Synthesis of Chalcone Derivatives and its Antimicrobial Activities

Dr. Arpitaben P. Desai, Patel Drashti K.

1Master of Sciences, Faculty Guide, Parul University, Faculty of Applied Science, P. o. Limda, Tal. Waghodia, dist. Vadodara - 391760, India

2M. Sc. Semester Iv, Parul University, Faculty of Applied Sciences, P. O. Limda, Tal. Waghodia, Dist. Vadodara – 391760, India

1. ABSTRACT

To investigate the in vitro antibacterial activity of these chalcones against Gram-positive and Gram-negative bacteria as well as fungi, a series of five chalcones that closely mimic those found in nature were created. By the Claisen-Schmidt condensation and a green chemistry procedure that used sodium hydroxide as a catalyst in clean water, chalcones were produced from acetophenone and substituted benzaldehydes with good yields. Amoxicillin and fluconazole were used as standards for the antimicrobial assessment, which was done using the filter paper disc plate technique on a set of chosen bacteria. The structure of the generated derivatives has been determined by employing FTIR Spectroscopy, NMR, and other physicochemical characteristics. These synthesized chalcones showed the expected antibacterial action after being tested for it. This green procedure is the most practical, effective, and environmentally friendly method for the synthesis of chalcones by the Claisen- Schmidt condensation because it is simple to synthesize and purify these chalcones and because high yields are observed. The future development of chalcone derivatives as commercial antibacterial medicines is quite promising.

2. KEYWORDS

Chalcone, Chalcone Synthesis, substituted chalcone derivatives, antimicrobial agents

3. INTRODUCTION

The therapeutic potential of natural product compounds is gaining more attention. An important class with nature compounds is chalcones. They are composed of open-chain flavonoids, which have two aromatic rings connected by a three-carbon unsaturated carbonyl system. It is revealed that the reactive, α,β unsaturated keto activity of chalcones gives rise to their antibacterial properties [1-2]. Recently, cytotoxic, anticancer, mutagenic, Research has been done on several chalcones' antibacterial, insecticidal,

and enzyme-inhibitory characteristics [3].

The chalcones have been found to be useful in providing the structure of natural products like phloretin, sakuranet, hemlocktanin, homoeriodictyol, ergodicity, cyanamaclurin [4-6] etc. Chalcone is an aromatic ketone and an Enone, and it is a key component of a variety of significant biological compounds that Harborne *et al.* refer to as chalcones or chalconoids [7]. Chalcones were researched for a very long time because of their high reactivity and strong kinship to flavanones, flavones, and dihydro flavonols. Its investigation as precursors to drugs with therapeutic value has been of significant interest.

The chalcones are useful starting materials for the synthesis of a variety of heterocyclic compounds, such as deoxybenzoins and hydantoins, which have some medicinal utility, as well as flavones, flavonols, pyrimidines, pyrazolines, anthocyanins, and benzal coumaranones. Bandgar, B. P., et al. [8-9]. Several biological actions, including antibacterial, anti- inflammatory, analgesic, antiplatelet, anti-ulcerative, anti-malarial, anticancer, and antiviral, have been documented for compounds using chalcones as their backbone. The existence the presence of a catalytic, α, β-unsaturated keto functionality is discovered to the root cause of the biological activities of chalcones.

Benzylideneacetophenone serves as the parent chemical for the chalcone series. Some other names for chalcone include phenyl styryl ketone, benzalacetophenone, phenylacrylophenone, diphenyl-, and phenyl-benzoyl ethylene. With sodium hydroxide acting as a catalyst, chalcones can be produced by performing an aldol reaction between acetophenone and benzaldehyde [10]. The release of chemical mediators, leukotriene B4 [11], and aldose reductase [12] activities are all inhibited by a variety of chalcones with hydroxy and alkoxy groups in various places, including those with antibacterial [13-15], anti-HIV agent [16], antifungal [17], antiulcer [18], antimalarial [19], antioxidant [20-21], vasodilatory [22], anticonvulsant [23],

anti-analgesic, antidiabetic [24-25] anti-tumour agent [26] antimitotic [27], and antileishmanial [28] properties.

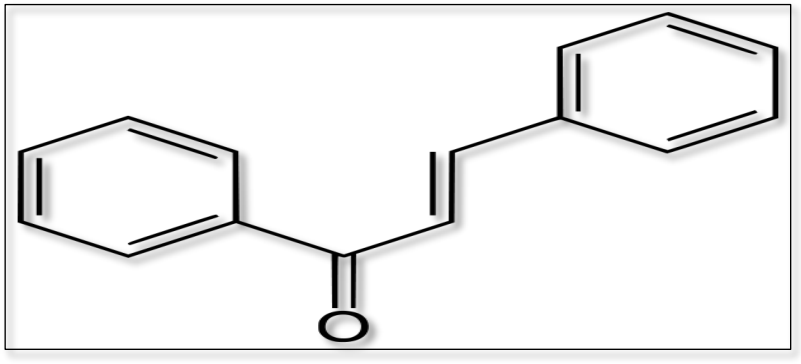


Figure 1. Structure of chalcone

Moreover, since many heterocycles exhibit a variety of biological activities, they are widely acknowledged as crucial intermediates in their chemical synthesis. [29]. There is increasing proof of the antibacterial action of chalcones. Several research teams were looking into isolating or creating chalcones with antibacterial characteristics. It was found that conjugate addition with a nucleophilic group was demonstrated by the, α, β -unsaturated ketone (Enone) reactive moiety in chalcones, which also contributed to their antibacterial activity. The type and arrangement of the substituents on the aromatic rings found in chalcones can vary, similar to a thiol group in an important protein, and this affects the antibacterial action of the chalcones. These results prompted us to create chalcones, which could serve as a model for future antibacterial drugs. The substitution pattern on the benzylideneacetophenones' nucleus is provided by the scaffold, it must be noticed.

