IN VITRO BIOLOGICAL SCREENING AND NOVEL SYNTHESIS OF CHALCONES USING REUSABLE POLYAMINE CATALYST

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A simple and efficient method for the synthesis of chalcone derivatives using polyamine catalyst has been described. The antibacterial activity of chalcones are also evaluated by using gram positive (*S. aureus, B. subtillus*) and gram negative bacteria (*Klebsiella pneumonia, P. aerugenosa*) microorganisms.

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INTRODUCTION

Flavonoids are the largest group of naturally occurring phenolic compounds, abundantly occurs in different plant both in free state and as glycosides. The presence of these pigments is responsible for the various colors and combination of such colors exhibited by bark, leaves, flowers, fruits and seeds of plants.[1] Flavonoids are present in vegetables, fruits, berries and beverages such as tea, red wine and fruit juices, having similar effects as antioxidants. Acyclic flavonoids include chalcones & aurones which also contain C6-C3-C6 backbone.[2]

They were formerly considered to be minor flavonoids. Chalcones are open chain flavonoids in which two aromatic rings are joined by three carbons α, β-unsaturated carbonyl system. They are the important intermediates of flavonoids synthesis.

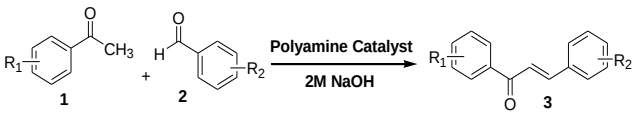
Aurones, (Z)-2-benzylidenebenzofuran-3-(2H)-ones are responsible for the bright yellow colour of some ornamental flowers.They are biosynthesized from chalcones by the key enzyme aureusidin synthase.[3] They contribute to the yellow flower color in several ornamental plants.

Chalcones are readily accessible via two well-established routes comprising a base catalyzed aldol condensation or acid mediated aldolisation of 2-hydroxyacetophenone and benzaldehyde. The base catalyzed aldol condensation is usually preferred route toward the chalcone formation.

Several methods have been reported for the synthesis of chalcones.[4] Under acidic conditions cyclization of chalcones leads to the formation of corresponding hydroxyl dihydrochalcone, 2-hydroxy heterochalcones. Chalcone derivatives are known as versatile physiologically active compounds.[5-6]

Chalcones have been reported to possess many useful properties, including anti-inflammatory,[7] anti-microbial,[8] antifungal,[9] antioxidant,[10] antitumor,[11] antimalarial,[12] antiprotozoal,[13] antibacterial properties,[14] antifilarial activity,[15] mosquito larvicidal activity,[16] anticonvulsant activity,[17] mammalian α-amylase inhibitory activity,[18] cyclo-oxygenase inhibitory activity,[19] monoamine oxidases inhibitory activity.[20]

In reported work, we developed a new methodology for the synthesis of biologically important chalcones using the mixture of aldehydes and acetophenones in presence of polyamine catalyst. The recovered polyamine catalyst was recycled and reused several times to carry out the same reaction, without any loss in its efficiency (Scheme 1).



Scheme 1. Synthesis of chalcones by using polyamine catalysts

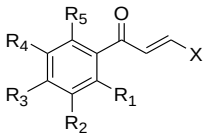
RESULTS AND DISCUSSION

Chalcones are synthesized using various catalysts like ionic liquid resins, clays, non-aqueous cation-exchange resin etc. Herein, we are reporting a simple and easy method for chalcone synthesis by using novel silica gel supported poly amine catalyst. The catalyst was prepared from solution of 3-aminopropyl-trimethoxy silane in dry toluene. The chalcone obtained using this catalyst is having more yields and it can be reusable. The activity of chalcones was found to be better. Structures of different chalcones synthesized are shown in Table 1.

Determination of minimum inhibitory concentration (MIC)

MIC of synthesized chalcone was determined using tube dilution technique against gram positive (*S. aureus,B. subtillus*) and gram negative bacteria (*Klebsiella pneumonia, P. aerugenosa*). The turbidity was measured by colorimeter at 620 nm. Colorimetrically, the MIC was found to be 125 μg mL^{-1}.

