Synthesis and screening for biological potential of some substituted chalcones

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Abstract
The present paper describes the synthesis and antibacterial as well as antioxidant activity of new chalcone derivatives derived from p-chloroacetopheneone. In the present work the reaction of p-chloroacetophenone with different substituted aromatic aldehyde. The newly synthesized chalcones (A-D) were analysed by their spectral data TLC, IR, UV and melting point. These newly synthesized compounds were evaluated for *in-vitro* antioxidant activity by Diphenyl Picryl Hydrazine (DPPH) model using ascorbic acid as standard and for antimicrobial activity by Cup Plate method on gram positive and gram negative bacterial strain using amoxicillin as standard.

Keywords: Chalcone, Antimicrobial, Antioxidant, P-chloroacetophenone, Aromatic aldehyde.

Introduction
Chalcones are well known intermediates for synthesizing various heterocyclic compounds and flavones. Chalcone is an aromatic ketone that forms the central core for many biological compounds. Chalcones are chemically 1, 3-diphenyl-2-propene-1-one which consists of two aromatic rings that are linked by an aliphatic three carbon α, β-unsaturated carbonyl system. Chalcones possess a completely delocalized π electron system on both the phenyl ring and a conjugated double bond. There is relatively low redox potential for the molecules that possess such system and also they have the greater probability of undergoing electron transfer reactionChalcones are also known as benzalacetophenone or beta-phenyl-alpha-benzoyl-ethylene.[1,2]

The compounds with the backbone of chalcones have been reported to possess various biological activities such as antimicrobial, anti-inflammatory, analgesic, antiplatelet, antiulcerative, antimalarial, anticancer, antiviral, antileishmanial, antioxidant, antitubercular, antihyperglycemic, immunomodulatory, inhibition of chemical mediators release, inhibition of leukotriene B, inhibition of tyrosinase and inhibition of aldose reductase activities. Some chalcones possess bactericidal, antifungal and insecticidal activity and some of their derivatives are reported to be antimutagenic. The presence of a reactive α, β-unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity.[3-5]

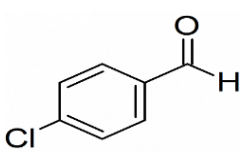
Chalcones having an α, β unsaturated carbonyl group are versatile synthons for various chemical transformations. The chemistry of chalcones has taken an important place in organic chemistry; the research in this area is encouraged because of development of bacterial resistance to widely used antibiotics of this type.

Materials and Method
General procedure for the synthesis of chalcones derivatives by Claisen– Schmidt condensation[1,6-7]

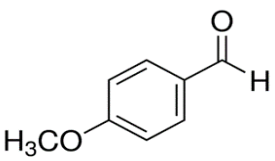
Equimolar quantities (12g, 0.1mol) of P-chloroacetophenone and respective aldehydes (10.6g, 0.7mol) were mixed and dissolved in minimum amount (15ml) of alcohol. To this, aqueous potassium hydroxide solution (0.003 mol) was added slowly and mixed occasionally for 1.5 h, at room temperature. After completion of the reaction, the reaction mixture was kept for 14-16 h at room temperature. The potassium salt of chalcone was separated by ice-cold HCl (10%, 30ml). The separated solid was filtered and washed with ice cold water till the washing was neutral to litmus. It was purified by recrystallization using ethanol and dried at room temperature.

Various Substituted Benzaldehyde:

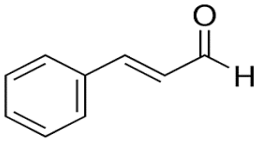
A = Anisaldehyde



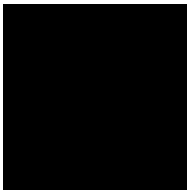
B = P-Chlorobenzaldehyde



C = Cinnamaldehyde



D = 3-Nitrobenzaldehyde



Characterization of Synthesized compounds

Melting point determination**:** The melting point was determined by filling the sample in the capillary, keeping it in the appropriate position in the melting point apparatus and the temperature at which the sample starts meting/got completely melted was noted.

TLC: The solutions of the compounds were prepared in methanol. The solvent system employed was pet ether: ethyl acetate (2:3). The solution of the compound was spotted on TLC plate using glass capillaries and after development spots were visualized in the UV chamber at 254nm.

