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# Synthesis of Some 4-Amino Chalcones and their Antimicrobial Activity

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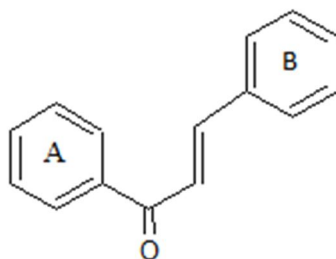
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**Abstract:** A series of some 4-amino chalcones were synthesized by Claisen-Schmidt condensation of 4-amino acetophenone with various substituted aromatic aldehydes. The synthesized 4-amino chalcones were characterized by IR, <sup>1</sup>H NMR and elemental analyses. When these 4-amino chalcones (A1-A8) were evaluated for their antibacterial and antifungal activities using cup plate method. Antibacterial activity was studied against three gram positive bacteria, *B. pumilis*, *B. subtilis* and *S. aureus* and two gram negative bacteria viz., *E. coli* and *P. vulgaris*, and antifungal activities against *A. niger*, *C. albicans* and *R. oryzae*. Some of them found to possess significant biological activity when compared to standard drugs.

**Keywords:** 4-Amino Chalcone, Antibacterial, Antifungal, Claisen Schmidt condensation, Cup plate method

## I. INTRODUCTION

Chalcones are pharmacologically valuable moieties possessing 1,3-diphenyl prop-2-ene-1-one (-CH=CH-CO-) as a core structure in which two aromatic rings are linked by first and third carbon of  $\alpha$ .  $\beta$  unsaturated carbonyl skeleton.



### 1) General Structure Of Chalcones (1,3-diphenyl Prop-2-Ene-1-One)

Due to the extended conjugation, the complete delocalisation of p electrons on both the benzene rings makes it good from bioactivity aspect. Recently many chalcones have been reported to have antimicrobial activity due the presence of a reactive  $\alpha$ ,  $\beta$  unsaturated keto skeleton[1]. In recent years a variety of chalcones have been reviewed for their cytotoxic, anticancer chemopreventive and mutagenic as well as antiviral, insecticidal and enzyme inhibitory properties[2,3]. A number of chalcones having hydroxyl, alkoxy groups in different position have been reported to possess antibacterial[4], antiulcer[5], antifungal[6], antioxidant[7], vasodilatory[8], antimutagenic[9], antimalarial[10], antileishmanial[11]. Appreciation of these findings motivated us to synthesize chalcones as a potential template for antimicrobial agents.

## II. EXPERIMENTAL

### A. Materials And Measurement

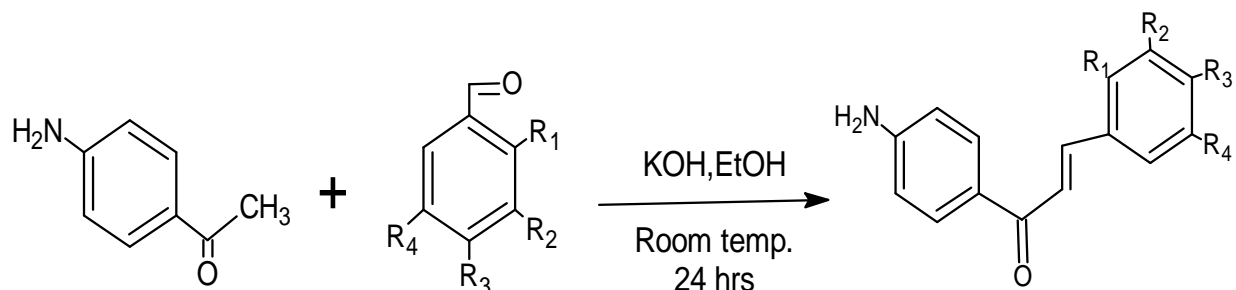
All the chemicals used for the synthesis of the compounds were of analytical grade and were purchased from reliable firms and institutes (Merck, SD Fine chemicals, Sigma etc.). Melting points were determined in an open capillary melting point apparatus and are uncorrected. <sup>1</sup>H NMR were recorded in CDCl<sub>3</sub> on Bruker WM 400 MHz spectrometer with TMS as internal standard. Infrared spectra were recorded (KBr) on a Perkin-Elmer 1650 FT-IR spectrophotometer. Microanalyses were performed on Carlo Erba EA-1108 element analyser and were within the  $\pm 0.4$  % of the theoretical values. Reaction completion was identified by TLC using Silica gel-G for TLC (Merck).

