclic compounds like isoxazoles, quinolinones, thiadiazines, benzofuranones, benzodiazepine, tetrahydro-2-chromens flavones etc

Chalcones are 1,3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α,β-unsaturated carbonyl system. These are abundant in edible plants and are considered to be precursors of flavonoids and isoflavonoids.

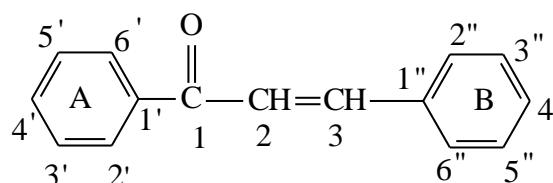


Figure 1: General structure of chalcone

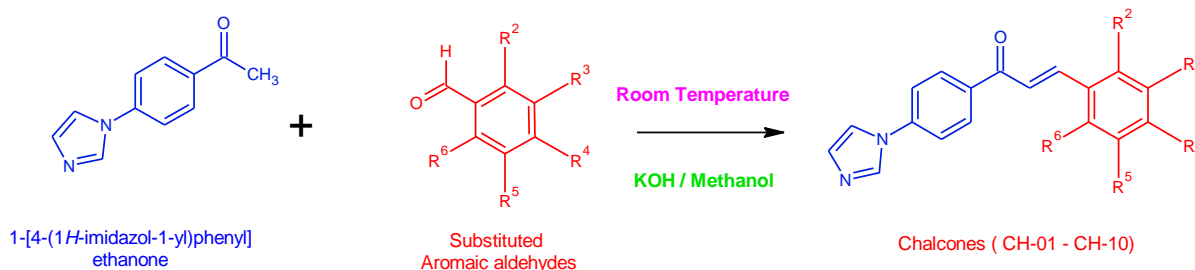
MATERIALS AND METHODS

4-(1H-imidazol-1-yl) Acetophenone, various aromatic aldehydes, alcoholic potassium hydroxide, conc. HCl, DMSO, DPPH reagent. All the reagents were purchased analytical grade. Open capillary tube method was used to determine melting point and are uncorrected. ¹H NMR spectra were recorded in the indicated solvent on Bruker WM 400 MHz spectrometer with TMS as internal standard. KBr pellet method was used to infrared spectral analysis on Perkin-Elmer AC-1 spectrophotometer. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

General method of preparation⁴

A mixture of 4-(1H-imidazol-1-yl) acetophenone (0.001 moles) and aryl aldehydes (0.001 moles) were dissolved in methanol (20 ml) and to it 3 millimoles of 15% KOH was added. The mixture was kept for 24 hours and it was acidified with 1:1 HCl and water, then it was filtered through vacuum by washing with water.

Chemical reaction



CHALCONE	RADICALS				
	R ₂	R ₃	R ₄	R ₅	R ₆
CH 01	-O-CH ₃	-H	-O-CH ₃	-H	-O-CH ₃
CH 02	-H	-O-CH ₃	-O-CH ₃	-O-CH ₃	-H
CH 03	-H	-H	-S-CH ₃	-H	-H
CH 04	-CH ₃	-H	-CH ₃	-H	-CH ₃
CH 05	-O-CH ₃	-O-CH ₃	-O-CH ₃	-H	-H
CH 06	-H	-H	-CF ₃	-H	-H
CH 07	-H	-H		-H	-H
CH 08	-CF ₃	-H	-H	-H	-H
CH 09	-Cl	-H	-H	-H	-F
CH 10	-H	-H		-H	-H

Biological evolution of compounds

Based on the literature, chalcones were reported to possess antimicrobial activity, anti-oxidant, anti-inflammatory, analgesic, anti-cancerous, etc. Therefore the present work performs the anti-microbial, anti-oxidant activities.