4. EXPERIMENTAL

4.1 Materials

1. Anisaldehyde
2. Acetophenone
3. Sodium hydroxide
4. Methanol
5. 4-dimethyl amino benzaldehyde

Solvent:

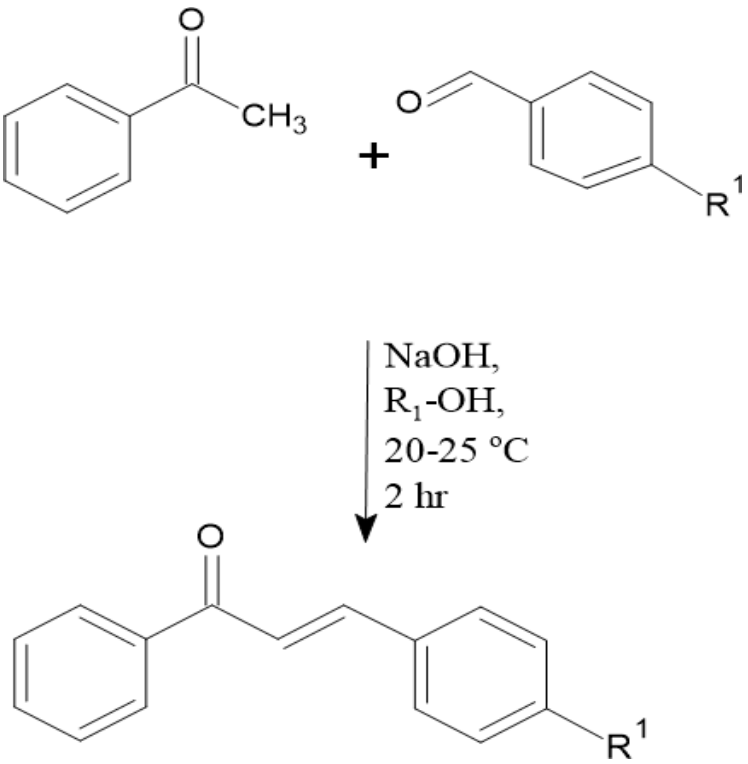
1. Ethyl acetate
2. n-hexane

4.2 METHOD/SYNTHESIS

The general procedure of chalcone:

Chalcones were produced using a core Condensation reactions chemical bond with the appropriate acetophenones and substitute benzaldehydes [16]. This method is widely known in the literature. The derivatives of acetophenone and benzaldehyde were mixed together and diluted in 10 ml of rectified spirit in a 250 ml shaped flasks with a magnetic stirring. Next, dropwise, 5.5 gm NaOH in 50 ml of

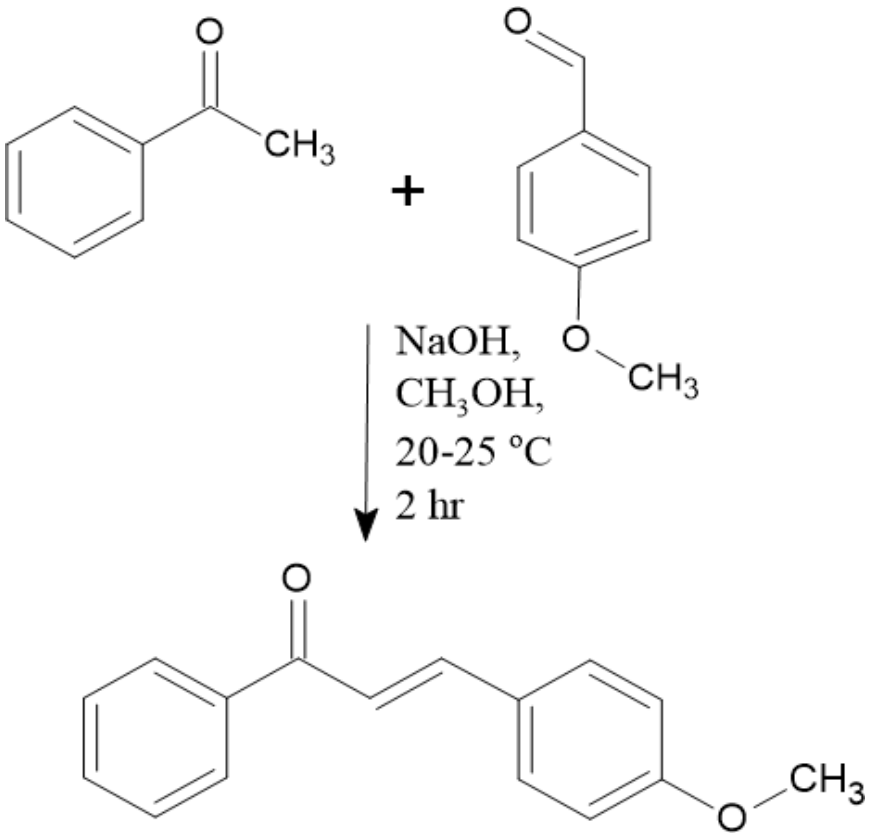
distilled water was added.when the reaction mixture had been vigorously stirred for 2 hours and the solution had turned turbid. A chilled water immersion on the magnetic stirrer was used to keep the reaction temperature between 20 and 25 C. The precipitation took place when 0.1–0.2N HCl was added to the reaction mixture after 2 hours of vigorous stirring. After being filtered out, the unrefined chalcones were air dried and then recrystallized using rectified spirit. To get pure chalcones, the residue underwent column chromatography purification (silica with 10% ethanol in n-hexane) (Scheme 1).



Scheme 1. Chalcone derivative

Procedure of chalcone using anisaldehyde

A mixture of acetophenone (5 ml), anisaldehyde (2.5 ml) and NaOH (2.5 ml) in methanol (15 mL) was kept at ambient temperature for 2 h followed by dilution with ice-cold water, acidification with cold dilute hydrochloric acid, and ether extraction.The purity of compound was evaluated using thin-layer chromatography using ethyl acetate/ n-hexane as an adhesive.And also evaluated by ^{1}H NMR, IR.