### B. General Procedure for the Preparation of 1-(4-Amino phenyl)-3-Phenyl Prop-2-en-1-ones (a1-a8):

Equimolar quantity (0.01 mole) of 4-amino acetophenone and respective aryl aldehyde were mixed and dissolved in minimum amount of ethyl alcohol. To this, aqueous potassium hydroxide solution (0.03 mole) was added slowly and mixed occasionally for

24 h, at room temperature and then poured into crushed ice and if necessary acidified with dil. HCl (10%) The precipitate was washed with EtOH and purified by recrystallization and chromatographic technique [12]. Reaction pathway is represented in scheme-1 and physical data in Table-1

Scheme-I



A1 R1=Cl, R2=R3=R4=H	A2 R3=Cl, R1=R2=R4=H
A3 R1=R3=Cl, R2=R4=H	A4 R3=f, R1=R2=R4=H
A5 R2=Br, R1=R3=R4=H	A6 R3=OCH3, R1=R2=R4=H
A7 R2=R3=OCH3, R1=R4=H	A8 R2=R3=R4=OCH3, R1=H

#### 1) Spectral data of the 4-amino chalcones A1-A8 synthesised

A-1; (2E)-1-(4-aminophenyl)-3-(2-chlorophenyl) prop-2-en-1-one

Pale Yellow solid, Yield 76%, m.p 109<sup>0</sup>C, R<sub>f</sub> 0.63, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3385, 3334 (N-H), 1649 (C=O), 1609 (CH=CH), 1342 (C-N), 1176 (C-Cl)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 4.08 (2H, br s, NH<sub>2</sub>), 6.64 (2H, d, J = 8.8 Hz, C-3' and 5' -H), 7.27-7.23 (2H, m, C-4 and 5-H), 7.38-7.36 (1H, m, C-3-H), 7.43 (1H, d, J = 15.6 Hz, -CO-CH=), 7.68-7.63 (1H, m, C-6- H), 7.86 (2H, d, J = 8.4 Hz, C-2' and 6' -H), 8.06 (1H, d, J = 15.6 Hz, Ar-CH=).

A-2; (2E)-1-(4-aminophenyl)-3-(4-chlorophenyl) prop-2-en-1-one

Pale Yellow solid, Yield 68%, m.p 157<sup>0</sup>C, R<sub>f</sub>0.72, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3460, 3343 (N-H), 1647 (C=O), 1631 (CH=CH), 1348 (C-N), 1178 (C-Cl)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 4.20(2H, br s, NH<sub>2</sub>), 6.74 (2H, d, J = 10 Hz, C-3' and 5' -H), 7.24 (1H, d, J = 16 Hz, -CO-CH=), 7.39 (2H, d, J = 8 Hz, C-3 and 5-H), 7.75 (2H, d, J = 8.8 Hz, C-2' and 6' -H), 7.95 (2H, d, J = 10 Hz, C-2 and 6-H), 8.04 (1H, d, J = 16 Hz, Ar-CH=).

