



ANTIMICROBIAL SCREENING OF SOME NOVEL SUBSTITUTED CHALCONE DERIVATIVES

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ABSTRACT

Chalcone is an aromatic ketone that forms the central core for the variety of important biological compounds, which are collectively known as chalcones. The derivatives of chalcones with an annular nitrogen atom in the phenyl ring were reported to have a wide range of biologically activities, such as antibacterial, anti-tuberculostatic and anti-inflammatory. The chalcones, two aromatic rings are linked by an aliphatic three carbon chain which bears a very good synthon so that variety of novel heterocyclics with good pharmaceutical profile can be designed. Chalcones have been considered as a magic moiety possessing myriad spectrum of medicinal activities. Diversity of biological response profile has attracted considerable interest of several researchers across the globe to explore this skeleton for its assorted therapeutic significance. The synthesized chalcone derivatives were screened for antimicrobial activity by comparing with the standard drug of ciprofloxacin on two gram positive and two gram negative strains, the synthesized compounds, by using Disc-diffusion method. Chalcone is a lead nucleus for future developments to get effective compounds.

Key words: Chalcone, Ciprofloxacin, *B. subtilis*, *Escherichia coli*, *S. aureus*

INTRODUCTION

Chalcone is an aromatic ketone that forms the central core for the variety of important biological compounds, which are collectively known as chalcones. The derivatives of chalcones with an annular nitrogen atom in the phenyl ring, were reported to have a wide range of biologically activities, such as antibacterial, anti-tuberculostatic and anti-inflammatory. In the countries such as china, Korea and Japan, butein (a tetra hydroxyl Chalcone) has been traditionally used for treatment of pain, thrombotic disease, gastric, stomach cancer and parasitic infections as well as food additives¹.

Liquorice (root of *Glycyrrhiza glabra*), from which a sweet flavor can be extracted and contain essential oils, alkaloids, polysaccharides, [polyamines, triterpenes and flavonoids) has been used in china for the treatment of gastric and duodenal ulcers, bronchial asthma, Addison's disease, food and drug poisoning and skin disease such as eczema and urticarial. Isoliquiritigenin, liquorice Chalcone, is currently in use as phosphodiesterase III inhibitor for the treatment of cardiovascular diseases. A large number of Chalcone derivatives have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase, aldose reductase, epoxide hydrolase, protein tyrosine kinase and quinine reductase².

The two major human malaria parasites plasmodium falciparum and plasmodium vivax, of which falciparum is responsible for most of the deaths and it has developed resistance to nearly all available drugs. Many chalcones have been described for their high antimalarial activity, probably as a result of Michael addition of nucleophilic species to the double bond of the enone³.

MATERIALS AND METHODS**Biological evaluation****Bacteria**

During the nineteenth century, the French scientist Louis Paster and German physician demonstrated the role of bacteria as pathogens the discovery of compound produce by

bacteria and fungi have shown their the lethal effect to other bacteria led to development of antibiotics¹².

Type of bacteria

Scientist use various system for classifying bacteria based on different, shapes, dependence on oxygen and by staining techniques.

Aerobic and anaerobic bacteria^{13, 15}

Bacteria can be classified according to need of the oxygen to survive. As aerobic bacteria require oxygen and anaerobic do not requires oxygen to survive

Eg. *Bacillus anthrax* aerobics, *Clostridium titani* anaerobics.

Autotrophic and heterotrophic bacteria¹⁰

All bacteria require carbon for growth and reproduction bacteria called autotrophes get their carbon from carbondioxide and heterotrophes from organic nutrient.

Gram positive and gram negative⁹

Bacteria can be classify by the Gram staining techniques which is identified as Gram positive and Gram Negative after staining they stains purple and pink respectively.

Eg. *S. Aureus*(Gram + ve), *E.coli*(gram -ve).

Antibacterial Agents

Anti microbial agents can be divided according to their mechanism of action.

- Agents that inhibit bacterial cell wall synthesis.
- Agents that interfere with DNA-RNA synthesis.
- Anti metabolites.
- Agents that interfere with protein synthesis.

Agents that inhibit bacterial cell wall synthesis

This includes B-lactamase antibiotics, Ciprofloxacin; B-lactamase inhibits D-alanyl-D-alanine transpeptidase activity by acylation, forming stable esters with opened lactum ring attached to hydroxyl group of the enzymes active site.