Scheme 2. 4-methoxy chalcone

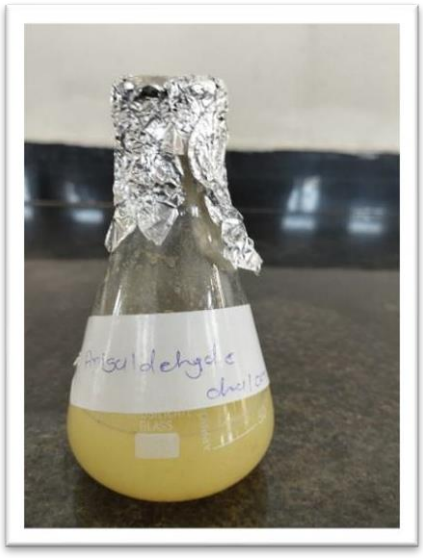
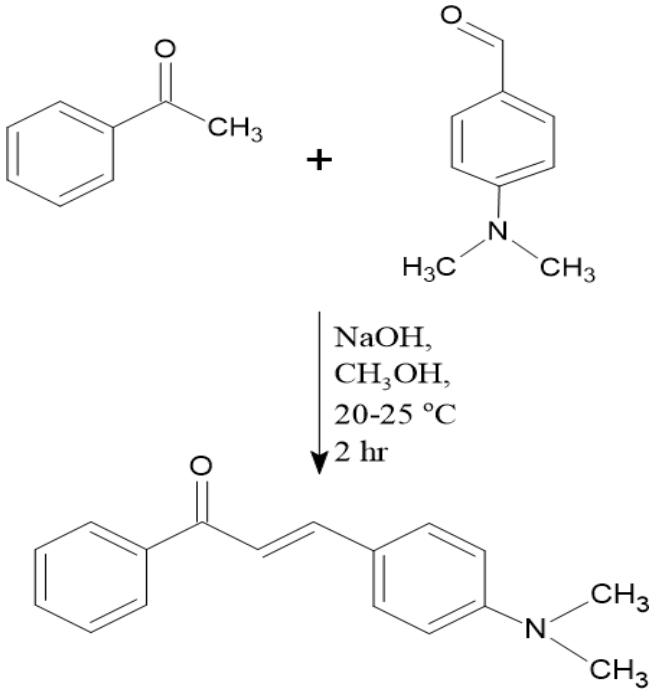


Figure 2. Anisaldehyde solution

Procedure of chalcone using 4-dimethyl amino benzaldehyde

A mixture of acetophenone (6.35 ml), 4-dimethyl amino benzaldehyde (5.5 gm), NaOH (2.75 gm), distilled water (25 ml), and methanol (15.25 mL) was kept at ambient temperature for 2 h followed by dilution utilising icy water, acidification with cold dilute hydrochloric acid, together with extraction ether. The purity of the compound was evaluated using thin-layer chromatography using ethyl acetate/ n-hexane as a purge. And also evaluated by ^{1}H NMR,IR.



Scheme 3. 4- dimethyl amino benzaldehyde

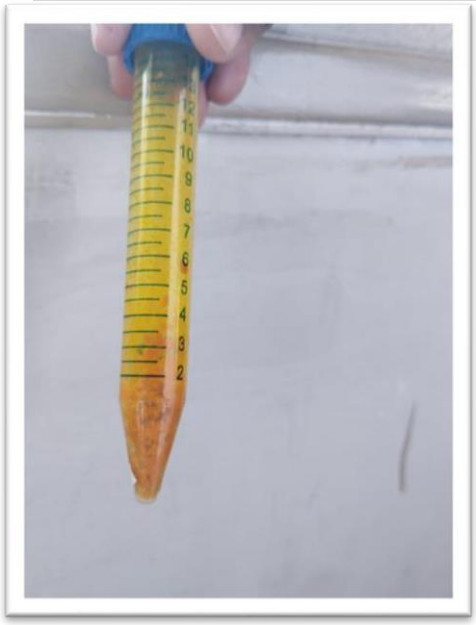


Figure 3.4-dimethyl amino benzaldehyde solution

Analysis of data

No.	Code No.	R	Molecular Formula	Molecular Weight (g/m)	Yield (%)	M.P. °C	C %	H %	N %
							Found		
1	1a	p-OCH_3	C_8H_8O2	136.15	73	-1	70.5%	5.8%	
2	1b	p-$N(CH3)_2$	$C_9H_{11}NO$	149.19	72	74	72.4%	7.3%	9.3%

5. RESULT AND DISCUSSION

The current study's findings showed that the green chemistry used was a potent tool that could be used to construct and make chalcones in distilled water accelerated by sodium hydroxide as well as to produce, -unsaturated ketones in good yield. The final watery reaction mixture is filtered once it has cooled to room temperature in order to separate the products in the almost pure state without the use of any solvents. By using IR, element microanalysis (CHN), and other physical standards, the structures of the produced compounds were confirmed The synthesised chalcone derivatives showed a number of distinct, sharp bands in the infrared spectra, with the lines around 1635 and 1660 cm-1 indicating the presence of the carbonyl C=O group of the produced ketone. was coupled to an alkene system as well as an aromatic system. The basic microanalysis showed good agreement between the result and the estimated percentages. The percentage disparities between the observed and calculated values were discovered to be within the bounds of accounting for at least. Uncorrected melting points were determined using the ThermoCal10 melting point device (Analab Scientific Pvt. Ltd.). Silica gel 60 F254 seeped through aluminium sheets as they underwent TLC.