A-3; (2E)-1-(4-aminophenyl)-3-(2, 4-dichlorophenyl) prop-2-en-1-one

Yellow solid, Yield 83%, m.p 181<sup>0</sup>C, R<sub>f</sub>0.65, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3438, 3363 (N-H), 1653 (C=O), 1611 (CH=CH), 1345 (C-N), 1176 (C-Cl)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 4.22 (2H, br s, NH<sub>2</sub>), 6.72 (1H, d, J = 15 Hz, -CO-CH=), 7.34 (1H, d, J = 8.5 Hz, C-6-H), 7.49 (2H, d, J = 10 Hz, C-3' and 5' -H), 7.59 (1H, d, J = 8.2 Hz, C-5-H), 7.73-7.66 (1H, m, C-3-H), 7.94 (1H, d, J = 16 Hz, Ar-CH=), 8.07 (2H, d, J = 8 Hz, C-2' and 6' -H).

A-4 (2E)-1-(4-aminophenyl)-3-(4-fluorophenyl) prop-2-en-1-one

Yellow solid, Yield 75%, m.p 143<sup>0</sup>C, R<sub>f</sub> 0.53, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3462, 3342 (N-H), 1630 (C=O), 1604 (CH=CH), 1346 (C-N), 1225 (C-F)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 4.22 (2H, br s, NH<sub>2</sub>), 6.63 (2H, d, J = 8.4 Hz, C-3 and 5-H), 7.04 (2H, d, J = 8.8 Hz, C-3' and 5' -H), 7.39 (1H, d, J = 15.6 Hz, -CO-CH=), 7.54 (2H, d, J = 10.5 Hz, C-2 and 6-H), 7.67 (1H, d, J = 15.6 Hz, Ar-CH=), 7.86 (2H, d, J = 8.4 Hz, C-2' and 6' -H)

A-5 (2E)-1-(4-aminophenyl)-3-(3-bromophenyl) prop-2-en-1-one

Yellow solid, Yield 88%, m.p 169<sup>0</sup>C, R<sub>f</sub> 0.72, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3415, 3327 (N-H), 1654 (C=O), 1628 (CH=CH), 1306 (C-N), 1178 (C-Br)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 4.22 (2H, br s, NH<sub>2</sub>), 6.72 (1H, d, J = 16 Hz, -CO-CH=), 7.28 (2H, d, J = 10 Hz, C-3' and 5' -H), 7.56-7.48 (3H, m, C-4, 5 and 6-H), 7.72 (1H, d, J = 15 Hz, Ar-CH=), 7.81 (1H, s, C-2-H), 7.96 (2H, d, J = 10 Hz, C-2' and 6' -H)

A-6 (2E)-1-(4-aminophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one

Yellow solid, Yield 65%, m.p 109<sup>0</sup>C, R<sub>f</sub>0.66, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3468, 3332 (N-H), 1632 (C=O), 1601 (CH=CH), 1344 (C-N), 1231, 1026 (C-O-C)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 3.86 (3H, S, OCH<sub>3</sub>), 4.22 (2H, br S, NH<sub>2</sub>), 6.73 (1H, d, J = 15.5 Hz, -CO-CH=), 6.95 (2H, d, J = 10 Hz, C-3' and 5' -H), 7.45 (2H, d, J = 9 Hz, C-3 and 5-H), 7.63 (2H, d, J = 8.8 Hz, C-2 and 6-H), 7.78 (1H, d, J = 15.5 Hz, Ar-CH=), 7.96 (2H, d, J = 10 Hz, C-2' and 6' -H)

A-7; (2E)-1-(4-aminophenyl)-3-(3, 4-dimethoxyphenyl) prop-2-en-1-one

Yellow solid, Yield 71%, m.p 140<sup>o</sup>C, R<sub>f</sub> 0.65, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3446, 3353 (N-H), 1643 (C=O), 1599 (CH=CH), 1318 (C-N), 1262, 1024 (C-O-C)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 3.85 (3H, S, C-3-OCH<sub>3</sub>), 3.86 (3H, S, C-4-OCH<sub>3</sub>), 4.22 (2H, br S, NH<sub>2</sub>), 6.72 (2H, d, J = 8 Hz, C-3' and 5' -H), 7.14-6.80 (3H, m, C-2, 5 and 6-H), 7.32 (1H, d, J = 15.5 Hz, -COCH=), 7.66 (1H, d, J = 15.6 Hz, Ar-CH=), 7.85 (2H, d, J = 8.4 Hz, C-2' and 6' -H)