UV Spectrum: The UV absorption spectrum of the synthesized compound was recorded using V-630 Jasco UV spectrophotometer by dissolving synthesized chalcone derivatives in methanol and the solution was scanned in the wavelength range 200-400 nm against blank.

IR spectral study: The IR spectra were recorded using the KBr pellet method in the range of 400-4000 cm^{-1}

Evaluation of biological potential

Antioxidant Activity[8]

In vitro antioxidant model 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity was used (as it is a model for lipophilic radicals that initiate lipid peroxidation.) Solvent used in the test was methanol.

DPPH free-radical scavenging activity

In-vitro antioxidant activity of newly synthesized compounds was performed by DPPH model. It based on determining the free radical inhibitory ability of different antioxidant by using very stable free radical such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. Stock solution of DPPH in methanol was prepared. Stock solution of DPPH 100 µL was added to 3.0 mL of methanol and absorbance was recorded at 516 nm. The various concentrations of synthesized compounds (12.5, 25, 50 and 100µg/mL) were also prepared. All sample solutions 1.0 mL each was diluted with 3.0 mL with methanol and 100 µL of stock solution of DPPH was added. Test tubes were kept for 30 min in light to complete the reaction. After 30 min, absorbance of each test tube was recorded at 516 nm on UV-VIS spectrophotometer against methanol as a blank.

The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Where;

a. Control is absorbance of a DPPH solution without compound
b. Test is the absorbance of the test compound with DPPH.

The degree of discoloration indicates the free radical scavenging efficiency of the compound. Ascorbic acid was used as the free radical scavenger reference compound.

Antimicrobial Activity[9]

Cup Plate Method: It is one of the common methods for the evaluation of antimicrobial activity, includes preparation of bore on the sterilized agar plate and pouring the test compound and measurement of zone of inhibition.

Microorganism

The following microorganisms were used to study the antibacterial activity.

a. Bacillus subtilis - Gram positive bacteria
b. Staphylococcus aureous - Gram positive bacteria
c. Escherichia coli - Gram negative bacteria
d. Pseudomonas aeruginosa- Gram negative bacteria

Standard: Amoxicillin

Solvent: Dimethylsulfoxide

All the test compounds were tested at 25 µg, 50 µg, 75 µg and 100 µg.

Preparation of the medium: The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water by heating on a water bath and were dispensed in 100 ml volume conical flasks. The conical flasks were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb. psig) for 15 minutes.

The contents of the conical flasks were poured aseptically into sterile petridishes are allowed to solidify. These sterilized Medias were used to subculture the bacterial culture.

Procedure

Each petridish was filled to a depth of 6-8 mm with a nutrient agar medium that was previously inoculated with suitable inoculums of suitable test organism, and then allowed to solidify. The petridish were specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium is in uniform thickness. The petridishes were sterilized at 160-170 °C in hot air oven for 30 mins before use. Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal diameter of approximately 6-8 mm and made of aluminium (or) stainless steel. Each plate was divided in to four equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. These four cavities are for test compounds and two plates are for the standard. The petridishes were incubated at 37 °C for 18 hours. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained by the test sample was compared with that produced by standard amoxicillin.

Result and Discussion

This study presents the synthesis and screening of antimicrobial and antioxidant activities of four chalcones (A-D). The syntheses of above chalcones were accomplished using p-chloroacetophenone with different aromatic benzaldehyde derivatives.

The alkaline condensation of p-chloroacetophenone with p-methoxybenzaldehyde, p-chlorobenzaldehyde, m-nitro benzaldehyde and cinnamaldehyde gives the corresponding chalcones. All the chalcones were purified by recrystallisation from ethanol. Chalcones A, B and C are yellow colored crystalline compound and the chalcone D is a brown colored powder. The purity of test compound was checked by melting point determination. The results are shown in Table 1 Further the UV spectra of all the synthesized compounds were taken in methanol as a solvent. The spectra reveals that almost all the chalcones shows UV absorption in the range of 300-350 nm. Generally it is known that the chalcones absorb light in UV region and transmit in the remaining region. The graph reveals that almost all synthesized chalcone derivatives show absorption in the UV range 300-350nm.This absorption is to be assigned to n-π transition and may be attributed to the excitation in the aromatic ring and carbonyl group. The data noted for peaks in UV spectra are recorded in Table 2. The Rf value calculated for the synthesized compounds are recorded in the Table 3