Antibacterial activity⁵⁻⁶:

The antibacterial activity was tested by determining inhibitory concentration by diffusion disc technique. The bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were *Staphylococcus aureus* (MTCC 737) *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 1687), *P. vulgaris* MTCC 1771

Procedure

The antimicrobial activity of the compounds was assessed by disc diffusion method Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, they were poured into a petridishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *P. vulgaris* were spread over the media with the help

of a sterile swab soaked in bacterium and is used for antibacterial study. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to produce a concentration of 500 µg/disc, 1 mg/disc and used for the study. Streptomycin 5 µg/disc was used as the standard. Then the sterile filter paper discs (6mm) having a capacity to hold 10 µl of solution were immersed in definite concentration of compounds and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petridishes were incubated at 37 °C for 24 hours. After the incubation period is over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicate.

Anti-oxidant activity evolution by DPPH radical scavenging method⁷⁻¹⁰

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radical reacts with various electron donating molecules (reducing agents or antioxidants). When electrons become paired off, bleaching of the DPPH solution is the result. This results in the formation of the

colourless 2, 2'-diphenyl-1-picryl hydrazine. Reduction of the DPPH radicals can be estimated quantitatively by measuring the decrease in absorbance at 517 nm.

Procedure

Equal volumes of 100 μM 2,2'-diphenyl-1-picrylhydrazyl (DPPH) in methanol was added to different concentrations of test compounds (0 – 200 $\mu\text{M}/\text{ml}$) in methanol, mixed well and kept in dark for 20 min. The absorbance at 517 nm was measured using the spectrophotometer UV-1650, Shimadzu. Plotting the percentage DPPH• scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0/A_1 \times 100$

Where as

A_0 was the Absorbance of control

A_1 was the Absorbance in presence of test or standard sample.

RESULTS AND DISCUSSION

Physical data of compounds

Physical Characterization, elemental analysis and spectral analysis were represented in table 1, table 2 and table 3 respectively

Table 1: Physical characterization of Novel Chalcones CH 01 – CH 10

Compound code	Compound Structure	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point ($^{\circ}\text{C}$)	Yield (%)
CH 01		$\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_4$	380.4	134-137	88
CH 02		$\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$	364.3	87-90	87
CH 03		$\text{C}_{19}\text{H}_{16}\text{N}_2\text{OS}$	320.4	121-124	88
CH 04		$\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}$	316.3	130-133	79
CH 05		$\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4$	364.3	110-113	75
CH 06		$\text{C}_{19}\text{H}_{13}\text{F}_3\text{N}_2\text{O}$	342.3	93-96	92

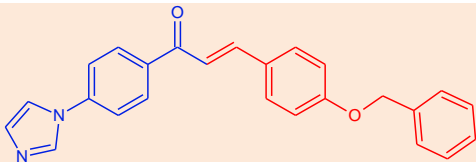
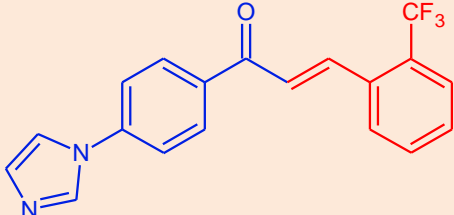
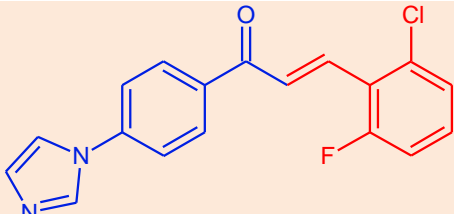
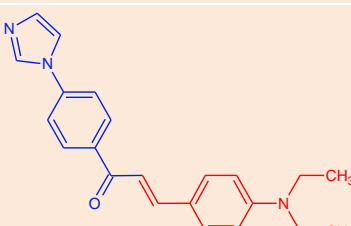
CH 07		$C_{25}H_{20}N_2O_2$	380.4	115-116	85
CH 08		$C_{19}H_{13}F_3N_2O$	342.3	125-126	87
CH 09		$C_{18}H_{12}ClF_2O$	326.7	128-129	83
CH 10		$C_{22}H_{23}N_3O$	345.4	135-137	85