A-8 ; (2E)-1-(4-aminophenyl)-3-(3, 4, 5-trimethoxyphenyl) prop-2-en-1-one

Yellow solid, Yield 72%, m.p 162<sup>o</sup>C, R<sub>f</sub> 0.58, FT-IR (ν<sub>max</sub> cm<sup>-1</sup>)

3469, 3344 (N-H), 1630 (C=O), 1604 (CH=CH), 1316 (C-N), 1219, 1026 (C-O-C)

<sup>1</sup>H NMR (CDCl<sub>3</sub>), δ ppm 3.92 (3H, S, C-4-OCH<sub>3</sub>), 3.94 (6H, S, C-3 and 5- OCH<sub>3</sub>), 4.22 (2H, br S, NH<sub>2</sub>), 6.73 (2H, d, J = 10 Hz, C-3' and 5' -H), 6.88 (2H, S, C-2 and 6-H), 7.45 (1H, d, J = 16 Hz, -CO-CH=), 7.74 (1H, d, J = 15.5 Hz, Ar-CH=), 7.96 (2H, d, J = 8 Hz, C-2' and 6' -H)

TABLE-1 Physical Data Of The 4-Aminochalcones (A1-A8) Synthesised

4-amino chalcone	m.p. (°C)	Yield (%)	R <sub>f</sub>	m.f. (m.w.)	Elemental Analysis % found(Calc. d)			Colour
					C	H	N	
A-1	109	76	0.63	C15H12NOCl (257.5)	69.90 (69.97)	4.66 (4.70)	5.43 (5.44)	Pale Yellow
A-2	157	68	0.72	C15H12NOCl (257.5)	69.90 (69.97)	4.66 (4.70)	5.43 (5.44)	Pale Yellow
A-3	181	83	0.65	C15H11NOCl <sub>2</sub> (292)	61.64 (61.70)	3.77 (3.79)	4.79 (4.79)	Yellow
A-4	143	75	0.53	C15H12NOF (241)	74.68 (74.76)	4.97 (5.01)	5.80 (5.81)	Yellow
A-5	169	88	0.72	C15H12NOBr (302)	59.60 (59.66)	3.97 (4.00)	4.63 (4.63)	Yellow
A-6	109	65	0.66	C16H15NO <sub>2</sub> (253)	75.88 (75.96)	5.92 (5.97)	5.53 (5.54)	Yellow
A-7	140	71	0.65	C17H17NO <sub>3</sub> (283)	72.08 (72.15)	6.00 (6.00)	4.94 (4.94)	Yellow
A-8	162	72	0.58	C18H19NO <sub>4</sub> (313)	69.00 (69.07)	6.07 (6.12)	4.47 (4.47)	Yellow

2) *Antimicrobial Activity*: Cup plate method [13, 14] using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of compounds, A1-A8, against three gram positive bacteria viz., *B. pumilis*, *B. subtilis* and *S. aureus* and two gram negative bacteria viz., *E. coli* and *P. vulgaris*. The agar medium was purchased from HI-Media laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (5 mg) was dissolved in DMSO (5 mL). Amikacin and Pencillin-G were employed as reference standards (1000 µg/mL of each) to compare the results. The pH of all the test solutions and control was maintained at 2-3 by using conc. HCl, because the compounds were not diffused through agar medium at pH below 3. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition.

Same cup plate method using potato dextrose agar (PDA) medium was employed to study the preliminary antifungal activity of chalcones, A1-A8 against *A. niger*, *C. albicans* and *R. oryzae*. The PDA medium was purchased from HI-Media laboratories Ltd., Mumbai, India. Preparations of nutrient broth, subculture, base layer medium and PDA medium were done as per the standard procedure. Each test compound (5 mg) was dissolved in DMSO (5 mL). Fluconazole employed as reference standard (1000 µg/mL)

to compare the results. The pH of all the test solutions and control was maintained at 2-3 by using conc. HCl, because the compounds were not diffused through agar medium at pH below 3. All the compounds were tested at a concentration of 0.05 mL (50 µg) and 0.1 mL (100 µg) level and DMSO as a control did not show any inhibition.

