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Synthesis and *in vitro* antimicrobial evaluation of some new chalcones containing vinyl ester group and substituted alkyl chain

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ABSTRACT

A novel series of chalconyl vinyl ester based derivatives were designed and synthesized. The newly synthesized compounds were studied for efficacy against several bacteria (*E.Coli, P.Aeruginosa, S.Aureus, S.Pyogenus*) and fungi (*C.Albicans, A.Niger, Clavatus*) using the broth dilution technique. Chalcones were prepared by treatment of 4-iodo acetophenone with 4-hydroxy benzaldehyde by Claisen-Schimidt condensation reaction. Various chalconyl vinyl ester derivatives (C_1-C_8) were prepared by reaction of chalcone with 4-n-alkoxy cinnamic acids derivatives. Compound C_8 shows the best bioactive desired antibacterial analogue with less MIC value against different tested strains. All the final synthesized compounds were characterized by IR, ^{1}H NMR, and elemental analysis.

***Keywords*:** Antibacterial; Antifungal; Chalcone; Vinyl ester

1. INTRODUCTION

Chalcones have been reported to possess various biological activities [1].They have also been reported as good chelating agents [2]. There is growing interest in the pharmacological potential of natural products is chalcones constitute an important group of natural products.

Chemically, they consist of open chain flavonoids in which the two aromatic rings are joined by a three carbon [3-8]. The presence of a reactive α, β unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity [9-10].

In recent years a variety of chalcones have been reviewed for their cytotoxic, anticancer chemoprevenive and mutagenic as well as antiviral, insecticidal and enzyme inhibitory properties [11-16]. A number of chalcones having hydroxyl, alkoxy groups in different position have been reported to possess anti-bacterial, antiulcer, antifungal, antioxidant, vasodilatory, antimitotic, antimalarial, antileshmanial and inhibition of chemical mediators release, inhibition of leukotriene B_4, inhibition of tyrosinase and inhibition of aldose reductase activities [17-22].

Appreciation of these findings motivated us to synthesize chalcones as a potential template for antimicrobial agents. Chalcones are product of condensation of substituted aromatic aldehydes with simple or substituted acetophenones in presence of alkali [23]. Many of them show antiinflammatory, anticonvulsant, immunotropic, hypolipidemic, antitumor, antiulcer and analgesic [24-26].

Cinnamic acid and its derivatives are used in various fields such as medicines**,** perfumery, polymer, cosmetics and agricultural fields [27-30]. They are also used as matrices for ultraviolet laser desorption mass spectrometry of protein and as useful intermediates for the synthesis of heterocyclic compounds [31-34].

Recently, we reported in our previous work to presently synthesized compound shows liquid crystalline property and also exhibits good thermal stability as well as mesophase length respectively [35-36]. Here in this present article, we have focused on the biological activity of present synthesized series and study the effect with varying side chain. So we have decided to synthesized chalcones derivatives and condensed with 4-n-alkoxy cinnamic acid.