Table 2 Elemental Analysis of Novel Chalcones CH 01 – CH 10

Compound		C	H	N	O	S	Cl	F
CH 01	%Calculated	69.22	5.53	7.69	17.56	-	-	-
	%Found	67.18	5.02	7.50	16.95	-	-	-
CH 02	%Calculated	69.22	5.53	7.69	17.56	-	-	-
	%Found	69.20	4.98	7.25	17.48	-	-	-
CH 03	%Calculated	71.22	5.03	8.74	4.99	10.01	-	-
	%Found	71.20	5.02	8.70	4.89	9.98	-	-
CH 04	%Calculated	79.72	6.37	8.85	5.06	-	-	-
	%Found	78.98	6.34	8.80	5.02	-	-	-
CH 05	%Calculated	69.22	5.53	7.69	17.56	-	-	-
	%Found	69.20	5.50	7.60	17.48	-	-	-
CH 06	%Calculated	66.66	3.83	8.18	4.67	-	-	16.65
	%Found	65.99	3.80	8.15	4.62	-	-	16.58
CH 07	%Calculated	78.93	5.30	7.36	8.41	-	-	-
	%Found	78.68	5.25	7.30	8.39	-	-	-
CH 08	%Calculated	66.66	3.84	8.18	4.67	-	-	16.65
	%Found	66.54	3.79	8.10	4.60	-	-	16.60
CH 09	%Calculated	66.16	3.70	8.57	4.90	-	10.85	5.81
	%Found	66.02	3.66	8.50	4.85	-	10.75	5.79
CH 10	%Calculated	76.49	6.71	12.16	4.63	-	-	-
	%Found	76.11	6.70	12.60	4.60	-	-	-

Table 3: Spectral Analysis of Novel Chalcones (CH 01 – CH 10)