The cups each of 8 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish which was inoculated with the organisms. The solutions of each test compound, control and reference standard(s) (0.05 and 0.1 mL) were added separately in the cups and petridishes were subsequently incubated at 37 ± 1 °C for 24 h for antibacterial activity and kept aside at room temperature for 48 h for antifungal activity. Zone of inhibition produced by each compound was measured in mm and the results are presented in Table-2 Figure 1a and 1b for antibacterial and in Table-3, Figure 2a and 2b for antifungal activity.

TABLE -2 Antibacterial Activity Of 4-Aminochalcones (A1-A8) Synthesised

Zone of inhibition (in mm)										
Conc. Of test compounds (in mL)										
Compd.	B. subtilis		B. pumilis		S. aureus		E. coli		P. vulgaris	
	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10
A1	16	21	16	21	15	21	13	19	16	19
A2	19	25	21	25	19	23	19	26	19	24
A3	17	21	17	21	17	21	16	19	15	20
A4	14	21	15	21	13	21	12	21	13	21
A5	19	25	21	26	19	23	19	25	21	25
A6	15	23	20	25	16	17	13	17	18	25
A7	21	24	22	25	20	24	18	19	20	24
A8	21	25	21	23	19	19	18	18	18	23
Amikacin	29	34	32	33	25	26	26	28	29	32
Pencillin-G	12	12	8	8	16	16	9	9	9	9