Agents that interfere with protein synthesis

This class includes, Tetra cyclones, which block and binds aminoacyl receptor site of tRNA, Chloramphenicol, and Erythromycines, binds p-sites of the 50S ribosomal subunit and inhibit translation.

Anti-bacterial activity⁸

All the compounds synthesized in the present investigation were screened for their anti-bacterial activity by cup plate

Method. Antibacterial activities were tested on nutrient medium against, *Staphylococcus aureus* and *Escherichia coli* which are representative types of gram positive and gram negative organisms respectively. The antibacterial activity of the compounds was assessed by disc-diffusion method.

Evaluation Techniques

The following conditions must be met for the screening of anti microbial activity

- There should be intimate contact between the test organisms and substance to be evaluated
- Required conditions should be provided for the growth of micro organisms.
- Conditions should be same through the study.
- Aseptic environment should be maintained

Various methods have been used from time to time by several workers to evaluate the anti microbial activity. The evaluation can be done by following methods;

- Turbid metric method
- Agar streak dilution method
- Serial dilution method
- Agar diffusion method

Following techniques are used as a agar diffusion method;

- Agar cup method
- Agar ditch method
- Paper disc method

We have used the paper disc method to evaluate the anti microbial activity

Preparation of nutrient agar media

Media Composition and Procedure:

The nutrient agar media was prepared by using the following ingredients.

- Peptone (Bacteriological) 20 gm
- Beef extract (Bacteriological) 5 gm
- Sodium chloride 5 gm
- Agar 20 gm
- Distilled water up to 1000 ml.

Weighed quantities of peptone and beef extract were dissolved in distilled water by gentle warming and then specified amount of agar was dissolved by heating on water bath. Then the pH of the solution was adjusted to 7.2 to 7.4 by adding the sodium chloride and the volume of the final solution was made up to 1000 ml with distilled water. Then it was transferred in to a suitable container, plugged with non-adsorbent cotton and the media was sterilized by in autoclave at 121°C for 20 minutes at 15 lbs pressure^{5,7}.

Preparation of test solutions

10 mg of the compound was dissolved in 10 ml of DMF. From this 1 ml of solution was taken and diluted up to 10 ml with DMF. Now the concentration of the test solution was 100 µg/ml. From the stock solution 1ml of solution was taken and diluted with 1ml of DMF now the concentration is 50µg/ml.

Preparation of standard antibiotic solution

Ciprofloxacin was used as standard antibiotics for comparison and solutions were prepared by using sterile water, as they were water-soluble. The solutions are diluted by using sterile water so that the concentrations of the solutions were 100 µg/ml and 50 µg/ml.

Method of testing

The sterilized media was cooled to 45°C with gentle shaking to bring about uniform cooling and then inoculated with 18-24 hrs old culture under aseptic conditions, mixed well by gentle shaking. This was poured in to sterile Petri dishes (properly labeled) and allowed the medium to set. After solidification all the Petri dishes were transferred to laminar flow unit. Then the discs which were previously prepared were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept as it is for one-hour diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters^{4,6}

Table-1

Compound	Zone of inhibition (mm)											
	Gram+ve						Gram-ve					
	S. aureus			B. subtitles			E. coli			P. psyringe		
Conc. (µg/ml)	25	50	150	25	50	150	25	50	150	25	50	150
I	2	0	0	1	2	4	0	0	0	0	1	1
II	0	0	0	1	2	3	2	2	2	0	0	0
III	0	0	0	3	2	1	0	2	0	4	3	0
IV	0	0	1	2	2	1	0	1	0	0	0	1
V	0	0	1	2	1	1	0	1	0	0	1	0
Control	0	0	0	0	0	0	0	0	0	0	0	0
Ciprofloxacin	06	07	12	6	7	9	0	0	0	0	0	14

RESULTS & DISCUSSION

The synthesized chalcone derivatives were evaluated for antibacterial studies. By comparing with the standard drug of ciprofloxacin on two gram positive and two gram negative strains, the synthesized compounds I, II, III have shown moderate activity and IV and V have shown mild antibacterial activity. I, II and III compounds shown moderate activity due to the the presence of high electronegative atom (chlorine), aromatic benzene rings, strong electron donating groups (-OCH₃).

CONCLUSION

By this study concluded that, the synthesized chalcone derivatives were screened for antibacterial studies. By comparing with the standard drug of ciprofloxacin on two gram positive and two gram negative strains, the synthesized

compounds, by using Disc-diffusion method. From the result of the above studies concludes that compounds I, II and III has moderate anti-bacterial activity when compared with standard drug.

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