

Available online on 30.08.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Designing and Synthesis of Flavonoids Derivatives and Screening of their Antimicrobial Activity

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ABSTRACT

Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). The *Streptococcus mutans* is a bacteria that found in the human mouth cavity. This bacterial strain produces plaque and acids that break down tooth enamel and cause dental caries. Gram positive cocci, facultatively anaerobic bacteria that forms rod-like chains. The chemical reaction of 2- hydroxyacetophenones with aromatic acylchloride occurs to form 1,3-diketones. This rearrangement reaction proceeds via enolate formation followed by acyl transfer. Then it cyclises into flavone.¹³ As the same of above scheme can be worked out as 2- Methoxybenzoyl Chloride is prepared by reaction of 2-methoxybenzoic acid with Thionyl chloride and DMF. 2-Methoxybenzoyl Chloride then added to mixture of 2- hydroxyacetophenone and pyridine, 2-[(2-Methoxybenzoyl)oxy]acetophenone thus obtained is treated with pyridine and KOH which gives 1-(2-Hydroxyphenyl)-3-(2-methoxyphenyl)-propan-1,3-dione. The result of study indicated that C5 [1-(2- hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is only inactive against *Streptococcus mutans*. All 3-hydroxyflavone derivatives exhibited their MIC to be in range of 250-125 µg/ml, 2,3-dihydroflavan-3-ol derivatives exhibited their MIC to be in range of 1000- 500 µg/ml. The chalcone derivatives exhibited their MIC to be at 250 µg/ml.

Keywords: *Streptococcus mutans*, flavonoids derivatives, MIC, 2,3-dihydroflavan-3-ol.

Article Info: Received 29 June 2019; Review Completed 04 Aug 2019; Accepted 22 Aug 2019; Available online 30 August 2019



Cite this article as:

Sharma M, Ahuja D, Jain A, Goyal R, Designing and Synthesis of Flavonoids Derivatives and Screening of their Antimicrobial Activity, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):916-920

DOI: <http://dx.doi.org/10.22270/jddt.v9i4-A.3966>

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INTRODUCTION:

Flavonoids are secondary metabolite. These are synthesized by phenylpropanoid pathway.

Series of enzyme Phenylalanine Ammonia Lyase (PAL), cinnamate 4- hydroxylase (C4H) & 4-Coumarate CoA ligase (4CL) catalyzes the conversion of phenylalanine to 4-coumaroyl-CoA, it then reacts with malonyl CoA. The flavonoid biosynthetic route starts with the condensation process of one molecule of 4-coumaroyl- CoA and three molecules of malonyl-CoA, and obtain tetrahydrochalcone. This reaction procedure is catalyzing by the chalcone synthase enzyme (CHS). Chalcone is converted into a flavanone (naringenin) by the help of enzyme chalcone f- isomerase (CHI). The central naringenin and the intermediate is converted into various side chains and each convert into different groups of flavonoids. Flavanone 3-hydroxylase (F3H) catalyzes the stereo- specific 3-beta-hydroxylation of (2S)-flavanones to dihydro-flavonols (dihydrokempferol).

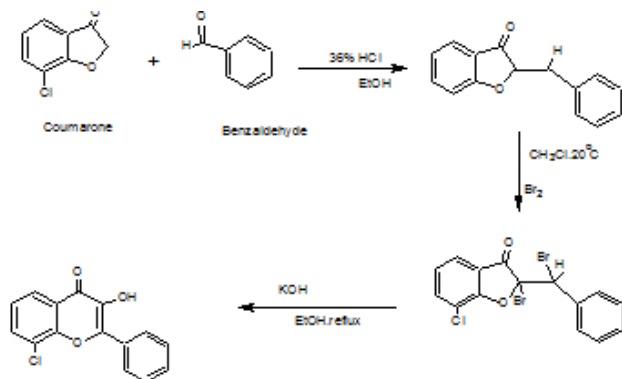
Antimicrobial Activity:

An antimicrobial is a substance that kills or inhibits the growth of microbes such as prokaryotic bacteria, viruses and fungus. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Since the flavonoids are secondary metabolites, they possess inherent ability to combat with infections. Thus they show antimicrobial activity. Lin reported a method of synthesis of flavone containing Beta fluoroethyl thiourea and screened for antimycobacterial activity. Sherif B. Abdel Ghani, synthesized flavone derivatives using a modified Baker- Venkataraman rearrangement and evaluated for antimicrobial activity against *S.aureus*, *E.coli* etc. Literature states that plant flavonoids in toothpastes are responsible for antiplaque action and works against tooth decay.¹ Plaque-related diseases are probably the most common bacterial diseases occurring in human society. The flavonoid derivatives which possess hydroxylated phenolic nucleus where the C6-C3 unit

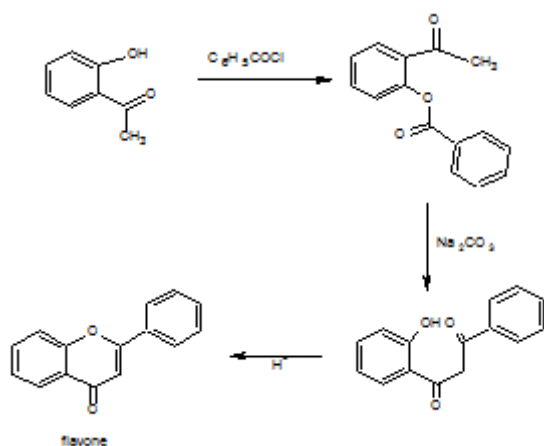
is linked to an aromatic ring, exhibit a broad range of antimicrobial activity. The catechins as found in green tea is the one of example of it.⁵⁴ The *streptococcus mutans* is a bacteria that found in the human mouth cavity. This bacterial strain produces plaque and acids that break down tooth enamel and cause dental caries. A gram positive cocci, facultatively anaerobic bacteria that forms rod-like chains.