Figure -1a

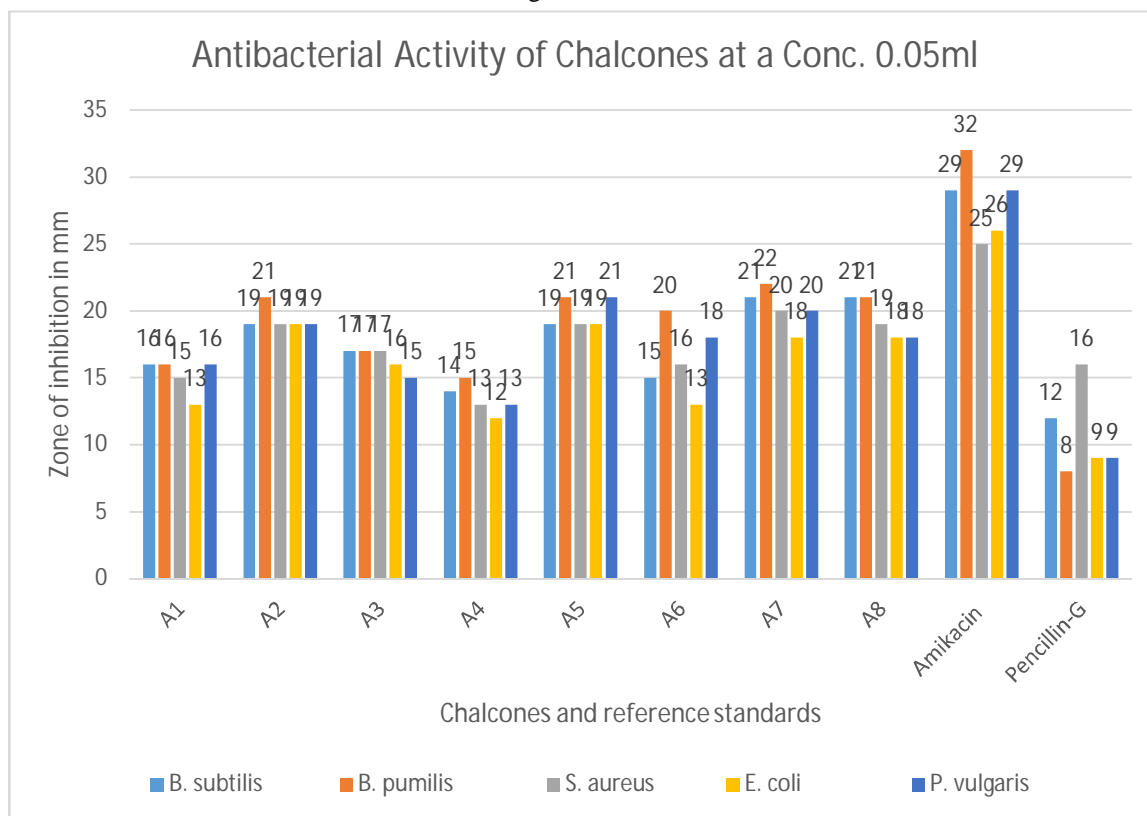


Figure 1b

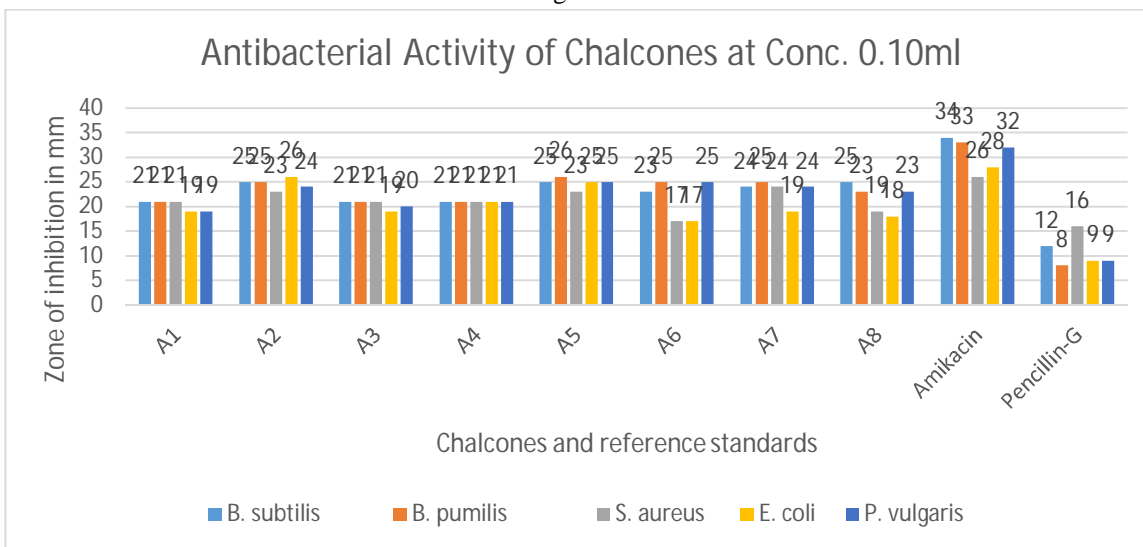


TABLE-3 ANTIFUNGAL ACTIVITY OF 4-AMINOCHALCONES (A1-A8) SYNTHESISED

Zone of inhibition (in mm)						
Conc. Of test compounds (in mL)						
Compd.	A. niger		C. albicans		R. oryzae	
	0.05	0.10	0.05	0.10	0.05	0.10
A1	14	16	16	17	15	16
A2	13	16	13	19	14	19
A3	20	26	22	27	24	26
A4	13	19	15	21	15	20
A5	16	16	15	17	15	18
A6	14	19	15	19	15	21
A7	11	15	13	13	13	15
A8	12	16	14	16	13	17
Fluconazole	25	29	25	29	23	28