2. CHEMISTRY

2. 1. Experimental

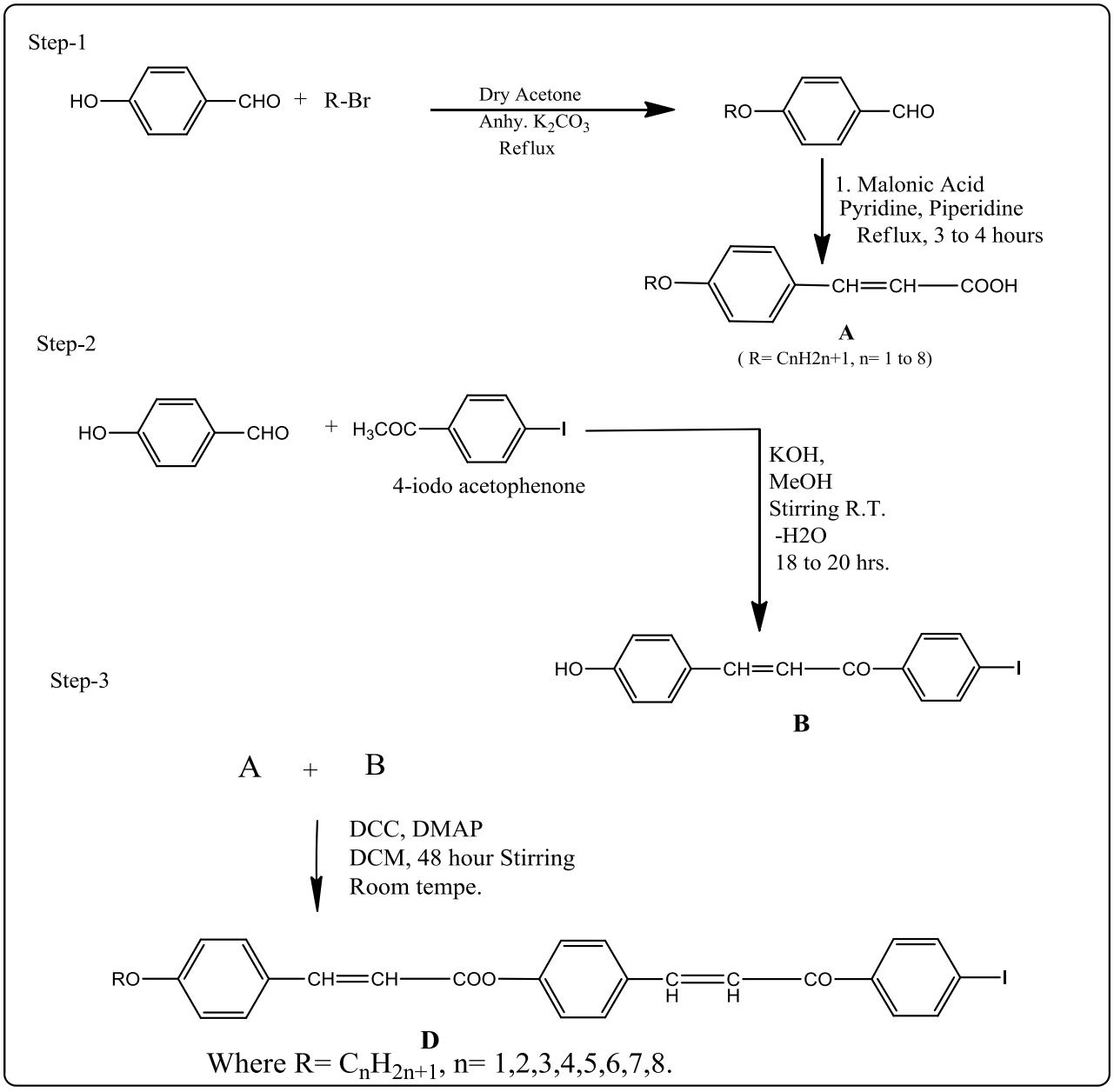
2. 1. 1 Synthesis

4-n-alkoxy cinnamic acids (A) were synthesized by the alkylation of 4-hydroxy benzaldehyde to form 4-alkoxy benzaldehyde and further reaction with malonic acid in pyridine in presence of few drops of piperidine as a catalyst [37], α-4-Hydroxy phenyl β-4'-iodo benzoyl ethylene (m.p. 167 °C, yield 76%) (B) was prepared by usual established method [23].

Final product was prepared by the esterification of (A) and (B) [38,39]. Thus, the ester chalconyl homologue derivatives were filtered washed with sodium bicarbonate solution followed by distilled water, dried and purified till constant transition temperatures obtained using an optical polarizing microscope equipped with a heating stage.