Before use, none of the chemical compounds were cleaned; they were all of the LR grade. Alkenes, ethanol, sodium hydroxides, and phenyl urea used in the experiment were supplied by Merck in Bombay, India. Every solvent was used exactly as it had been delivered by Lockheed Martin in Mumbai, India.

Antimicrobial activity

As chemists, pharmacists, and doctors first refined the active ingredients from plant and animal tissues, then subsequently from microorganisms and their fermentation by-products, the chemistry of

therapeutically relevant compounds was born. Several Certain substances have been linked to therapeutic benefits for frequently poorly understood medical conditions. Several of the chemicals in question have been linked to therapeutic benefits for frequently poorly understood medical conditions.

Principal:

The test microorganism is injected onto agar plates in a consistent amount. The paper discs are placed on the agar surface and have a diameter of about 6 mm. They contain the test substance at the desired concentration. A suitable environment is used to incubate the Petri dishes.

Typically, antimicrobial drugs permeate into the agar, inhibiting the test microorganism's germination and development, after which the diameters of the inhibitory growth zones are determined. Incubation results in the formation of a clean zone or ring around a disc if the chemical prevents bacterial growth.

Requirements

1. Gram positive Organism Subtilis Bacillus

A. Staphylococcus

2. Gram negative Organsim

E. coli

Pseudomonas aeruginosa

3. Test Compound
4. Alcohol
5. Sterile Nutrient Agar
6. Glass Spreader
7. Sterile Petri Plates
8. Paper discs
9. Sterile Forceps

Inoculum preparation

An appropriate broth was used to emulsify 4-5 colonies of the indicator organism from the slope of a stock culture to create the inoculum. The inoculation broth was heated to 37 °C until it reached the McFarland standard of 0.5 turbidity. This occurs in 2 to 8 hours.

Procedure

- Take 4 Sterile Petri dishes and pour Nutrient Agar (Autoclaved) on jars of Petri dishes.
- Take 0.1 mL of Your 24-48 hours old bacterial culture and spread it onto the exterior of nutrient Agar dishes using a glass Spreader.

The disc holding the chemical should be inserted into the top layer of the agar plates using sterile pipette or a disc dispenser.

- Right away, softly push it down with the tool to make sure the disc and the upper surface of agar are completely in touch. Never move a disc once it has been exposed to the surface of the agar because some drug diffusion happens instantly.
- Incubate plates at 37°C or at an optimum growth temperature day and night.
- Observe the zone of inhibition after the incubation period & The size of the inhibitory zones should be read and noted.

BACTERIAL IMAGE

Figure 4. Antimicrobial of E.coli (-)

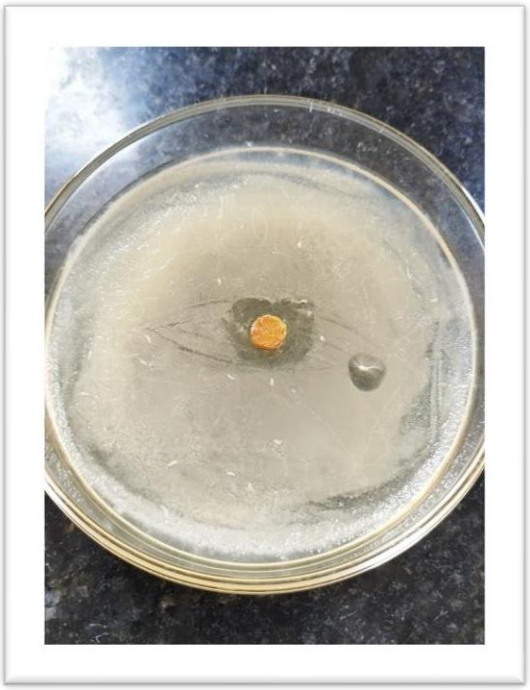


Figure 5. Antimicrobial of Bacillus (+)

Figure 7. Antimicrobial of E.coli (-)

Figure 8. Antibacterial of Bacillus (+)

5.1 CHARACTERISATION

Melting points were calculated without correction using ThermoCal10 (Analab Scientific Pvt. Ltd.) melt point equipment. Aluminum sheets that had silica gel 60 F254 percolated through them underwent TLC. All reagents utilized were of LR grade & utilized without any purification. Various aldehydes, phenyl urea, ethanol, and sodium hydroxides were utilised in the form that Merck, Mumbai, India, supplied.All the solvents were used as received from Merck, Mumbai, India.

Infrared Spectroscopy

Infrared spectroscopy is considered one among the most important technique used in organic chemistry for elucidating the composition of various organic compounds according to their characteristic absorption frequencies pertaining to the operational teams and type of bonds present in compound.
The most potent analytical approach is infrared spectroscopy, which can identify all types of organic and many types of inorganic substances as well as find functional groups in pure molecules. IR spectrometers may take a range of types of samples, include gases, liquids, and solids, using various

sampling techniques. IR spectroscopy is a vital and well-liked tool for deciphering structures and identifying chemicals.

Infrared spectra of synthesis derived products have scanned in KBr pellets by utilising Shimashu 1801 FTIR. Spectrophotometer instrument in area from 4000 cm-I to 667 cm-I . Roughly 100 mg of dry potassium bromide powder is completely combined with the sample (0.5 to 1.0 mg), which is coarsely pulverised. Grinding and combining can be accomplished with a mortar and pestle. The finely ground mixture was transferred to the mould, and high transparency pellets were formed by pressing on the hydraulic press. The produced pellets were used to scan the sample's infrared spectra.

1. Synthesis of chalcone using anisaldehyde

Examination of IR spectra reveals that all the compounds of chalcone showed a stronge absorption near at 1644-1618 cm^{-1} and at 1685-1666 cm^{-I} demonstrates the existence of - CH=CH- and C—O group. For the FT-IR Spectra of anisaldehyde revelved that the broad band at 1656 cm^{-1} observed for the OCH_3 group, and the stretching vibration shows the existence of OCH_3 group. Also, the shifting of band confirms that the OCH_3 group presence in the chalcone for chalcone derivative.