The structure of synthesized compounds was established by IR spectral data. All the compounds gave satisfactory IR data (Fig. No. 5,6,7,8 with respect to compound A-D) correlation with the assigned structures are as follows:

Compound A

The IR peak at 1656 cm^{-1} suggesting the presence of C = O (a, β- unsaturated ketone group). The peak at 2841 cm^{-1}, 2972cm^{-1}, 3005cm^{-1} , 3035cm^{-1}, 3068cm^{-1} indicates that the presence of =C-H (aliphatic and aromatic) stretching. The IR peak at 800cm^{-1} indicates the presence of C-Cl stretching.

Compound B

The IR peak at 1656 cm^{-1} suggesting the presence of C=O (Str) group. The IR peak at 2848 cm^{-1}, 2916cm^{-1}, 3001cm^{-1}, 3028cm^{-1}, 3071cm^{-} indicates the presence of aliphatic and aromatic C-H stretching. The IR peak at 742cm^{-1} indicates the presence of aromatic bending. The IR peak at 800 cm^{-1} indicates the presence of C-Cl streching.

Compound C

The IR peak at 1668 cm^{-1} suggesting the presence of C=O (Str) group The IR peak at 2864 cm^{-1}, 2972cm^{-1}, 3010cm^{-1}, 3039cm^{-1}, 3064cm^{-1} suggesting the presence of aromatic and aliphatic C-H stretch. The IR peak at 1668 cm^{-1} indicates the presence of C=C alkene stretching. The IR peak at 723 cm^{-1} indicates the presence of aromatic bending. The IR peak at 800 cm^{-1} indicates the presence of C-Cl stretching.

Compound D

The IR peak at 1668 cm^{-1} suggesting the presence of C=O (Str) group. The IR peak at 2864 cm^{-1}, 2972cm^{-1}, 3010cm^{-1}, 3039cm^{-1}, 3064cm^{-1} suggesting the presence of aromatic and aliphatic C-H stretch. The peak at 1398 cm^{-1} indicates presence of NO_2 stretching group.

DPPH (1, 1–diphenyl-2-picrylhydrazyl) radical scavenging activity was measured for all the chalcones (**A-D**) (Table 4). Ascorbic acid, the well-known antioxidant was used in the test for comparing the results, compound A appears to be the best among all the tested compounds. It was found that the chalcone **A** posseses more antioxidant activity as compare to ascorbic acid. Chalcone **C** was found to be more free radical scavenger in the concentration of 12.5ug/ml.

Statistical significance for antioxidant activity of synthesized compound was evaluated. Fig 1 shows that compound C exhibit significant anti-oxidant activity on the minimal concentration i.e. 12.5 ug/ml as compared with standard drug ascorbic acid whereas compound A

and D Shows inhibition on 25 ug/ml, compound D on 50ug/ml and A, C and D on 100ug/ml when compared at same dose of ascorbic acid

The four synthesized chalcones were screened for their antimicrobial activity (Table 5a and 5b). They were tested against four bacterial species namely S.aureus, B. subtilis (gram positive bacteria) and E.coli, P.aeruginosa (gram negative bacteria). The technique used was agar cup plate method using amoxicillin as standard.

It was found that all the four chalcones possess less antimicrobial activity as compare to standard towards the gram positive bacteria (B.subtilis). Only the chalcone A showed the zone of inhibition in S.aureus at a concentration of 100 ug/ml.

Antimicrobial activity against P.aeruginosa was found to be more satisfactory in all the four compounds. The antimicrobial activity against E. coli was found more in all the four compounds at a concentration of 75ug/ml. So we can say that the synthesized chalcones are more active against the gram negative bacteria and thus can be used for treating infections caused by gram negative bacteria.