Alkyl halides, EtOH, KOH, Acetone, DCM, 4-Iodo acetophenone, 4-Hydroxy benzaldehyde, dicyclohexyl carbodimide, Dimethyl amino pyridine, Malonic acid etc., required for synthesis were used as received except solvents which were dried and distilled prior to use. The synthetic route to the series is mentioned below as Scheme-1.

2. 1. 2. Reaction Scheme



Scheme 1. Synthesis route to the series.

2. 1. 3. Characterization

Representative homologues of a series were characterized by elemental analysis, Infrared spectroscopy, ^{1}H NMR spectra. IR spectra were recorded on Perkin-Elmer spectrum GX, ^{1}H NMR spectra were recorded on Bruker using $CDCl_3$ as solvent. Microanalysis was performed on Perkin-Elmer PE 2400 CHN analyser (Table 1).

2. 1. 4. Analytical data

Table 1. Elemental analysis for (1) Hexyloxy (2) Heptyloxy (3) Octyloxy (4) Ethoxy (5) Methoxy (6) Propyloxy (7) Pentyloxy (8) Butyloxy derivatives.

Sr. No.	Molecular formula	% Elements found			% Elements calculated		
		C	H	I	C	H	I
1	$C_{30}H_{29}O_4I$	62.41	4.98	21.80	62.50	5.03	21.87
2	$C_{31}H_{31}O_4I$	62.66	5.18	21.20	62.73	5.22	21.24
3	$C_{34}H_{39}O_4I$	64.00	6.08	19.72	64.05	6.12	19.78
4	$C_{42}H_{53}O_4I$	67.39	8.03	16.82	67.46	7.09	16.86
5	$C_{25}H_{19}O_4I$	57.98	3.60	24.68	58.93	3.73	24.75
6	$C_{27}H_{23}O_4I$	60.24	4.22	23.38	60.33	4.28	23.46
7	$C_{29}H_{27}O_4I$	61.43	4.72	22.20	61.59	4.77	22.30
8	$C_{28}H_{25}O_4I$	60.74	4.98	22.78	60.98	4.53	22.86

3. RESULT AND DISCUSSION

3. 1. Synthetic route

3. 1. 1. Synthesis of Trans-4-n-alkoxy cinnamic acid (A)

The resulting 4-n-alkoxybenzaldehydes were reacted with Malonic acid (1.2 equiv.) in the presence of 1-2 drops piperidine as catalyst and pyridine as solvent, refluxing the reaction mixture 3 to 4 hours to yield corresponding Trans 4-n-alkoxy cinnamic acids (B), which was confirmed by IR study [36].

3. 1. 2. Synthesis of Chalcone (3-(4-hydroxyphenyl)-1-(4-iodophenyl) prop-2-en-1-one) (B)

Chalcone (B) was prepared by usual established method reported in literature [23].

3. 1. 3. Synthesis of Ester derivatives (D)

3. 1. 3. 1. C_1 Derivative

The compound has been prepared by esterification of the appropriate 4-methoxy cinnamic acid (A) (2.02 mmol) and chalcone (C) (0.246 g, 2.02 mmol), dicyclohexylcarbodiimide (DCC) (0.457 g, 2.22 mmol) and dimethylaminopyridine (DMAP) in catalytic amount (0.002 g, 0.2 mmol) in dry CH_2Cl_2 (DCM) (30mL) was stirred at room temperature for 48 h. The white precipitate of DCU is obtained which was isolated by

filtration and discarded, while the filtrate was evaporated to dryness. The resultant crude residue was purified by column chromatography on silica gel eluting with dichloromethane, recrystallization from methanol: chloroform (2:3) until constant transition temperatures were observed [37].

3. 1. 3. 2. C_2 Derivative

The compound has been prepared by esterification of the appropriate 4-ethoxy cinnamic acid (A) (2.02 mmol) and chalcone (C) (0.246 g, 2.02 mmol), dicyclohexylcarbodiimide (DCC) (0.457 g, 2.22 mmol) and dimethylaminopyridine (DMAP) in catalytic amount (0.002 g, 0.2 mmol) in dry CH_2Cl_2 (DCM) (30 mL) was stirred at room temperature for 48 h. The white precipitate of DCU is obtained which was isolated by filtration and discarded, while the filtrate was evaporated to dryness. The resultant crude residue was purified by column chromatography on silica gel eluting with dichloromethane, recrystallization from methanol: chloroform (2:3) until constant transition temperatures were observed [37].