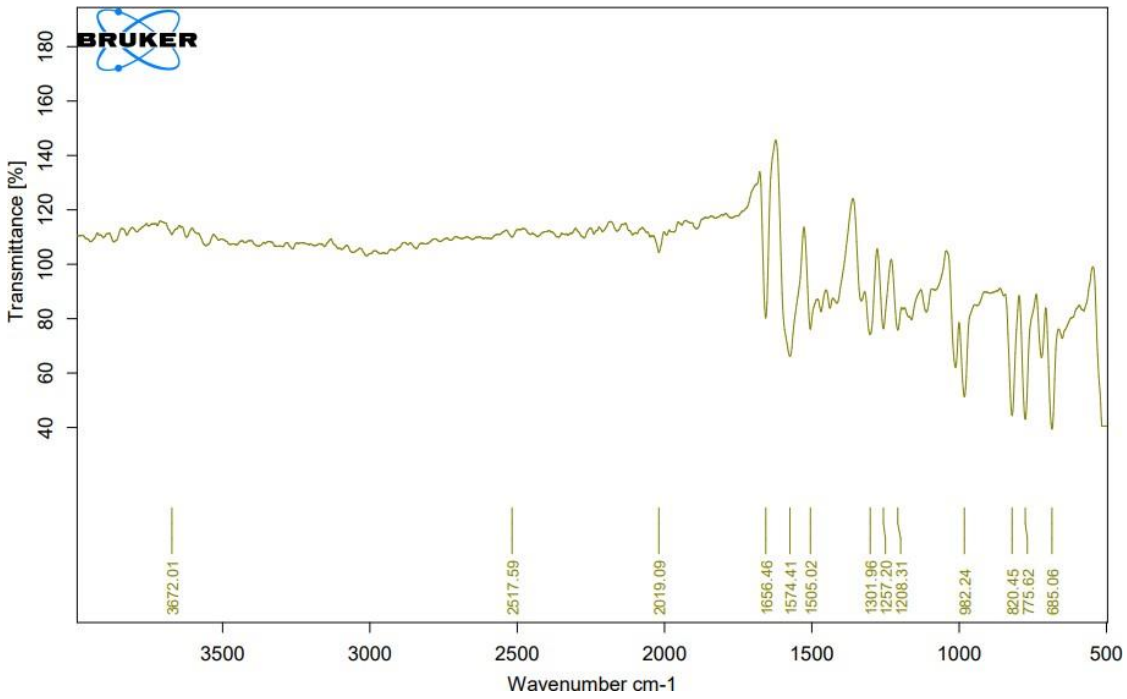


Figure 9. FT-IR spectra of anisaldehyde

2. Synthesis of chalcone using 4-dimethyl amino benzaldehyde

IR spectra reveals that all the derivatives of chalcone were displayed. A stronge absorption near at 1644-1618 cm^{-1} and at 1685-1666 cm^{-I} indicates being present -CH=CH- and C-O group. From figure 8. The IR bands at 1669 cm-1 indicates that the 4-dimethyl amino group presence in the chalcone-derived substance and its due to the shifting of bands at the region of chalcone derivative.

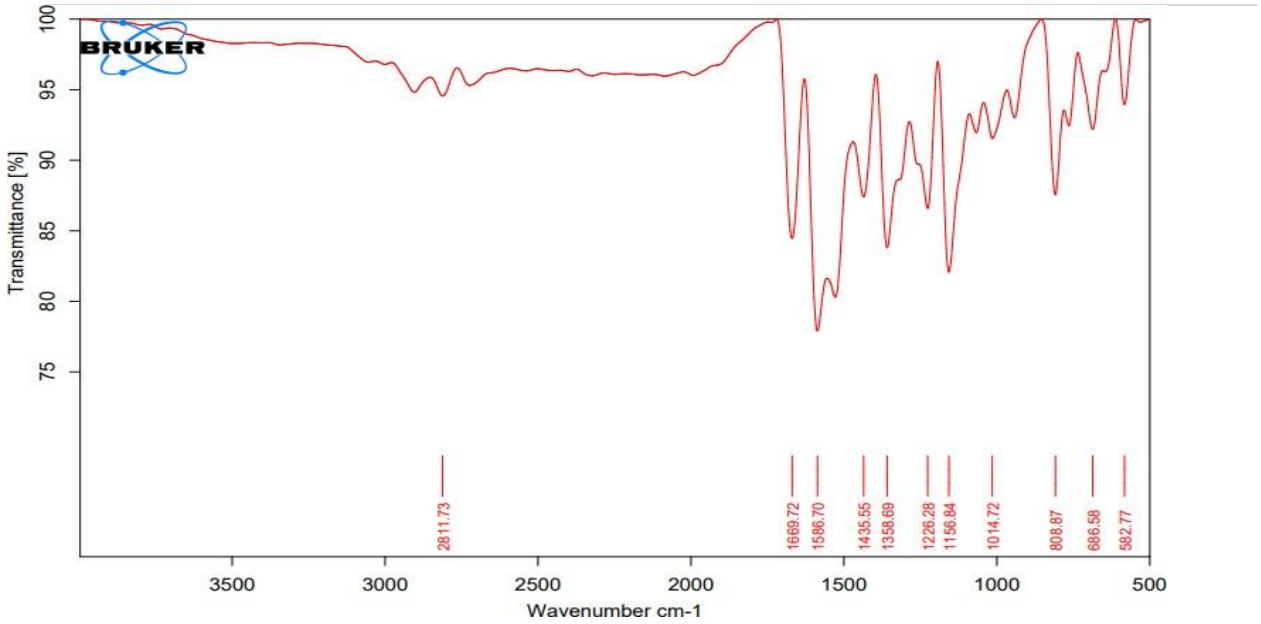


Figure 10. FT_IR spectra of 4-dimethyl amino benzaldehyde

NMR:

Nuclear magnetic resonance spectroscopy (NMR) is one of the most recent physical approaches for studying organic molecules. It permits the qualitative and quantitative identification of distinct atom-to-atom bonds. High resolution 1H NMR is another effective technology that has been used in the analysis of organic structure. The 1H-NMR spectra were acquired on a BRUKER AVANCE 11 400 NMR spectrometer using DMSO as the solvent and TMS as the internal reference.