Compound	IR, NMR data
CH-1	C=O, str. – 1660.76cm ⁻¹ ; C=C, str. – 1602.33cm ⁻¹ , N-H stretching :3365.01 cm ⁻¹ , C-H stretching: 3105 cm ⁻¹ , C-H stretching:3048.5 cm ⁻¹ , C-H stretching: 2936.16 cm ⁻¹ , C-C stretching:1583.83 cm ⁻¹ , C-N stretching: 1461.77cm ⁻¹ C-N stretching: 1371.05 cm ⁻¹ , C-N stretching:1318.09 cm ⁻¹ :(¹ H NMR(CHCl ₃):7.05 (1H, s, C-2 of imidazole), 7.58 (1H, d, C-4 of imidazole), 7.44-7.89 (6H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (9H, s, 3-OCH ₃)
CH-2	C=O, str. – 1661.12cm ⁻¹ , C=C str. – 1588.75cm ⁻¹ C-Cl str. – 828.23cm ⁻¹ ; N-H stretching : 3364.71 cm ⁻¹ , C-H stretching:3364 cm ⁻¹ C-H stretching: 3119.04 cm ⁻¹ , C-H stretching: 2937.74 cm ⁻¹ C-C stretching: 2834.04 cm ⁻¹ , C-N stretching: 1587.08 cm ⁻¹ , C-N stretching: 1486.59 cm ⁻¹ , C-N stretching: 1420.91 cm ⁻¹ : ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (6H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (9H, s, 3-OCH ₃)
CH-3	C=O: str. – 1657.87cm ⁻¹ C=C str. – 1600.46 cm ⁻¹ C-F str. – 1333.18 cm ⁻¹ ; N-H stretching :3404 cm ⁻¹ , C-H stretching: 3144.43 cm ⁻¹ C-H stretching: :3051.37 cm ⁻¹ , C-H stretching:2926.41 cm ⁻¹ , C-C stretching:1588.82 cm ⁻¹ , C-N stretching: 1491.32 cm ⁻¹ , C-N stretching: 1491.32 cm ⁻¹ , C-N stretching:1426.08 cm ⁻¹ ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), -CH ₃), 2.53 (3H, s, -CH ₃)
CH-4	C=O: str.- 1649.38cm ⁻¹ C=C str. – 1598.05cm ⁻¹ C-O str.- 1376.52cm ⁻¹ ; N-H stretching : 3410.25 cm ⁻¹ , C-H stretching: 3137.68 cm ⁻¹ C-H stretching: 3043 cm ⁻¹ , C-H stretching:2962.79 cm ⁻¹ C-C stretching: 1601.15 cm ⁻¹ , C-N stretching:1469.96cm ⁻¹ , C-N stretching: 1379.91 cm ⁻¹ , C-N stretching: 1304.04 cm ⁻¹ ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (6H, m, Ar-H), 7.20 (1H, d, α-H), 8.05 (1H, d, β-H), 2.34-2.48 (9H, s, 3-CH ₃)
CH-5	C=O: str.- 1658.95cm ⁻¹ C=C str.- 1594.05cm ⁻¹ C-S str.- 756.23cm ⁻¹ ; N-H stretching : 3392.75 cm ⁻¹ , C-H stretching: 3123.41 cm ⁻¹ C-H stretching: 2942.19 cm ⁻¹ , C-H stretching: 2829.8 cm ⁻¹ C-C stretching: 1602.2 cm ⁻¹ , C-N stretching: 1486.58 cm ⁻¹ , C-N stretching:1587.10 cm ⁻¹ : ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 6.50-7.89 (6H, m, Ar-H), 7.42 (1H, d, α-H), 8.33 (1H, d, β-H), 3.83 (9H, s, 3-OCH ₃)
CH-6	C=O, str. – 1661.12cm ⁻¹ , C=C str. – 1588.75cm ⁻¹ C-Cl str. – 828.23cm ⁻¹ ; N-H stretching : 3379.04 cm ⁻¹ , C-H stretching:2971.04 cm ⁻¹ C-H stretching: 2922.0 cm ⁻¹ , C-H stretching: 2866.04 cm ⁻¹ C-C stretching: 1603.2 cm ⁻¹ , C-N stretching: 1455.99 cm ⁻¹ , C-N stretching: 1325.03 cm ⁻¹ : ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.44-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H)
CH-7	C=O: str. – 1657.87cm ⁻¹ C=C str. – 1600.46 cm ⁻¹ C-F str. – 1333.18 cm ⁻¹ ; N-H stretching : 3330.60 cm ⁻¹ , C-H stretching: 3115.98 cm ⁻¹ C-H stretching: 3034 cm ⁻¹ , C-H stretching: 2931.65 cm ⁻¹ C-C stretching: 1595.88 cm ⁻¹ , C-N stretching: 1451.59 cm ⁻¹ , C-N stretching: 1422.70 cm ⁻¹ , C-N stretching: 1347.88 cm ⁻¹ , ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.38-7.89 (13H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 3.83 (2H, s, -OCH ₂ -)
CH-8	C=O: str.- 1649.38cm ⁻¹ C=C str. – 1598.05cm ⁻¹ C-O str.- 1376.52cm ⁻¹ ; N-H stretching :3368.84 cm ⁻¹ , C-H stretching: 2971.98 cm ⁻¹ C-H stretching: 2834.04 cm ⁻¹ , C-H stretching: 11587.08 cm ⁻¹ , C-C stretching: 1486.59 cm ⁻¹ , C-N stretching: 1420.91 cm ⁻¹ , C-N stretching: 1370.02 cm ⁻¹ , C-N stretching:1326.02 cm ⁻¹ , ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.31-7.89 (8H, m, Ar-H), 7.42 (1H, d, α-H), 8.33 (1H, d, β-H)
CH-9	C=O: str.- 1649.38cm ⁻¹ C=C str. – 1598.05cm ⁻¹ C-O str.- 1376.52cm ⁻¹ ; N-H stretching : 3077.88 cm ⁻¹ , C-H stretching: 3116.5 cm ⁻¹ C-H stretching: 3025.22 cm ⁻¹ , C-H stretching: 2969.61 cm ⁻¹ C-C stretching: 1599.52 cm ⁻¹ , C-N stretching: 1479.27 cm ⁻¹ , C-N stretching: 1370.88 cm ⁻¹ , C-N stretching: 1308.03 cm ⁻¹ ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 7.07-7.89 (7H, m, Ar-H), 7.42 (1H, d, α-H), 8.33 (1H, d, β-H)
CH-10	C=O: str.- 1649.38cm ⁻¹ C=C str. – 1598.05cm ⁻¹ C-O str.- 1376.52cm ⁻¹ ; N-H stretching : 3675.56 cm ⁻¹ , C-H stretching: 2972.21 cm ⁻¹ C-H stretching: 2867.73 cm ⁻¹ , C-H stretching: 2844.02 cm ⁻¹ , C-C stretching: 1591.62 cm ⁻¹ , C-N stretching: 1468.47 cm ⁻¹ , C-N stretching:1430.37 cm ⁻¹ , C-N stretching: 1349.69 cm ⁻¹ , C-N stretching: 1349.69 cm ⁻¹ ¹ H NMR(CHCl ₃): 7.15 (1H, s, C-2 of imidazole), 7.16 (1H, d, C-4 of imidazole), 6.71-7.89 (8H, m, Ar-H), 7.59 (1H, d, α-H), 8.06 (1H, d, β-H), 1.15 (6H, t, 2-CH ₃), 3.41 (4H, q, 2-CH ₂ -)