3. 1. 3. 3. C_4 Derivative

The compound has been prepared by esterification of the appropriate 4-butoxy cinnamic acid (A) (2.02 mmol) and chalcone (C) (0.246 g, 2.02 mmol), dicyclohexylcarbodiimide (DCC) (0.457 g, 2.22 mmol) and dimethylaminopyridine (DMAP) in catalytic amount (0.002 g, 0.2 mmol) in dry CH_2Cl_2 (DCM) (30 mL) was stirred at room temperature for 48 h. The white precipitate of DCU is obtained which was isolated by filtration and discarded, while the filtrate was evaporated to dryness. The resultant crude residue was purified by column chromatography on silica gel eluting with dichloromethane, recrystallization from methanol: chloroform (2:3) until constant transition temperatures were observed [37].

3. 1. 3. 4. C_5 Derivative

The compound has been prepared by esterification of the appropriate 4-pentyloxy cinnamic acid (A) (2.02 mmol) and chalcone (C) (0.246 g, 2.02 mmol), dicyclohexylcarbodiimide (DCC) (0.457 g, 2.22 mmol) and dimethylaminopyridine (DMAP) in catalytic amount (0.002 g, 0.2 mmol) in dry CH_2Cl_2 (DCM) (30 mL) was stirred at room temperature for 48 h. The white precipitate of DCU is obtained which was isolated by filtration and discarded, while the filtrate was evaporated to dryness. The resultant crude residue was purified by column chromatography on silica gel eluting with dichloromethane, recrystallization from methanol: chloroform (2:3) until constant transition temperatures were observed [37].

IR Spectra in cm^{-1} for Pentyloxy, Butyloxy, Hexyloxy, Propyloxy and Methoxy Derivatives

Pentyloxy (C_5): 2960 (C-H str. of alkane), 2848 (C-H str. of –(CH_2-)n group of $–OC_5H_{11}$ group, 1760 (C=O str. of carbonyl carbon of ester), 1658 (C=O str. of carbonyl carbon of ester), 1600 (C=C str. of alkene), 1448, 1510, 1571 (C=C str. of aromatic ring), 952, 1024 (C-H bending of alkene), 1170 (C-O str. of ether linkage), 1251 (C-O str. of ester group), 1359 (C-H bending of alkene), 648, 570 (C-I str.). IR data confirms the molecular structure.

Butyloxy (C_4): 2960 (C-H str. of alkane), 2870 (C-H str. of –(CH_2-)n group of –OC_4H_9 group, 1730 (C=O str. of carbonyl carbon of ester), 1600(C=C str. of alkene), 1467, 1533, 1579 (C=C str. of aromatic ring), 1004, 1062 (C-H bending of alkene), 1143, 1199 (C-O str. of ether linkage), 1251 (C-O str. of ester group), 1309 (C-H bending of alkene), 690, 570 (C-I str.). IR data confirms the molecular structure.

Hexyloxy (C_6): 2918 (C-H str. of alkane), 2870 (C-H str. of –(CH_2-)n group of –OC_6H_{13} group, 1730 (C=O str. of carbonyl carbon of ester), 1610 (C=C str. of alkene), 1467, 1533, 1579 (C=C str. of aromatic ring), 1004, 1062 (C-H bending of alkene), 1143, 1199 (C-O str. of ether linkage), 1256 (C-O str. of ester group), 1309 (C-H bending of alkene), 690, 572 (C-I str.). IR data confirms the molecular structure.

