Quest Journals
Journal of Research in Pharmaceutical Science
Volume 7 ~ Issue 11 (2021) pp: 56-65
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

In-Vitro Antibacterial Activity of Some Substituted Chalcone Derivatives

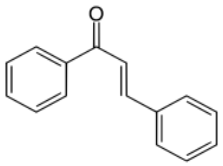
Pritam Deka[1], Madhusmita Kumari[2], Ansumita Borsaikia[3], Harshita Baruah[4]
Faculty of pharmaceutical science, Assam Down Town University, Guwahati-781026, Assam, India

ABSTRACT:
The chalcones, are precursor of open chain flavonoids and isoflavonoids which are present in several nature products, and their derivatives have been showed increasing attention due to numerous potential biological and pharmacological activities such as anti-inflammatory, anti-tuberculosis, anti-fungal, anti-malarial, anti-microbial, anti-bacterial, anti-cancer. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents having improved potency and lesser toxicity.
In this report we present the synthesis of a series of α,β- unsaturated carbonyl compounds (chalcones), and also report the evaluation of this synthesis by Eco Scale, which shows anti-bacterial activity of some selected pathogens according to their efficacy by semi quantitative analysis base on safety, economic and ecological features.
Chalcones were synthesized via Claisen Schmidt condensation reaction with acetophenone and p-hydroxy benzaldehyde, m-nitro benzaldehyde, p-chloro benzaldehyde, p- dimethyl amino benzaldehyde, aqueous NaOH in ehanol, at room temperature. After completion of the reaction, the mixture was filtered to collect the precipitates and purification by recrystallization affords the pure chalcones. The green chemistry foments the use of auxiliary substances should be unnecessary wherever possible and innocuous when used, due to we use ethanol instead methanol, because it obtained from renewable resources.

KEYWORDS:
- *P-hydroxy benzaldehyde*
- *M-nitro benzaldehyde*
- *P-chloro benzaldehyde*
- *P-dimethyl amino benzaldehyde*
- *Antibacterial activity*

Received 14 November, 2021; Revised: 27 November, 2021; Accepted 29 November, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION:



1.1. CHALCONE is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Chalcones are also known as benzyl acetophenone or benzylideneacetophenone. Benzylideneacetophenone is the parent member of the chalcone series. The alternative name given to chalcone are phenyl styryl ketone, benzalacetophenone, *β*-phenylacrylophenone, *γ*-oxo-*α*,*γ*-diphenyl-*α*-propyleneand *α*-phenyl-*β*-benzoylethylene. Chalcones and their derivatives demonstrate wide range of biological activities beneficial for anti-inflammation. The presence of enone functionality in chalcone moiety confers biological activity upon it, like anti-inflammatory, anti-fungal, anti-oxidant, anti-malarial, anti-tuberculosis, analgesic, anti- HIVand anti-tumouractivities. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcones (trans-1, 3-diaryl-2-propen-1-ones) are α, β-unsaturated ketones consisting of two aromatic rings having diverse array of