Table 1: Observations of Melting point

Compounds	Melting Point(°C)
A	110-114
B	120-124
C	126-130
D	140-144

Table 2: UV analysis of synthesized compounds

Compound	Peaks In (Nm)	Absorbance
A	245 343	0.571 1.174
B	244 315	0.457 0.994
C	260 340	0.588 1.117
D	237 267	0.399 0.617

Table 3: Observation of TLC analysis

Compound	R_f
A	0.82
B	0.84
C	0.81
D	0.78
P-chloroacetophenone	0.64

Table 4: Antioxidant Activity: Percentage Inhibition of Free Radicals Using DPPH Method

Compound	12.5 µg/ml		25 µg/ml		50 µg/ml		100 µg/ml	
	Absorb-ance	% Inhibition	Absorb-ance	% Inhibition	Absorb-ance	% Inhibition	Absorb-ance	% Inhibition
A	0.0189	82.81	0.0214	80.54	0.041	62.72	0.031	71.81
B	0.0165	85.00	0.0860	21.81	0.067	39.09	0.078	29.09
C	0.0084	92.36	0.0359	67.36	0.029	73.63	0.038	65.45
D	0.0693	37.00	0.0220	80.00	0.005	95.45	0.010	90.90
Ascorbic acid	0.0175	84.09	0.0268	75.63	0.0231	79.00	0.0566	48.54

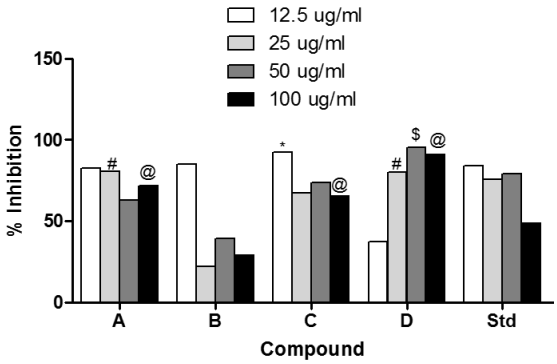


Fig. 1: Graph shows % inhibition of free radicals

Where, * 12.5 ug/ml vs Ascorbic acid 12.5 ug/ml
25 ug/ml vs Ascorbic acid 25 ug/ml
$ 50 ug/ml vs Ascorbic acid 50ug/ml
@ 100 ug/ml vs Ascorbic acid 100 ug/ml

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Table 5a: Zone of Inhibition Gram Positive Bacteria

Compound	Zone of inhibition(mm)							
	B.subtilis				S.aureus			
	25µg	50 µg	75 µg	100 µg	25µg	50 µg	75 µg	100 µg
A	9.2	8.2	10.0	9.2	-	-	-	13.2
B	8.0	8.2	8.7	9.5	-	-	-	-
C	11.7	9.2	9.5	9.75	-	10.2	-	-
D	9.0	9.5	1.07	1.02	-	-	-	-
Amoxicillin	11.0	12.7	14.2	16.0	10.1	11.5	11.9	12.8

(-) indicate no zone of inhibition

Table5b: Observations of Zone of inhibition (Gram -ve)

Compound	Zone of inhibition(mm)							
	E.coli				P.aeroginosa			
	25 µg	50 µg	75 µg	100 µg	25 µg	50 µg	75 µg	100 µg
A	10.2	11.7	12.7	10.5	11.0	13.0	14.7	15.5
B	-	-	12.0	10.5	11.2	10.7	11.0	13.5
C	-	-	12.5	8.2	11.2	11.0	11.7	14.5
D	-	11.2	10.5	-	11.7	13.7	12.0	13.2
Amoxicillin	11.5	11.9	12.8	14.2	14.5	15.5	15.2	20.2

(-) indicate no zone of inhibition

Conclusion

The four synthesized chalcones were screened for their antioxidant and antimicrobial activity. The antioxidant study results show that all the compounds show moderate to high antioxidant activity as compare to the reference compound that is ascorbic acid. It was found that the chalcones posseses more antioxidant activity as compare to ascorbic acid.

The antimicrobial study shows that all the four chalcones possess less antimicrobial activity as compare to standard towards the gram positive bacteria (B.subtilis). The synthesized chalcones have more activity against the gram negative bacteria.

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