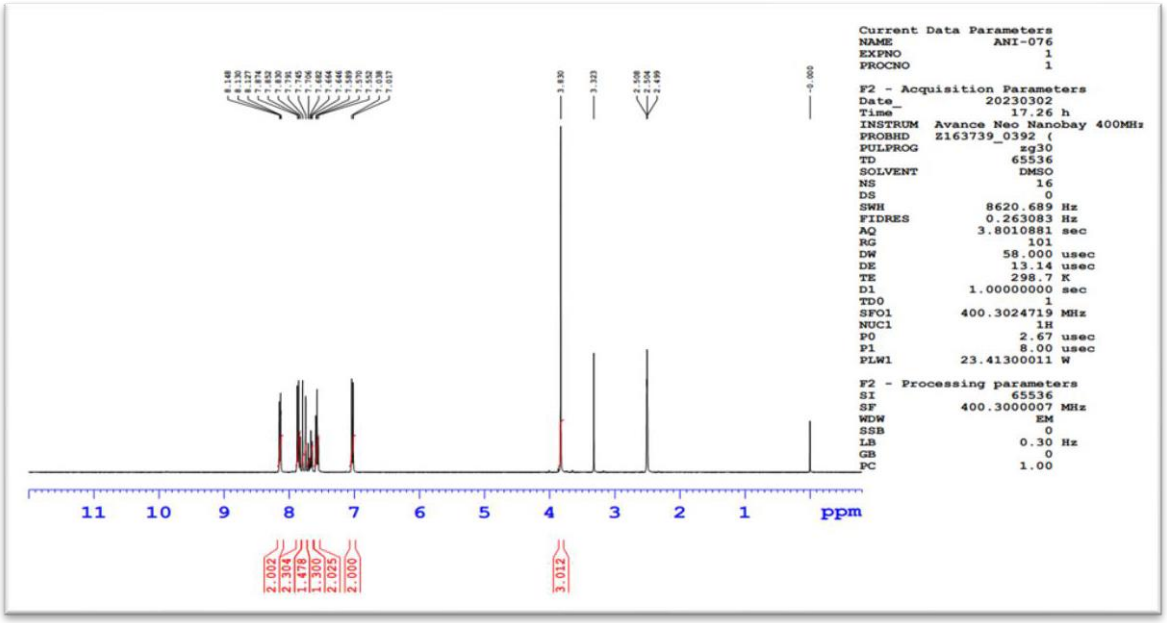
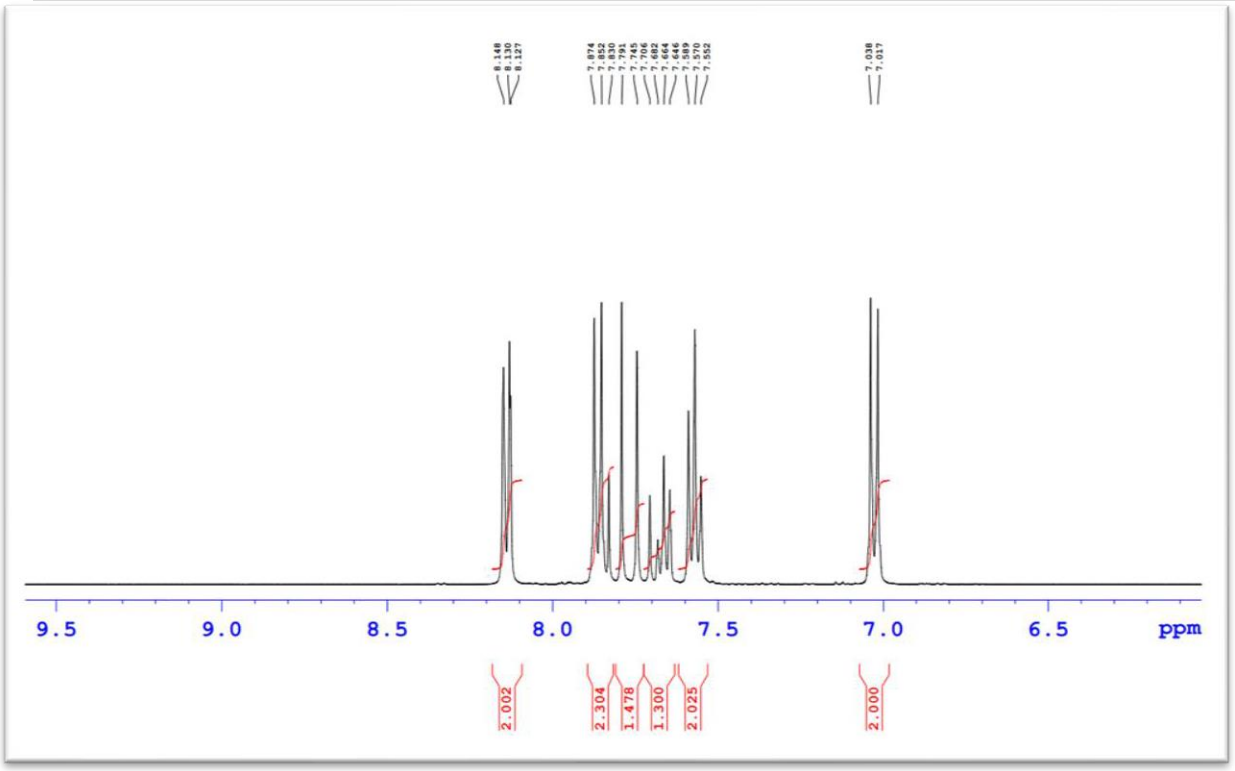
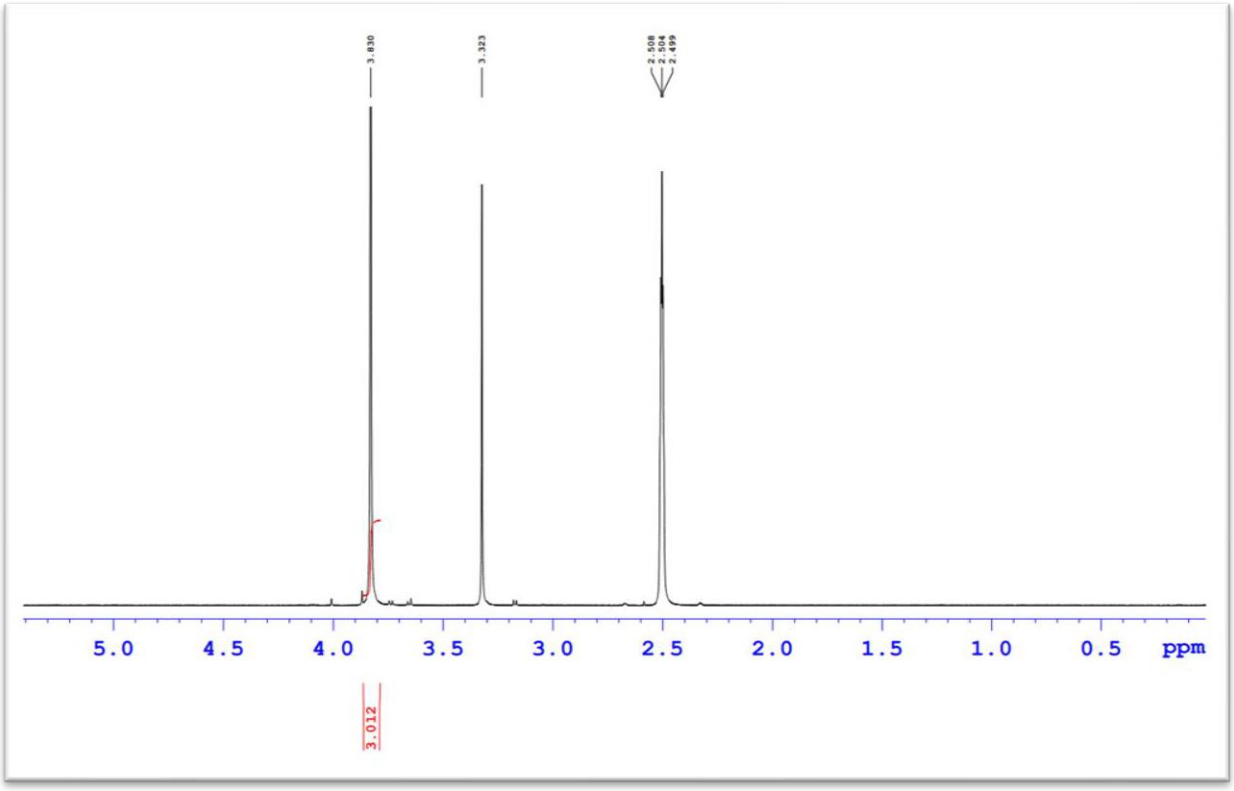