Table 1. Synthesis of chalcones using N,N-dibasic polyamine catalyst



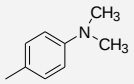
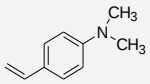
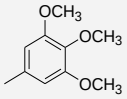
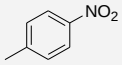
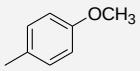
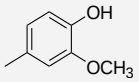
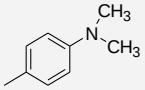
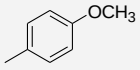
Comp. Codes	Substituent groups						M. p. (°C)	Yields (%)
	X	R1	R2	R3	R4	R5		
C1		OH	H	OH	H	H	187	93.32
C2		OH	H	OH	H	H	138	95.67
C3		OH	H	OH	H	H	124	87.45
C4		OH	H	OH	H		174	88.67
C5		OH	H	OH	H	H	96	76.45
C6		OH	H	OH	H	H	110	78.56
C7		OH	H	OH	H	H	5	73.45
C8		H	H	H	H	H	122	67.34
C9		H	H	H	H	H	94	87.78
C10		H	H	NO_2	H	H	118	68.78

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Table 2. MIC Results of C9 compound against gram positive and gram negative bacteria

Sr. No.	Concentration, µg mL^{-1}	Transmittance, %			
		S. aureus	*K. pneumonia*	*B. subtillus*	*P. aeruginosa*
C9a	1000	99	100	97	99
C9b	500	96	99	98	97
C9c	250	95	93	94	95
C9d	125	84	83	91	91
C9e	62.5	27	31	47	51

Table 3. MIC Results of C10 compound against gram positive and gram negative bacteria

Sr. No.	Concentration	Transmittance			
		S. aureus	*K. pneumonia*	*B. subtillus*	*P. aeruginosa*
C10a	1000	99	100	98	99
C10b	500	97	99	97	97
C10c	250	95	97	96	93
C10d	125	96	94	93	92
C10e	62.5	28	52	52	54

This concentration was used to study zone of inhibition of synthesized compounds. MIC results of chalcone C9 are shown in Table 2, Figure 1 and MIC results of chalcone C10 are shown in Table 3, Figure 2.

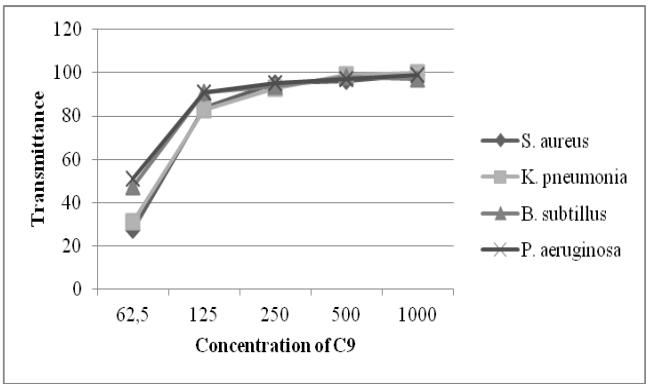

Figure 1. Graph indicating MIC of C9 against gram positive and gram negative bacteria

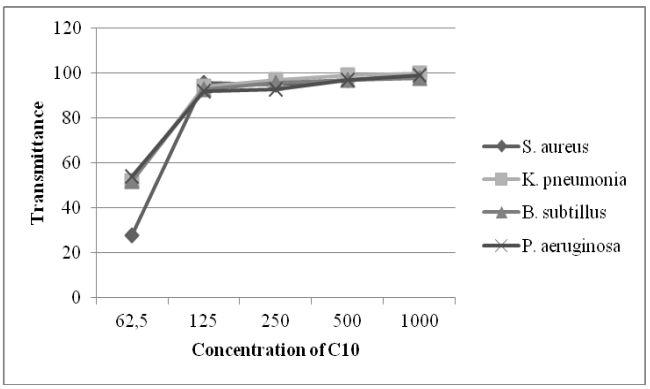

Figure 2. Graph indicating MIC of C10 against gram positive and gram negative bacteria

The results of MIC confirmed that the chalcone derivatives C9 and C10 showed inhibition growth of gram positive (*S. aureus, B. subtillus*) and gram negative bacteria (*K. pneumonia, P. aerugenosa*). MIC of 125µg/ml of testing compound is the concentration used to determine zone of inhibition against various microorganisms.[21]

Antibacterial activity of novel chalcone compounds

The chalcone are found to be antibacterial in nature. Various food borned pathogens are common agents responsible for various diseases. Several gram negative bacteria are harmful for human beings. The chalcone were used for bacterial growth inhibition. Compounds C9 and C10 are found to be antibacterial and showed a broad range of antibacterial activity. [22]

Zone of Inhibition

As per MIC results, it was founds that 125 µg/ml concentration is the minimum inhibitory concentration to inhibit further microbial growth. This concentration of compounds were used for the study of zone of inhibition on various microorganism. 100 µg of Ciprofloxacin was taken as standard and DMF were used as control.