Biological Evaluation

All the synthesized ten novel chalcones were subjected for their antibacterial, antioxidant evaluation and represented their results in the table 4-5.



Table 4: Anti-bacterial evaluation:

	Concentration (µg/ml)	<i>B.subtilis</i>	<i>S.aureus</i>	<i>E.coli</i>	<i>P.vulgaris</i>
Zone of inhibition (mm)					
CH 01	50	14	13	13	10
	100	16	16	14	15
	150	18	18	17	16
CH 02	50	16	14	15	16
	100	18	18	18	18
	150	20	21	20	19
CH 03	50	6	8	9	6
	100	9	11	11	9
	150	10	14	10	10
CH 04	50	10	10	9	9
	100	13	13	11	11
	150	14	14	14	12
CH 05	50	14	14	14	14
	100	18	17	16	16
	150	20	20	18	18
CH 06	50	20	20	20	18
	100	22	18	18	20
	150	28	28	26	24
CH 07	50	8	9	8	8
	100	11	12	10	10
	150	12	13	12	11
CH 08	50	22	22	24	22
	100	24	26	28	24
	150	26	28	32	30
CH 09	50	18	18	16	16
	100	22	24	18	18
	150	24	26	22	24
CH 10	50	12	11	10	9
	100	15	14	12	13
	150	17	16	15	14
Standard (streptomycin)	50	24	24	26	28
	100	26	28	30	34
	150	30	34	36	34
Control (DMSO)	50	-	-	-	-
	100	-	-	-	-
	150	-	-	-	-

Table 5: Anti-oxidant activity:

S.No	Compound	Concentration (µM)	DPPH Screening (µM)
1	CH-1	100	91.54± 0.24
		200	170.24±0.58
		300	278.31±0.75
		400	341.24±0.58
		500	462.24±0.59
2	CH-2	100	56.24± 0.20
		200	125.24±.47
		300	185.24±0.25
		400	256.36±0.35
3	CH-3	100	92.74± 0.24
		200	175.24±0.58
		300	282.31±0.75
		400	348.24±0.58
4	CH-4	100	83.54± 0.24
		200	150.1±0.24
		300	260.1±0.25
		400	325.4±0.84
5	CH-5	100	61.27± 0.34
		200	135.24±0.24
		300	210.36±0.42
		400	286.36±0.28
6	CH-6	100	93.74± 0.24
		200	178.24±0.58
		300	284.31±0.75
		400	346.24±0.58
7	CH-7	100	84.54± 0.24
		200	152.24±0.58
		300	265.31±0.75
		400	328.24±0.58
8	CH-8	100	94.74± 0.24
		200	180.24±0.58
		300	286.31±0.75
		400	348.24±0.58
9	CH-9	100	92.21± 0.33
		200	166.33±0.58
		300	270.24±0.64
		400	342.55±0.25
10	CH-10	100	75.34± 0.33
		200	148.31±0.25
		300	258.24±0.24
		400	320.54±0.56
11	Ascorbic acid	100	48.63 ± 0.18
		200	98.31±0.33
		300	140.25±0.32
		400	210.41±0.54
		500	310.24±0.45