substituents. Rings are interconnected by a highly electrophonic three carbon α, β-unsaturated carbonyl system that assumes linear or nearly planar structure. They contain the ketoethylenic group (–CO–CH=CH-). Chalcones possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings. Chalcones have been used as intermediate for the preparations of compounds having therapeutic value. Chalcones have been identified as interesting compounds that are associated with several biological activities. The most common chalcones found in foods are phloretin and its glucosidephloridzin (phloretin 2′-0-β-glucopyranoside), and chalconaringenin. Chalcones are also intermediates in the biosynthesis of flavonoids which are substances widespread in plants and with an array of biological activities. Throughout the ages mankind is dependent on nature, particularly on plants as source of carbohydrates, proteins and fats for food and shelter. In addition, plants are valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, bio pesticides and food additives. With the presence of a wide variety of secondary metabolites, plants have formed the basis of the traditional medicine systems that have been in existence for phenolic compounds, including tannins and derived poly-phenols and their different derivatives form one major group of phytochemicals. It has been found that in many plants flavonoids protect them against their pathogenic bacteria and fungi. The basic flavonoid structure is the flavan nucleus, which consists of fifteen carbon atoms arranged in three rings (C6-C3-C6). Chalcones (1, 3-diphenyl-2-propen 1-one, 2) are the biogenetic precursor of flavonoids abundant in edible plants in different chemical forms. Chalcone based compounds both natural and synthetic are very versatile as physiologically active compounds with a diverse array of biological activities associated with them. The therapeutic potential of the chalcone based compounds is supported by their ease of preparation, potential of oral administration, safety and profound natural abundance. The last decade witnessed the devotion of tremendous effort around the world to elucidate the mechanisms of these chalconoids for their unparallel array of biological activities. Consequently, a number of synthetic methods have also been developed for the synthesis of this very important class of molecules including the structural modification of the core chalcone moiety. Chemically, chalcones are open chain flavonoids where two aromatic rings are joined by a three carbon α, β unsaturated carbonyl system. Variation of the core chalcone moiety is mainly based on the modification of these three carbon α, β unsaturated carbonyl system, many plants used as traditional medicine for different ailments have been reported to contain a substantial amount of these chalconoids. In addition to their numerous biological activities, chalcones find a pronounced application in synthetic organic chemistry. Application of chalcones in the synthesis of many heterocycles and as intermediate in the synthesis of many pharmaceuticals.

II. MATERIAL AND METHOD:-

2.1. CHEMICAL USED:-

SI.NO	Chemical Name	Quantity Required	Manufacturer Pack Size	Manufacturer
1	m-nitrobenzaldehyde	500gm	500gm	Sisco Research laboratories Pvt.Ltd.
2	p-chlorobenzaldehyde	200gm	250gm	Sisco Research laboratories Pvt.Ltd.
3	p-hydroxybenzaldehyde	1000gm	500gm	Sisco Research laboratories Pvt.Ltd.
4	P-dimethylaminobenzaldehyde	200gm	100gm	HiMedia laboratories Pvt.Ltd.
5	Acetophenone	100ml	500gm	S.d. Fine Ltd.
6	Sodium Hydroxide	16gm	500gm	S.d. Fine Ltd.
7	Ethanol	200ml	500gm	S.d. Fine Ltd.

2.2. INSTRUMENT USED:- Melting Point Apparatus, TLC Chamber, Reflux Condenser, Hot air oven, UV visible spectrophotometer, Beaker, Glass rod, Funnel, Pipette, Round bottom flask, Condenser, China dish, Thermometer, Digital balance, Heating Mantle.

SCHEME

COMPOUND	CODE
M-Nitro benzaldehyde	A1
P-dimethyl amino benzaldehyde	A2
p-chloro benzaldehyde	A3
Hydroxy benzaldehyde	A4
M-nitro benzaldehyde + p-dimethyl amino benzaldehyde	A5
M-nityro benzaldehyde + p-chloro benzaldehyde	A6
M-nitro benzaldehyde + Hydroxy benzaldehyde	A7
Standard drug (Amoxicillin)	S

2.3. PROCEDURE:

The synthesis of chalcone was carried out via Claisen Schmidt condensation of commercially available Acetophenone and benzaldehyde in presence of NaOH as a base in ethanol. The reaction was initiated by removal of a proton from the a-carbon of Acetophenone to form a resonance stabilized enolate ion by the base. This was followed by the nucleophilicenolate attacks on the electrophilic carbonyl carbon of benzaldehyde resulting in a new carboncarbon bond formation. This reaction joined the a-carbon of acetophenone to the carbonyl carbon of benzaldehyde to form intermediate. The final step of this reaction was protonation and deprotonation by hydroxide ion to form an a.ß-unsaturated ketone. % yield was determined by using following form: % yield = Practical yield/ theoretical yield x100

2.3.1. TLC was performing to find out only the purity of the compound by taking suitable solvent as mobile phase and silica gel as stationary phase. The R_f value was determined by using following formula.:-

R_f Value = Distance travel by solute/ Distance travel by solvent

2.3.2. INFRARED SPECTROPHOTOMETRY:

The infrared absorption spectrum is unique for any given chemical compound with the exceptionof optical isomers, which have identical spectra. However, polymorphism may something show difference in the infrared spectrum of a given compound in the solid state. Because of the number of maxima in an infrared absorption spectrum, it is sometimes possible to measure quantitatively the individual components of a mixture of known qualitative composition without prior separation.