Figure 2a

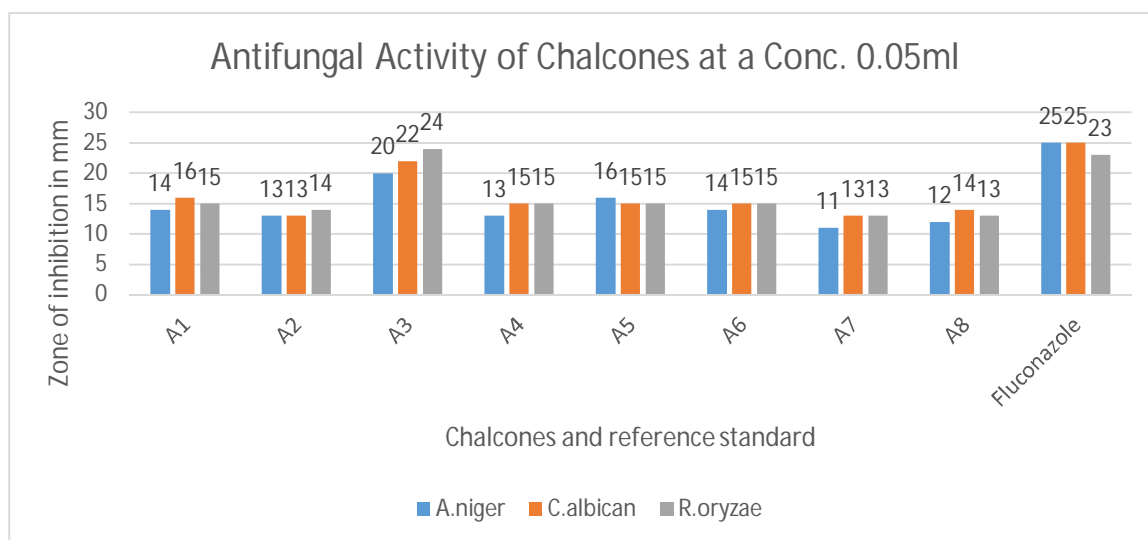
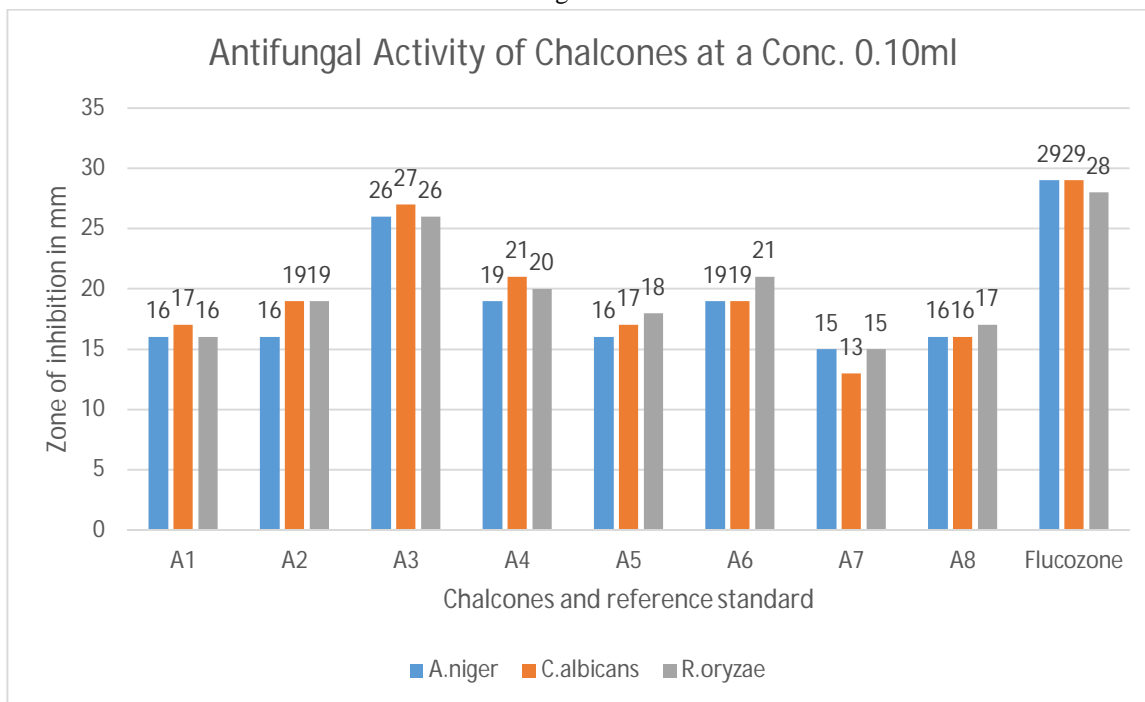