Propyloxy (C_3): 2966 (C-H str. of alkane), 2870 (C-H str. of –(CH_2-)n group of –OC_3H_7 group, 1730 (C=O str. of carbonyl carbon of ester), 1600 (C=C str. of alkene), 1467, 1533, 1579 (C=C str. of aromatic ring), 1004, 1062 (C-H bending of alkene), 1143, 1199 (C-O str. of ether linkage), 1251 (C-O str. of ester group), 1309 (C-H bending of alkene), 690, 570 (C-I str.). IR data confirms the molecular structure.

Methoxy (C_1): 2926 (C-H str. of alkane), 2860 (C-H str. of –(CH_2-)n group of –OCH_3 group, 1730 (C=O str. of carbonyl carbon of ester), 1660 (C=C str. of alkene), 1467, 1533, 1579 (C=C str. of aromatic ring), 1004, 1062 (C-H bending of alkene), 1143, 1199 (C-O str. of ether linkage), 1251 (C-O str. of ester group), 1309 (C-H bending of alkene), 690, 570 (C-I str.). IR data confirms the molecular structure.

^{1}HNMR spectra in $CDCl_3$ in δ ppm for Heptyloxy, Octyloxy, Butyloxy, Propyloxy and Ethyloxy Derivative

Heptyloxy (C_7): 0.82 (t, 3H, -CH_3 of –C_7H_{15}), 1.29 (t, 4H, CH_3-CH_2-CH_2-CH_2-CH_2-OC_7H_{15}), 1.43 (p, 5H –CH_2-CH_2-CH_2- of –OC_7H_{15}), 1.31 (q, 4H, -CH_2-CH_3), 1.73(t, 2H,-CH_2-CH_2-of -OC_7H_{15}), 4.06 (t, 3H, -OCH_2-CH_2-of –OC_7H_{15}), 6.51 & 7.59 (d, 2H, -CH=CH), 7.2 &7.74 (4.01 H, middle phenyl ring), 7.65 & 7.97 (4H, third phenyl ring), 6.93 & 7.61 (4H, phenyl ring with alkoxy chain). NMR data confirms the molecular structure.

Octyloxy (C_8): 0.82 (t, 3H, -CH_3 of –C_8H_{17}), 1.75 (t, 4H, CH_3-CH_2-CH_2-CH_2-CH_2-CH_2 -OC_8H_{17}), 1.29 (p, 5H –CH_2-CH_2-CH_2- of –OC_8H_{17}), 1.31 (q, 4H, -CH_2-CH_3), 1.73(t, 2H,-CH_2-CH_2-of -OC_8H_{17}), 3.93 (t, 3H, -OCH_2-CH_2-of –OC_8H_{17}), 6.51 & 7.59 (d, 2H, -CH=CH), 7.21 &7.74 (4.01 H, middle phenyl ring), 7.56 & 7.97 (4H, third phenyl ring), 6.93 & 7.61 (4H, phenyl ring with alkoxy chain). NMR data confirms the molecular structure.

Butyloxy (C_4): 0.82 (t, 3H, -CH_3 of –C_4H_9), 1.75 (t, 4H, CH_3-CH_2-CH_2-CH_2 – OC_4H_9), 1.31 (q, 4H, -CH_2-CH_3), 1.73(t, 2H,-CH_2-CH_2-of -OC_4H_9), 6.57 & 7.56 (d, 2H, -CH=CH-),7.23 &7.74 (4.01 H, middle phenyl ring), 7.52 & 7.96 (4H, third phenyl ring), 6.92 & 7.61 (4H, phenyl ring with alkoxy chain). NMR data confirms the molecular structure.

Propyloxy (C_3): 0.82 (t, 3H, -CH_3 of –C_3H_7), 1.29 (t, 4H, CH_3-CH_2-CH_2- -OC_3H_7), 1.30 (q, 4H, -CH_2-CH_3), 4.06 (t, 2H, -OCH_2-CH_2-of –OC_3H_7), 6.54 & 7.56 (d, 2H, -CH=CH-), 7.22 &7.74 (4.01 H, middle phenyl ring), 7.66 & 7.98 (4H, third phenyl ring), 6.93 & 7.61 (4H, phenyl ring with alkoxy chain). NMR data confirms the molecular structure.