The infrared region of the electromagnetic spectrum may be divided into three main sections

- Near infrared (overtone region) 0.8-2.5 um (12500-4000 cm-1)
- Middle infrared (vibration rotating region) 2.5-50 um (4000-200 cm-1)

> Far infrared (rotation region) 50-1000 µm (200-10 cm 1)

The main region on interest for analytical purpose is form 2.5 to 25 um i.e. 4000 to 400 wave numbers. Normal optical materials such as glass or quarts absorb strongly in the infrared, no Instruments for carrying our measurement in this region differ from those used for the electronicregion.Infrared spectra originate from the different modes of vibration and rotation of a molecule. Atwavelength below 25 µm the radiation has sufficient energy to cause changes in the vibrationalenergy in the rotational energy levels. The pure rotational spectra of molecules occur in theinfrared region and the used for determining molecular dimensions by treating the molecule as aharmonic oscillator.

2.3.3. ANTIMICROBIAL ACTIVITY:-

Chalcone derivatives were dissolved in distilled water i.e; required amount of chalcone derivative were dissolved in distilled water to make the final concentration 100 ml and 1000 ml. antibacterial test were carried out by disk diffusion method using nutrient agar media . Bacteria cultivated at 37 degree Celsius overnight for 24 hour were used as inoculums.

Nutrient agar was prepared in sterile conical flask, autoclaved for 30 minutes and poured into sterile petriplates. The plates were then inoculated with sensitive bacteria (s.aureas). Disk of uniform sizes were made and dipped in chalcone derivative solution (100mg/ml & 1000mg/ml) and placed on the culture media . The plates were incubated at 37 degree Celsius for 24 hours.The test is done against standard antibacterial agent (amoxicillin)

Test bacteria are inoculated in sterilized nutrient broth and incubated at 37 degree Celsius.

⬇

Uniform sized disks were dipped in the different concentrations of respective chalcone derivatives.

⬇

Plates were incubated at 37 degree Celsius for 24 hours.

⬇

Result is observed and zone of inhibition is measured after 24 hours.

III. RESULTS OF DISCUSSION:

3.1. PHYSIOCHEMICAL PROPERTIES OF SYNTHESIZED COMPOUNDS:

From all the synthesized compound "A1" having the highest % yield and A1 + A3 have lowest % yield. Melting point of synthesized compound is given in a table and the entire compound shows the sharp melting point.

Compound	%Yield	Melting Point (^{0}C)	Solubility	Colour
A1	94%	100-102	Water/Methanol	Cream
A2	82%	52-56	Water	Brown
A3	88%	43-45	Methanol	White

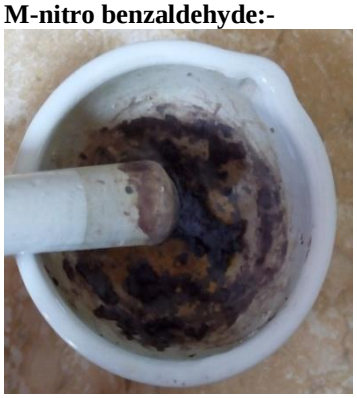
A4	76%	71-74	Methanol	Yellow
A5	71.15%	116-120	Methanol	Brown
A6	59.15%	97-100	Water/ Methanol	Cream
A7	67.25%	87-90	Methanol	Yellow