Figure 11. NMR of Anisaldehyde





6. CONCLUSION

The synthesised chalcone analogues have moderate antibacterial activity, including those against Staphylococcus. The findings, which were based on the potentially active chalcone skeleton, highlighted the importance of the positions of the electron releasing groups in the B ring, specifically the methoxy and hydroxy groups or compounds 2 and 4, in achieving superior antibacterial activity when compared to the reference standard amoxicillin at both the 0.5 ml (500 g) and 1 ml (1000 g) concentration levels. Evaluating chalcones with pharmacophores like nitro groups to the reference standard fluconazole at concentrations of 0.1 ml (100 g), 0.5 ml (500 g), and 1 ml (1,000 g), fungicidal screening data showed that nitro group-containing chalcones had stronger antimicrobial properties than other chalcones. values of concentration at 1 ml (1000 g). These findings imply that perhaps the chalcone derivatives might serve as a good design and development template for novel commercial antibacterial compounds. To clarify their mode of action, more research is required. While reviewing the antimicrobial findings, an intriguing structure-activity link was found, showing that particle groups tended to reduce the efficacy of the antifungal activity while exciton groups increased it.

7. Acknowledgement

I would like to sincerely thank the following for their support, assistance, and encouragement throughout my course of research. And I am extremely thankful to my mentor and guide Dr. Arpitaben Desai who helped me throughout my research work and also solved my several queries so I would complete my work with ease. I would also like to mention our principal Dr. Trilok Akhani sir and Hod Dr. Kushan Parikh sir for providing this golden opportunity to work on this project.

8. REFERENCE

1. Hsieh, Hsin., Lee, Tai-Hua., Wang, Pyang., Wang, Jeng., Lin, Chun. Synthesis and Anti-inflammatory Effect of Chalcones and Related Compounds, *Pharm Res.*, **1998**, 15, 39–46

2. Wong E., Foetschr, *Chem. org. Naturs.*, 28, **1970**, 1.

3. Patil, C. B.; Mahajan, S. K.; Katti, S. A., Chalcone: A Versatile Molecule. *J. Pharm. Sci. Res.* **2009**, 1, 11-22.

4. Claisen, L.; Claparéde, A., Condensationen von Ketonen mit Aldehyden. *Chem. Ber.* **1881,** *14*, 2460-2468

5. Schmidt, J., Ueber die Einwirkung von Aceton auf Furfurol und auf Bittermandelöl bei Gegenwart von Alkalilauge. *Chem. Ber.* **1881,** *14*, 1459-1461.