Ethyloxy (C_2): 0.82 (t, 2H, -CH_3 of –C_2H_5), 1.43 (p, 5H –CH_2-CH_2-CH_2- of –OC_7H_{15}), 1.31 (q, 3H, -CH_2-CH_3), 6.51 & 7.60 (d, 2H, -CH=CH-), 7.16 & 7.72 (4.01 H, middle phenyl ring),

7.65 & 7.94 (4H, third phenyl ring), 6.93 & 7.61 (4H, phenyl ring with alkoxy chain). NMR data confirms the molecular structure.

Thus, keeping in view the antimicrobial potential of chalcones and cinnamate ester, it was envisaged that the synthesis and antibacterial and antifungal evaluation of newly chalconyl vinyl ester hybrids is worth the attempt. Figure 1 shows the representative structures of potent antibacterial compounds that contain chalcone (–CH=CH–CO–) and cinnamate (–CH=CH–COO–) group with terminal iodo group and left side alkoxy group to form the basis of our designed prototype. The geometrical shape of present synthesized compound is rod type and also its exhibited LC property with good thermal stability [40-46].

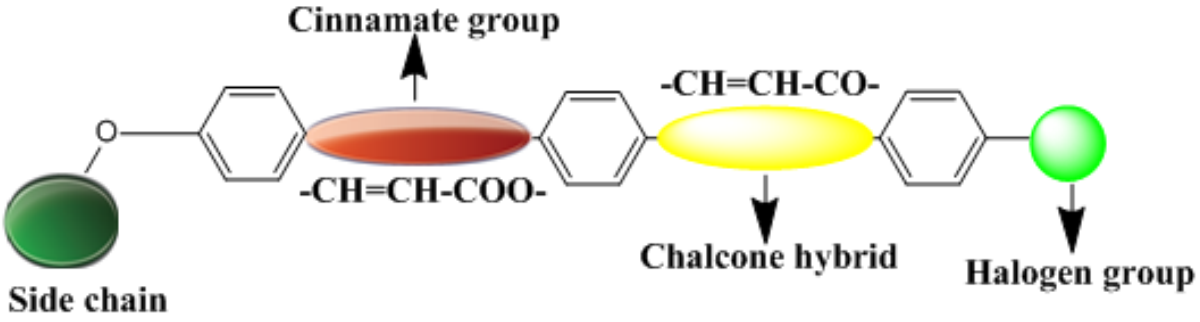


Figure 1. Designing of chalcone and cinnamate based hybrids

3. 2. Biological Evaluation

In the present work, the focus has been drawn on designing new structural entities of chalcones by incorporating para iodo acetophenone and para substituted aldehydes into chalcone scaffolds to evaluate the prospective effect on biological activity, particular antibacterial and antifungal. The antibacterial activity of the synthesized compounds C_1 to C_8 was determined *in-vitro* using MIC (Broth dilution method) against four pathogenic micro-organism viz. *E. coli*, *P. aeruginosa* (Gram –ve) and *S. aureus*, *S. pyogenus* (Gram +ve) and three fungal strains *C. albicans*, *A.niger* and *A. clavatus* at various concentrations. Synthesized compounds showed good activity results against *E. coli*, *P. aeruginosa* (Gram –ve) and *S. aureus*, *S. pyogenus* (Gram +ve), which was comparatively nearer to the standard drug Ampicillin. While, in antifungal activity of comp. C_7 showed good results in *C. albicans* at 500 µg/mL which was equivalent to standard drug Greseofulvin.