P-hydroxybenzaldehyde:-

Initial

Final

M-nitro benzaldehyde:-



Initial

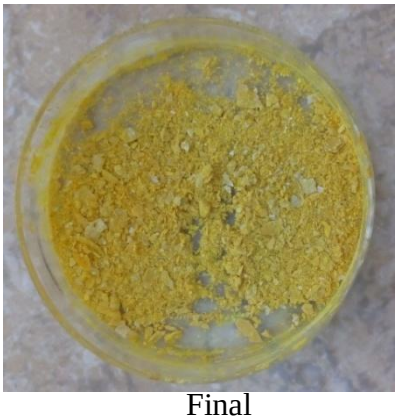
Final

Initial

Final

P-dimethyl amino benzyldehyde:-

Initial



Final

3.2. THIN LAYER CHROMATOGRAPHY:-

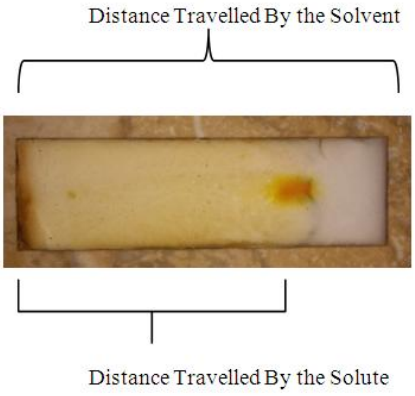
TLC was performing to find out only the purity of the compound by taking suitable solvent as mobile phase and silica gel as stationary phase.

R_f value = distance travelled by solute / distance travelled by solvent

TLC was performed by taking solvent system –

water soluble = **methanol**

methanol soluble =**Ethyleacetate:methanol: chloroform = 1 : 4 : 5**

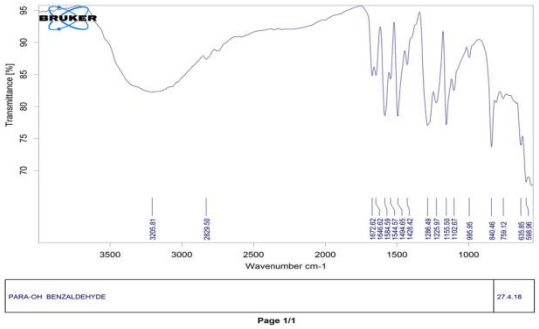


Single spot was observed in TLC which confirms the purity of the compounds. R_f value are given below:-

Compound	R_f Value
A1	0.75
A2	0.76
A3	0.71
A4	0.69
A5	0.72
A6	0.67
A7	0.73

3.3. SPECTRAL ANALYSIS:

3.3.1. P-hydroxybenzaldehyde(A1):-



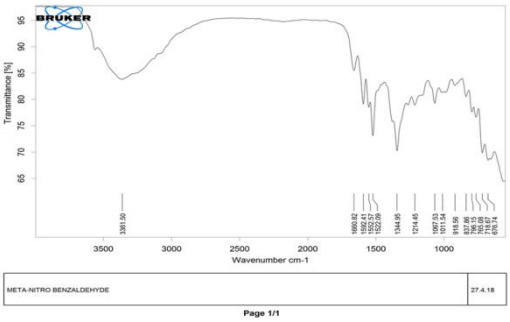
O-H – stretching (3205)

N-H - bending (1564.59)

C-O – stretching (1286.49)

C-C – stretching (1155.5)

3.3.2. M- nitro benzaldehyde (A2):-

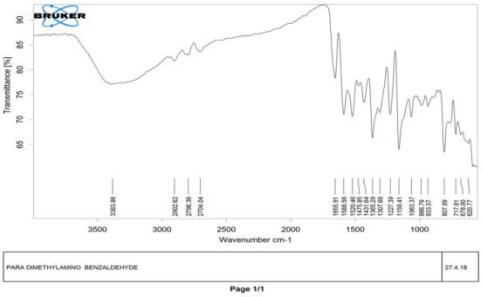


C=O – Stretching (3361.5)

N-H – Bending (11522.09)

O-H – Bending (1344.95)

3.3.3. P- chlorobenzaldehyde(A3):-

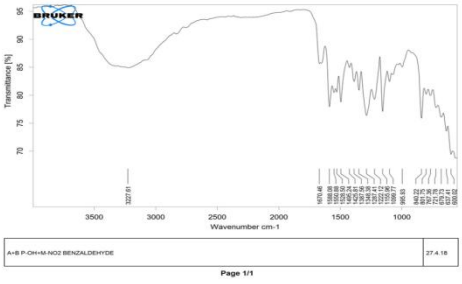


O-H – stretching (3318)

