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SYNTHESIS, CHARACTERIZATION, ANTI MICROBIAL AND ANTIOXIDANT ACTIVITY OF CHALCONES FROM 3-METHOXY ACETOPHENONE

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ABSTRACT

Chalcones were important starting materials for the synthesis of various classes of five, six and seven member heterocyclic compounds. In the present work Chalcones were synthesized by base catalysed Claisen–Schmidt condensation, of 3-mehoxy acetophenone with appropriate aldehydes followed by dehydration. Six Chalcones were synthesized and structures were confirmed by spectral evidence. The compounds were tested for anti microbial activity and antioxidant activity using diffusion method by measuring the Zone of the inhibition and DPPH measuring by measuring the percentage of inhibition. In that compound B_3 shows potent activity than compare with other compoundswith Staphylococcus aureus zone of inhibition 16, 22 mm at 500 µg/ml, 1mg/ml, with Pseudomonas aeruginosa zone of inhibition 14, 22 mm at 500 µg/ml, 1mg/ml, with Escherichia coli the zone of inhibition 14, 26 mm at 500 µg/ml, 1mg/ml. compounds B3 shows potent activity than other compounds at concentration of 51. 24± 0.27 µM.

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 activity.[1] 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 chemically it is an important bio-molecule for synthesis of various molecules like flavones and isoflavones.

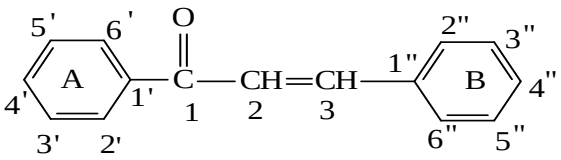


Figure. 1: General structure of chalcone.

Experimental Work

MATERIALS AND METHODS

3- Methoxy Acetophenone, various aromatic aldehydes, alcoholic potassium hydroxide, conc. HCl, DMSO, DPPH reagent. all the reagents were purchased analytical grade. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded in the indicated solvent on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded in KBr on Perkin-Elmer AC-1 spectrophotometer. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

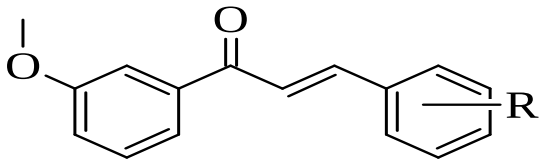
General method of preparation[4]: A mixture of 3- methoxy acetophenone(0.001moles) and aryl aldehydes(0.001moles) were dissolved in methanol(20ml) and to it 3millimoles of 15%KOH was added. The mixture was kept for 24hours and it was acidified with 1:1 HCl and water, then it was filtered through vacuum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

Chemical reaction

3-Mehoxy Acetophenone + Aromatic aldehydes → Chalcone derivatives

S. No.	Sample code	R
1.	B1	-H
2.	B2	4-Cl
3.	B3	4-F
4.	B4	4-OH
5.	B5	4-SCH3
6	B6	2-Cl
7	B7	2-F
8	B8	2-OH
9	B9	3-OH

Physical data of compounds



Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (^{o}C)	Yield %
B_1	-H	$C_{16}H_{14}O_2$	238.2	134-137	88
B_2	4-Cl	$C_{16}H_{13}ClO_2$	272.7	87-90	87
B_3	4-F	$C_{16}H_{13}FO_2$	256.2	121-124	88
B_4	4-OH	$C_{16}H_{14}O_3$	254.2	130-133	79
B_5	4-SCH_3	$C_{17}H_{16}O_2S$	284.3	110-113	75
B_6	2-Cl	$C_{16}H_{13}ClO_2$	272.7	93-96	92
B_7	2-F	$C_{16}H_{13}FO_2$	256.2	115-116	85
B_8	2-OH	$C_{16}H_{14}O_3$	254.2	125-126	87
B_9	3-OH	$C_{16}H_{14}O_3$	254.2	128-129	83

Table. 3: Elemental Composition.

Compound	%Calculated			%Found		
	C	H	O	C	H	O
B_1	80.6	5.92	13.4	79.8	5.88	13.34
B_2	70.4	4.8	11.7	70.38	4.79	11.65
B_3	74.9	5.1	12.4	74.85	5.08	12.38
B_4	75.5	5.55	18.8	75.48	4.48	18.79
B_5	71.8	5.6	11.2	71.79	5.55	11.18
B_6	70.4	4.8	11.7	70.38	4.75	11.65
B_7	74.9	5.1	12.4	74.85	5.05	12.38
B_8	75.5	5.5	18.8	75.4	5.4	18.7
B_9	75.5	5.5	18.8	75.45	5.45	18.75

Table. 4: Spectral data of compounds.