Table 4. Zone of inhibition of compounds

Compound code	Zone of inhibition(mm)			
	S. aureus	*K. pneumonia*	*B. subtilis*	*P. aeruginosa*
C4	20	22	19	21
C5	22	23	21	20
C9	22	24	22	19
C10	19	24	19	13
Ciprofloxacin	31	30	32	32

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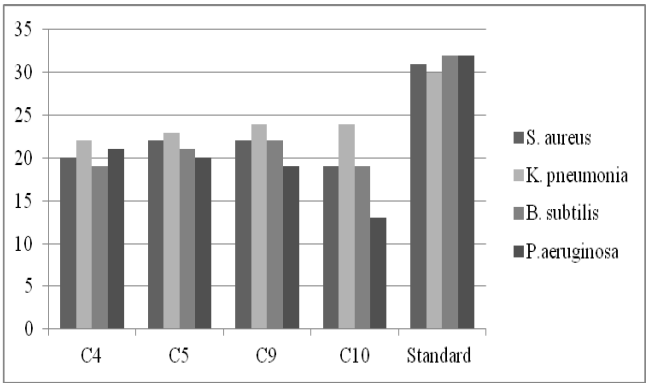

Figure 3. Zone of inhibition of compounds

EXPERIMENTAL SECTION

Melting point of the synthesized compounds were determined in melting point apparatus made by viggo. The IR was monitored in the range of 400-4000 cm^{-1} IR spectra of final derivatives were recorded using a Shimadzu FT-IR spectrometer, MASS analyses of synthesized compounds were done on Micromass TOF mass spectrometer, and NMR analysis by VARIAN USA Mercury plus 300 MHz NMR spectrometer.

Preparation of silica gel supported polyamine catalyst

Silica gel supported polyamine catalyst was prepared by known literature processes. Silica gel was synthesized under acidic conditions. To control the population density of hydroxyl groups in the silica gel, it was calcinated at 600°c for 6 hours. Surface fictionalization of the silica gel was carried out by suspending the gel in solution of 3-aminopropyl-trimethoxy silane in dry toluene and refluxed at boiling temperature for 24 hours.[23]

General procedure for synthesis of chalcones derivatives

The substituted chalcone derivatives were prepared by stirring a mixture of substituted acetophenone (0.01 mol) and aromatic benzaldehyde (0.01 mol) in catalytic amount of polyamine catalyst in adequate amount of ethanol for 3-4 hrs in presence of 2M NaOH. The reaction mixture was poured on crushed ice and neutralized with dilute HCl. The precipitated product was filtered and purified by recrystallisation in ethanol.

Spectral data of selected compounds from Table 1.

(2E)-1-(2,4-dihydroxyphenyl)-3-[4-(dimethylamino)-phenyl]prop-2-en-1-one (C1): M.F. $C_{17}H_{17}NO_3$; FT-IR (KBr): ν=1629.85, 1693.50, 852.54, 852, 1300.02, 1261.45, 3552.88 cm^{-1}, ^{1}HNMR (300 MHz, $CDCl_3$, δppm): 3.04(6H,s,-$N(CH_3)_2$), 6.85(1H,d, α-H), 7.90 (1H,d, β-H), 7.05-7.80(7H,m, Ar-H), 9.25(1H,s, Ar-OH), 9.20(1H,s, Ar-OH), MS (EI, 70 eV): (M^+) 283.32, M+1= 284.10

(2E)-3-[4-(dimethylamino)phenyl]-1-phenylprop-2-en-1-one (C9): M.F. $C_{17}H_{17}NO$, FT-IR (KBr): γ =1602.85, 1641.42, 819.75, 850.61, 1230.58, 1307.74 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$, δppm): 7.90 (1H,d, β-H), 6.85(1H,d,αH), 3.05(6H,s, -$N(CH_3)_2$), 7.05-7.80(8H,m, Ar-H). MS (EI, 70 eV): (M^+) 251.32, M+1= 252.50

CONCLUSIONS

A new methodology for the synthesis of biologically important chalcones using the mixture of aldehydes and acetophenones and novel catalyst silica gel supported polyamine has been developed. The advantages of this catalyst is environmental friendly approach, recycled and reused several times, mild condition, etc. In biological screening C9 and C10 compounds were found to be potential antibacterial against gram positive and gram negative bacteria.

Compounds C9 and C10 were showed better zones of inhibition which confirms their effectiveness against the test microorganisms. These zone of inhibitions were measured in mm scale as shown in Table 4 and the respective graphs were plotted (Figure 3).

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