N-H – bending (1589)

C-Cl – stretching (767)

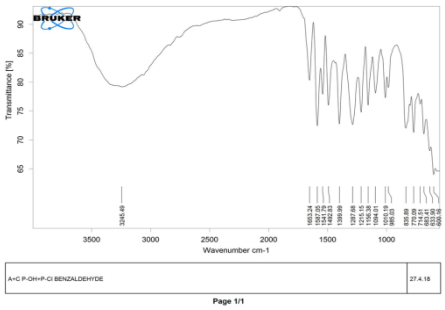
3.3.4. P- dimethyl amino benzaldehyde(A4):-



O-H – stretching (3383)

C-C – stretching (1159)

3.3.5. A1 + A2(A5):-



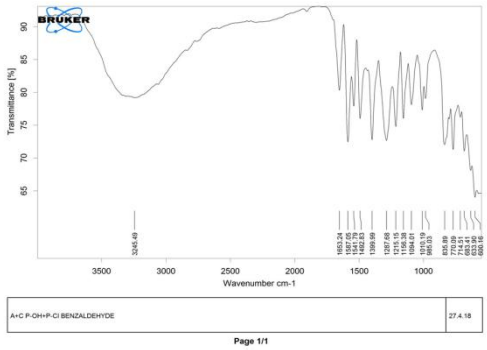
O-H – stretching (3227)

N-H – bending (1588)

C-C – stretching (1155)

C-O – stretching (1287)

3.3.6. A1+ A3(A6):-



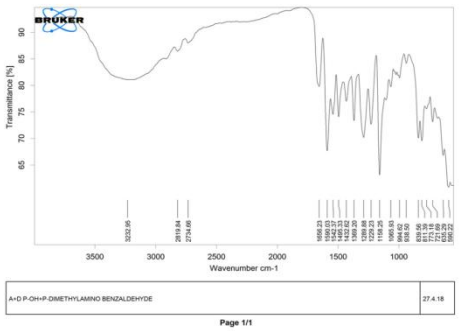
O-H – stretching (3245.49)

N-H – bending (1587.05)

C-O – stretching (1287.68)

C-C – stretching (835/770)

3.3.7. A1+ A4 (A7):-



O-H – stretching (3232)

N-H – bending (1590)

C-C – stretching (1158)

C-O – stretching (1289)

3.4.1. Zone of inhibition(mm) formed by "A" (m-nitro benzaldehyde):-

BACTERIA	CONCENTRATION	ZONE OF INHIBITION
S. aureas	1000 ml (A1) 100 ml (A2) **STANDARD (S)**	9 mm 11 mm 13 mm

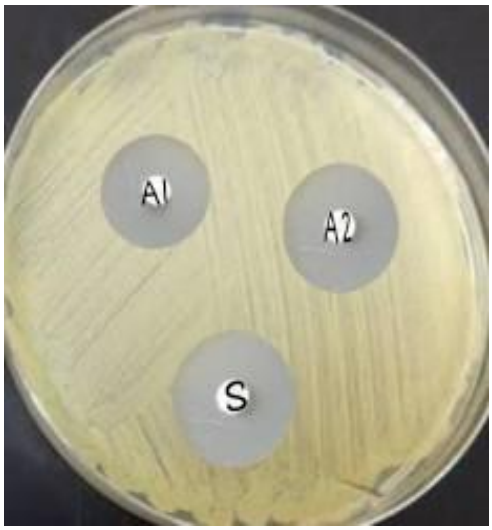


Fig:- zone of inhibition for s. aureas by m-nitro benzaldehyde against standard (amoxicillin)

3.4.2. Zone of inhibition (mm) formed by ‘’B’ ’(p- dimethyl amino benzaldehyde):-

BACTERIA	CONCENTRATION	ZONE OF INHIBITION
S. aureas	1000 ml 100 ml **STANDARD (S)**	7 mm 10 mm 13 mm