6. Harborne, J. B.; Williams, C. A., Advances in Chalcone research since 1992. *Phytochemistry* **2000,** *55*, 481-504.

7. Bandgar, P.; Gawande, S.; Bodade, G.; Totre, V.; Khobragade, N., Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti- inflammatory and antioxidant agents. *Bioorg. Med. Chem.* **2010,** *18*, 1364-1370

8. Oyamada, B., *J. Chem. Soc. Japan,* **1934,** *55*, 1256

9. Torssell, G., Natural Product Chemistry. A Mechanistic, Biosynthetic, and Ecological Approach. K.B.G. *Torssell., Plant Gro. Regu.*, **1999**, 29, 203

10. Horng K, LoTT, Kun Y, Cheng L, Jih W, Chun L. Structure–activity relationship studies on chalcone derivatives: the potent inhibition of chemical mediator's release. *Bio. & Medi. Chem.*, **2003**, 11, 105-111.

11. Fabio S, Stefania B, Luca C, Gabriella V, Michele M., Luciano A. Synthesis and activity of a new series of chalcones as aldose reductase inhibitors. *Euro. Jour. of Medi. Chem.*, **1998**; 33, 11, 859-866.

12. Liu L, Xu J, Go L. Functionalized chalcones with basic functionalities have antibacterial activity against drug-sensitive Staphylococcus aureus. *Euro. Jour. of Medi. Chem.*, **2008**, 43, 681-1687.

13. Raj, Chenna., Sarojini, Balladaka., Hegde, Subramanya., Sreenivasa, Swamy., Ravikumar, Yalega., Bhanuprakash, Veerakyathappa., Revanaiah, Yogisharadhya., Ragavendra, Ramappa., *Med Chem Res*, **2013**, 22:2079–2087

14. Ahmed N., Brahmbhatt K.G., Sabde S., Mitra D., Singh I.P., Bhutani K.K., Synthesis and evaluation of beta-carboline derivatives as inhibitors of human immunodeficiency virus. *Bioorg. Med. Chem.*, **2010**, 18, 2872-2879.

15. Lahtchev KL, Batovska DI, Parushev SP, Ubiyvovk VM, Sibirny AA. Antifungal activity of chalcones: A mechanistic study using various yeast strains. *Euro. Jour. of Medi. Chem.***2008**; 43: 2220-2228.

16. Jeffrey JA, Pamela EO, Jared LR, Jeffrey NJ, Peter DM, Linda MO, Pamela SW, and Beth LE. Synthesis and biological evaluation of flavonoids and related compounds as gastro-protective agents. *Bio. And Medi. Chem. Letters*., **1996**; 6 (8): 995-998.

17. Ram VJ, Saxena A, Srivastava S., Chandra S. Oxygenated chalcones and bristlecones as potential antimalarial agents. *Bio. And Medi. Chem. Letters*., **2000**;10: 2159-2161

18. Detsi A, Majdalani Maya, Christos AK, Dimitra HL, Panagiotis K. Natural and synthetic 20-hydroxy-chalcones and aurones: Synthesis, characterization, and evaluation of the antioxidant and soybean lipoxygenase inhibitory activity. *Bio. And Medi. Chem.*, **2009**; 17: 8073–8085

19. Lahsasni S.A., Korbi F.I--I. and Aljaber N.A., Synthesis, characterization and evaluation of antioxidant activities of some novel chalcones analogues, *Chem. Cent. Jour.*, **2014**, 8, 32, 1-10.

20. Dong X, Chen J, Jiang C, Liu T, Hu Y. Design, synthesis, and biological evaluation of prenylated chalcones as vasorelaxant agents. *Arch Pharm (Weinheim)*, **2009**; 342, 7, 428-32.

21. Ozdemir Zuhal., Kandilici Burak., Gumusel Bulent., Calis Unsal. and Bilgin Altan., *Eur. J.Med. Chem.*, **2007**, 42, 373-379.

22. Wong E., Foetschr, *Chem. org. Naturs.*, 28, **1970**, 1.

23. Krishna R.B., Panade R., Bhaithwal S.P. and Parmar S.S., *Eur. J. Med.Chem.*, 15, **1980**, 567.

24. Kumar Srinivas, Hagar Erin., Pettit Catherine., Gurulingappa Hallur., Davidson Nancy., Khan, Saeed, Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents, *J. Med. Chem.*, **2003**, 46, 2813.

25. Rao YK, Fang SH, Tzeng M. Synthesis and biological evaluation of 3,4,5 trimethoxychalcone analogs as inhibitors of nitric oxide production and tumor cell proliferation. *Bio. And Medi. Chem.*,

2009; *17*, 7909–7914.

26. Liu M, Wilairat P, Croft SL, Choo AL., Goa L. Structure–Activity Relationships of antileishmanial and antimalarial Chalcones. *Bio. And Medi. Chem.* ,**2003**;11: 2729– 2738.

27. Geiger Walton., Conn Jean., The Mechanism of the Antibiotic Action of Clavacin and Penicillic Acid, *J. Am. Chem. Soc*., **1945**, 67, 1, 112–116.

28. Paramesh M, Niranjan M, Niazi S, Shivaraja S, and Rubbani MS, Synthesis and antimicrobial study of some chlorine-containing chalcones, *Int J Pharm Pharm Sci.* **2010**, 2, 113.

29. Zhang, Xue-Wu., Zhao, Dong-Hai., Quan, Ying-Chun., Sun, Liang-Peng., Yin, Xiu- Mei., Guan, Li-Ping., Synthesis and evaluation of antiinflammatory activity of substituted chalcone derivatives, *Medi. Chem. Res.*,**2010**, 19, 403–412