Compound	IR, NMR data
B_1	C=O,str. – 1660.76cm^{-1} ;C=C, str. – 1602.33cm^{-1}, 2H; 7.4ppm doublet, 2H; 4.08ppm doublet, 1H; 1.6ppm doublet, 2H; 7.5ppm doublet, 1H; 1.6ppm doublet, 2H; 7.39-8.01ppm doublet.
B_2	C=O,str. – 1661.12cm-1, C=C str. – 1588.75cm-1 C-Cl str. – 828.23cm-1; 2H; 7.4ppm doublet, 2H; 7.6ppm doublet, 1H; 7.8ppm doublet, 2H; 7.5ppm doublet, 2H; 7.9ppm doublet, 2H; 7.6-7.5ppm doublet.
B_3	C=O: str. – 1657.87cm-1 C=C str. – 1600.46 cm-1 C-F str. – 1333.18 cm-1; 4H; 8.4-8.0ppm two doublets, 2H; 8.0-7.8ppm. doublet, 2H; 7.8-7.6ppm doublet, 3H 7.4-7.2ppm doublet.
B_4	C=O: str.- 1649.38cm-1 C=C str. – 1598.05cm-1 C-O str.- 1376.52cm-1; 1H. 9.0ppm singlet, 1H;. 7.8ppm singlet, 1H; 7.5ppm singlet, 1H; 7.3ppm singlet, 4H; 7.0ppm triplet.
B_5	C=O: str.- 1658.95cm-1C=Cstr.- 1594.05cm-1 C-S str.- 756.23cm-1; 1H; 8.5ppm singlet, 2H; 8.2ppm doublet, 2H; 8.0ppm triplet, 2H; 7.6ppm doublet, 3H; 7.4ppm triplet.
B_6	C=O,str. – 1661.12cm-1, C=C str. – 1588.75cm-1 C-Cl str. – 828.23cm-1; 2H; 7.4ppm doublet, 2H; 7.6ppm doublet, 1H; 7.8ppm doublet, 2H; 7.5ppm doublet, 2H; 7.9ppm doublet, 2H; 7.6-7.5ppm doublet.
B_7	C=O: str. – 1657.87cm-1 C=C str. – 1600.46 cm-1 C-F str. – 1333.18 cm-1; 4H; 8.4-8.0ppm two doublets, 2H; 8.0-7.8ppm. doublet, 2H; 7.8-7.6ppm doublet, 3H 7.4-7.2ppm doublet.
B_8	C=O: str.- 1649.38cm-1 C=C str. – 1598.05cm-1 C-O str.- 1376.52cm-1; 1H. 9.0ppm singlet, 1H;. 7.8ppm singlet, 1H; 7.5ppm singlet, 1H; 7.3ppm singlet, 4H; 7.0ppm triplet.
B_9	C=O: str.- 1649.38cm-1 C=C str. – 1598.05cm-1 C-O str.- 1376.52cm-1; 1H. 9.0ppm singlet, 1H;. 7.8ppm singlet, 1H; 7.5ppm singlet, 1H; 7.3ppm singlet, 4H; 7.0ppm triplet.

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 activity[5,6]

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* (NCIM 2079), *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 2931).

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* were spread over the media with the help of a sterile swab socked 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 method[7,10]

DPPH is a stable free radical that can accept anelectron or hydrogen radical to become a stable diamagnetic molecule. Due to its oddelectron, 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 µM/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.[6] 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 = $Ao - A1/Ao \times 100$

Where A0 was the Absorbance of control reaction

A1 was the Absorbance in presence of test or standard sample.

RESULT

S. No	Compound	DPPH Screening (µM)
1	B_1	NA
2	B_2	66.24± 0.22
3	B_3	51.24± 0.27
4	B_4	115± 0.33
5	B_5	101.22± 0.33
6	B_6	61.27± 034
7	B_7	85.34± 0.33
8	B_8	94.54± 0.24
9	B_9	111.22± 033
10	Ascorbic acid	48.63 ± 0.18

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 B3 shows maximum activity than compare with other compounds, with Staphylococcus aureus zone of inhibition 16,22 mm at 500 µg/ml, 1mg/ml, with Pseudomonas aeruginosa zone of inhibition 14,22 mm at 500 µg/ml, 1mg/ml, with Escherichia coli the zone of inhibition 14,26 mm at 500 µg/ml, 1mg/ml. the compound B_2 shows activity against Staphylococcus aureus zone of inhibition 14,20 mm at 500 µg/ml, 1mg/ml, with Pseudomonas aeruginosa zone of inhibition 14,22 mm at 500 µg/ml, 1mg/ml, with Escherichia coli the zone of inhibition 15,24 mm at 500 µg/ml, 1mg/ml. compound B_4 moderately shows results against the Staphylococcus aureus zone of inhibition 12,16 mm at 500 µg/ml, 1mg/ml, with Pseudomonas aeruginosa zone of inhibition 12.20 mm at 500 µg/ml, 1mg/ml, with Escherichia coli the zone of inhibition 16,22 mm at 500 µg/ml, 1mg/ml.

Compound	***Staphylococcus aureus***		***Pseudomonas aeruginosa***		***Escherichia coli***	
	Zone of inhibition (mm)					
	500µg/ml	**1mg/ml**	**500µg/ml**	**1mg/ml**	**500µg/ml**	**1mg/ml**
B_1	**12**	**18**	**12**	**16**	**14**	**18**
B_2	**14**	**20**	**14**	**22**	**15**	**24**
B_3	**16**	**22**	**14**	**22**	**14**	**26**
B_4	**12**	**16**	**12**	**20**	**16**	**22**
B_5	**12**	**16**	**14**	**18**	**12**	**18**
B_6	**14**	**20**	**14**	**20**	**14**	**20**
B_7	**16**	**19**	**14**	**20**	**14**	**22**
B_8	**12**	**16**	**12**	**16**	**12**	**16**
B_9	**11**	**15**	**12**	**16**	**12**	**16**
Streptomycin	**16**	**20**	**18**	**22**	**20**	**26**
Control(DMSO)	**-**	**-**	**-**	**-**	**-**	**-**

The above anti oxidant activity of synthesized compounds were evolved using DPPH assay method. In the compounds B3 shows potent activity than other compounds at concentration of 51.24 µM and the latter compounds B6, B2, B7, B8 shows activity. here compound B1 shows no activity.

CONCLUSION

The above results we concluding the compound B_3 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. we concluding the compound B_3 is may be best fit molecule against microbes, a having the anti-oxidant activity it may became an lead to discover anti microbial molecule, good anti oxidant molecule.

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