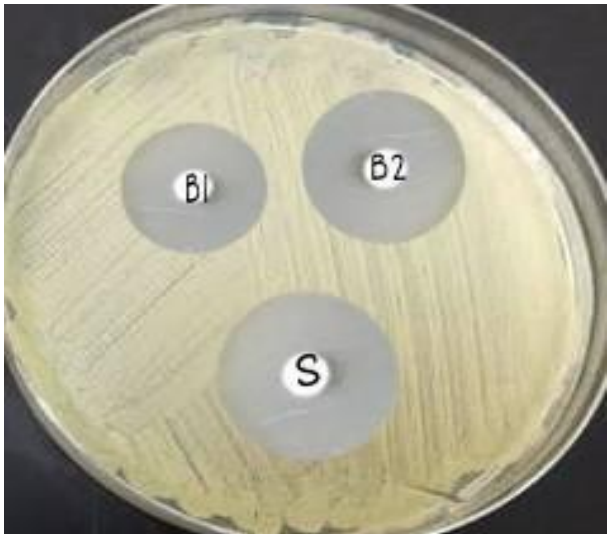


Fig:-Zone of inhibition for s. aureas by p- dimethyl amino benzaldehyde against standard (amoxicillin)

3.4.3. Zone of inhibition(mm) formed by ‘’C’’(p- chlorobenzaldehyde):-

BACTERIA	CONCENTRATION	ZONE OF INHIBITION
S. aureas	1000 ml (A1) 100 ml (A2) **STANDARD (S)**	6 mm 8 mm 13 mm

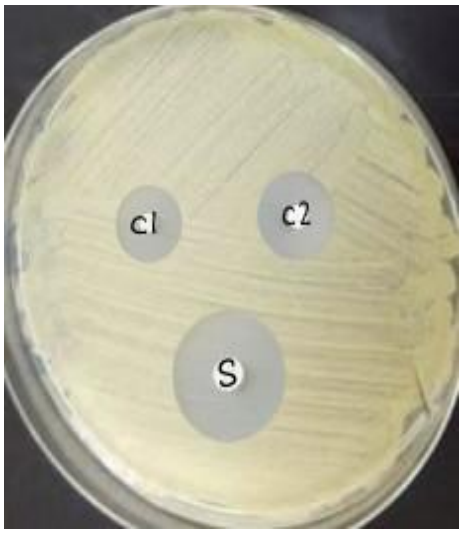


Fig:-Zone of inhibition for s. aureas by p-chloro benzaldehyde against standard (amoxicillin)

3.4.4. Zone of inhibition(mm) formed by A + B & B + C:-

BACTERIA	CONCENTRATION	ZONE OF INHIBITION
S. aureas	100 ml (A+B) 100 ml (B+C) **STANDARD (S)**	12 mm 11 mm 13 mm

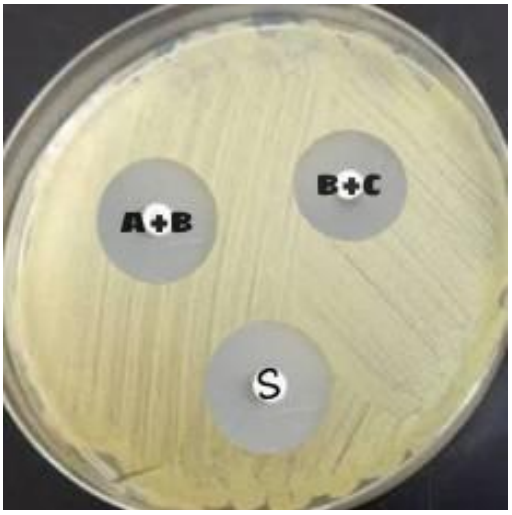


Fig:- Zone of inhibition for s. aureas by A+B & B+C against standard (amoxicillin)

IV. CONCLUSION:-

The study has confirmed that chalcone derivatives;
A: M-nitro benzaldehyde:Inhibit the activity of s.aureas
B: P- dimethyl amino benzaldehyde:Inhibit the activity of s.aureas
C: P- chlorobenzaldehyde: Shows reasonable activity against s.aureas.
A+B: Inhibit the growth of s.aureas and prevents activity
B+C:shows reasonable activity against s.aureas
It can be used to treat infection caused by bacteria such as urinary tract infection, diarrhea, sepsis and meningitis.

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