3. 2. 1. *In vitro* antibacterial activity

Table 2 shows that all the newly synthesized compounds were found to exhibit good to moderate activity against specific microbial strains. Initially, we screened all the synthesized compounds (C_1 to C_8) for their antibacterial activity *in vitro* by using both dilution method. The *in vitro* antibacterial results confirmed that some of the chalconyl-ester hybrids exhibited good results against various strains of *E. coli*, *P. aeruginosa* (Gram–ve) and *S. aureus*, *S. pyogenus* (Gram +ve) as shown in Table 2. Antibacterial results was comparatively nearer to the standard drug ampicillin as compare to other drug. Compound C_6 and C_8 (62.5 µg/ml

MIC) gives results for *E. coli* at lower concentration as compare to standard drug ampicillin. While, C_7 and C_8 showed activity against *S. aureus* (62.5 µg/ml MIC) which is lower than standard drug ampicillin. Furthermore, compound C_3 to C_8 having growth inhibition at (100 µg/ml MIC). The result obtained is nearer to standard drug. Compound C_1 to C_3 showed activity against *Gram +ve and Gram –ve* at higher concentration as compare to higher side chain compound. From this result, we studied that antibacterial and antifungal activity of compound C_4 to C_8 is good as compare to compound C_1 to C_3.

Table 2. Result of antibacterial activity of the synthesized compounds.

ANTIBACTERIAL ACTIVITY					
Minimal Inhibition Concentration					
Sr.No	**Code**	***E. coli***	***P. aeruginosa***	***S. aureus***	***S. pyogenus***
	No.	**MTCC 443**	**MTCC 442**	**MTCC 96**	**MTCC 442**
Microgramm / ML					
1	C_1	125	120	200	200
2	C_2	125	125	250	200
3	C_3	125	100	250	200
4	C_4	100	100	200	100
5	C_5	100	100	250	100
6	C_6	62.5	100	100	100
7	C_7	100	100	62.5	100
8	C_8	62.5	100	62.5	100
Standard	**Ampicillin**	**100**	**100**	**250**	**100**
Standard	**Gentamycin**	0.05	1	0.25	0.5
Standard	**Chloramphenicol**	50	50	50	50
Standard	**Ciprofloxacin**	25	25	50	50

3. 2.2. *In vitro* antifungal activity

Antifungal activity data (Table 3) displayed that the synthesized compound C_1 to C_8 showed adaptable degrees of inhibition against the tested fungi *C. albicans*, *A. niger*, *A. clavatus*. Fungi was inhibited by C_2, C_5, C_8 at 500 µg/ml MIC which is equal to the concentration of standard drug Greseofulvin. While inhibiting against *A. Niger*, *A. clavatus*

fungi at higher MIC value. Compound C_6, C_7 shows good inhibiting against C.Albicans. Compound C_6 shows good activity profile against *C. albicans*, *A. niger*, and *A. clavatus*. Rest of the derivatives exerted moderate to poor activity profiles.

Table 3. Result of antifungal activity of the synthesized compounds.

ANTIFUNGAL ACTIVITY				
Minimal Fungicidal Concentration				
Sr. No	**Code. No.**	***C. albicans***	***A. niger***	***A. clavatus***
		MTCC 227	**MTCC 282**	**MTCC 1323**
Microgramm / ML				
1	C_1	1000	>1000	>1000
2	C_2	500	>1000	>1000
3	C_3	1000	250	250
4	C_4	1000	250	250
5	C_5	500	1000	500
6	C_6	250	250	500
7	C_7	250	500	500
8	C_8	500	1000	1000
Standard	**Nystatin**	100	100	100
Standard	**Greseofulvin**	500	100	100

4. CONCLUSION

In conclusion, we have designed and synthesized a newly chalconyl vinylester based series and The majority of these hybrid compounds, especially, exhibited promising in vitro antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenus*. Furthermore, the antifungal screening of some compounds found to be active due to presence of long side chain. The newly synthesized chalconyl ester derivative shows LC property as well as antimicrobial activity. All the synthesized compounds were confirmed by spectral analysis.

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