Each value is expressed as mean ± SD of three replicates, NA- No Activity

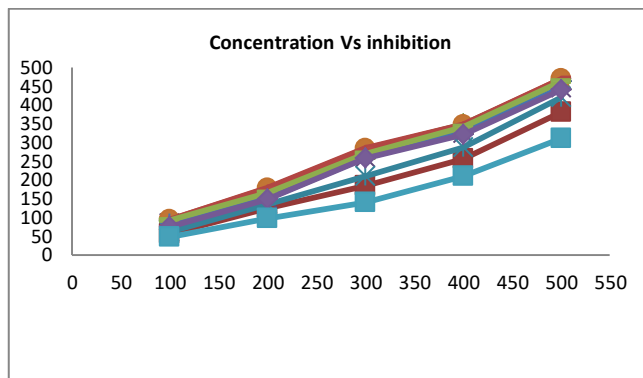


Figure 1: Concentration Vs Inhibition

DISCUSSION

The above synthesized compounds anti microbial evolution were performed by using Diffusion method by the calculation of Zone of inhibition against the test organisms, the compounds shows that compound CH-08 shows maximum activity as shown in Fig-1 than compare with other compounds, with *Bacillus subtilis* zone of inhibition 22,24,28 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml , *Staphylococcus aureus* zone of inhibition 22,26,28 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml , with *Pseudomonas vulgaris* zone of inhibition 22,24,30 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml, with *Escherichia coli* the zone of inhibition 24,28,32 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml. the compound CH-6 shows activity against with *Bacillus subtilis* zone of inhibition 20,22,28 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml , *Staphylococcus aureus* zone of inhibition ,20,18,28 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml , with *Pseudomonas vulgaris* zone of inhibition 20,18,26 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml, with *Escherichia coli* the zone of inhibition 18,20,24 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml. compound CH-9 with *Bacillus subtilis* zone of inhibition 18,22,24 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml , *Staphylococcus aureus* zone of inhibition 18,24,26 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml , with *Pseudomonas vulgaris* zone of inhibition 16,18,22 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml, with *Escherichia coli* the zone of inhibition 16,18,24 mm at 50 µg/ml ,100 µg/ml , 150 µg/ml.

The above anti oxidant activity of synthesized compounds were evolved using DPPH assay method. In the compounds CH-02 shows inhibition at 56.24± 0.20,125.24±.47, 185.24±0.25, 256.36±0.35, 380.36±0.36 at concentration of 100, 200, 300 ,400, 500 µg/ml respectively than other compounds ,and the latter compounds CH-05,CH-01, CH-10 shows activity .here compound CH-03 shows less activity than other compounds at concentration of 100, 200, 300 ,400, 500 µg/ml. the compound CH-02 potency was compare with the standard compounds ascorbic acid at similar concentration of test compounds.

CONCLUSION

The above results are concluding that the compound CH-08 was showing the better anti-microbial activity against both gram positive and gram negative the organism. The reason is due that compound contain more electron with drawing group than that of other compounds. In case of anti-oxidant compound CH-02 shows better anti-oxidant than other compounds due to less electron releasing tendency of the molecules. We concluding the compound CH-08 is may be best fit molecule against microbes, CH-02 having best anti-oxidant activity.

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