Figure 2b



### III. RESULTS AND DISCUSSION

As shown in Scheme-I, 4-aminochalcones (A1-A8) were synthesized by a base catalyzed condensation of appropriately substituted aldehydes and 4-amino acetophenone [15]. A high concentration of KOH was used for this reaction [15]. 4-aminochalcones were obtained by neutralization of the reaction mixture followed by washing with ethanol and chromatographic purification. Structures of all the synthesized chalcones were characterized by  $^1\text{H}$  NMR spectra which showed double doublet in the range of  $\delta$  6.72-7.45 ppm indicating that prop-2-ene linkage was formed. In the  $^1\text{H}$  NMR spectral analysis singlets at  $\delta$  3.85, 3.86, 3.92 and  $\delta$  3.93 ppm were assigned to methoxy protons on aromatic ring. Also  $^1\text{H}$  NMR spectrum showed the disappearance of the singlet at  $\delta$  2.47 corresponding to keto group of 4-aminoacetophenone indicate formation of chalcone linkage. The IR spectra of synthesized compounds exhibited absorption bands of C=O and CH=CH of chalcone linkage at  $1630\text{ cm}^{-1}$  -  $1654\text{ cm}^{-1}$  and  $1599\text{ cm}^{-1}$  -  $1631\text{ cm}^{-1}$  respectively. Further, structures of the entire compound were supported by molecular ion peaks corresponding to the molecular formula. The structures of various synthesized chalcones were characterized on the basis of elemental analyses, IR and  $^1\text{H}$ NMR spectral data. The synthetic Chalcones were obtained as pale yellow or yellow crystals with melting points ranging from  $108^\circ\text{C}$  to  $180^\circ\text{C}$ . Percentage yield and the Physical properties of the synthesized chalcones is summarized in Table 1. Chalcone A5 displayed the highest Percentage yield (88%) followed by A3 (83%), A1 (76%), A4 (75%), A8 (72%), A7 (71%), A2 (68%) and A6 (65%). From the results. Compounds A1-A8 showed significant antibacterial activity at both 0.05 mL (50  $\mu\text{g}$ ) and 0.1 mL (100  $\mu\text{g}$ ) concentration level when compared with standard amikacin and penicillin-G. In particular compounds A2, A5, A6 and A8 possessed maximum activity which may be due to the presence of chlorine at C-4, bromine at C-3, methoxyl at C-3 and 4 and also methoxyl at C-3, 4 and 5, respectively on aromatic ring-B. The results of antifungal activity revealed that the compounds, A1 -A8 exhibited moderate to considerable activity when compared with reference standard, fluconazole at both 0.05 mL (50  $\mu\text{g}$ ) and 0.1 mL (100  $\mu\text{g}$ ) concentration level. Compounds A3, A5 and A6 carrying chlorine at 2- and 4- position (A3), bromine at 3- position (A5) and methoxyl at 4-position (A6) on the aromatic ring-B showed remarkable activity.

### IV. CONCLUSION

From the results it can be concluded that amino chalcones synthesised have significant antibacterial activity against both gram positive and gram negative bacteria as compared to reference standard amikacin and penicillin-G which may be attributed to the presence of chlorine, bromine or methoxyl group as substituents. The synthesised amino chalcones showed moderate to considerable antifungal activity as compared to reference standard fluconazole.

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