EXPERIMENTAL WORK:

Synthetic Scheme:



The first step in this mechanism is an acid catalyzed aldol condensation between benzaldehyde and a Coumarone to an ortho-hydroxychalcone. Bromination of the alkene group gives a dibromo-adduct which rearranges to the flavonol by reaction with potassium hydroxide, which yield flavonol.^{13,14}



The Baker-Venkataraman rearrangement is the chemical reaction of 2-hydroxyacetophenones with aromatic acylchloride occurs to form 1,3-diketones. This rearrangement reaction proceeds via enolate formation followed by acyl transfer. Then it cyclises into flavone.¹³ As the same of above scheme can be worked out as 2-Methoxybenzoyl Chloride is prepared by reaction of 2-methoxybenzoic acid with Thionyl chloride and DMF. 2-Methoxybenzoyl Chloride then added to mixture of 2-hydroxyacetophenone and pyridine, 2-[(2-Methoxybenzoyl)oxy]acetophenone thus obtained is treated with pyridine and KOH which gives 1-(2-Hydroxyphenyl)-3-(2-methoxyphenyl)-propan-1,3-dione. This is then treated with conc. H₂SO₄ and acetic acid which gives flavone.

Antibacterial Activity

The synthesized organic compounds were analysed in vitro for their antibacterial activity against *Streptococcus mutans* which is pathogenic in human beings.

Method: the cup plate agar diffusion method using Nutrient

agar.

Preparation of Nutrient broth:

Nutrient broth (3.8 gm) was dissolved in 100 ml distilled water and pH was maintained to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 min.

Preparation of Inoculums:

24 hours prior to these testing inoculations of the above bacterial cultures was made in the Nutrient broth and incubated at 37°C for 18-24 hrs.

Preparation of medium:

2.8 gm Nutrient agar was dissolved into 100 ml distilled water. It was sterilized by autoclaving at 15 psi for 20 min.

Preparation of test solution:

Each test compound (5 mg) was dissolved in dimethylformamide (5 ml) to give stock solution of concentration 1000 mcg/ml. Then the 0.1 ml of this solution was used for testing.

Method of testing:

Nutrient agar plates were prepared by pouring 15-20 ml of medium into each sterilised petridish and were allowed to set at the room temperature. The cell suspension was inoculated over the surface of agar medium using sterile cotton. The three cups were scooped in each plate using cork borer of 6mm diameter. Then the solution of test compounds (0.10 ml) was added in cups by using micropipettes and these plates are incubated at 37°C for 48 hrs. The zone of inhibition is measured in mm.

Determination of Minimum inhibitory concentration (MIC) Method: Tube dilution / Broth dilution method

Tube dilution technique

One ml of double strength nutrient broth was added to a set of presterilized 5 test tubes (numbered from 1-5). To the first test tube, 1 ml complex solution (conc. = 2000 µg/ml) was added. After mixing, a solution (1 ml) from test tube no.1, it was transferred to test tube no.2 so as to obtain concentration of 1000 µg/ml. The same procedure (serial dilution) was followed for the remaining test tubes from no. 3 to no. 5 to get the concentration of compounds in the 500 µg/ml, 250 µg/ml and 125 µg/ml. From the 5th test tube 1 ml of solution was discarded so as to get the equal volume in each test tube. Thus each tube having concentration of (2000, 1000, 500, 250, 125 µg/ml). To each test tube 20 µl of *Streptococcus mutans* suspension was added (inoculation). All test tubes were incubated at 37°C for 24 hours and observed the turbidity. This set of test tube were compared for determining the MIC.

Table 1: MIC experimental set up

| | |
|-------------------------------------|-----------------------------|
| Micro-organism | <i>Streptococcus mutans</i> |
| Media | Double strength nutrient |
| Conc. of Compounds | 100 µg/ml |
| Loaded volume of compounds | 1 ml |
| Loaded Volume of media | 1 ml |
| Volume of microbial suspension load | 20 µl |
| Incubation temperature | 37°C |
| Incubation period | 24 Hrs |

RUSLTS AND DISCUSSION

Antimicrobial (Antiplateque) Screening

Antimicrobial activity against *Streptococcus mutans* has shown satisfactory results.

Zone of Inhibition

The diameters of ZOI with streptococcus mutans were found to be satisfactory in all derivatives. The observation of ZOI suggested the antibacterial activity of tested compounds J1, J2, J3, J4, J6, V1, V2, V4 V5, R1, R2, R3, R4, R5, R6 against *Streptococcus mutans*.

Minimum inhibitory concentration

On observation of positive results of antimicrobial activity, the MICs of synthesized compounds were determined by tube dilution method. Studies indicated the requirement of inhibitory concentration in order "chalcone derivatives > 2,3-dihydroflavan-3-ol derivatives > 3-hydroxyflavone derivatives. The result of study indicated that C5 [1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is only inactive against *Streptococcus mutans*. All 3-hydroxyflavone derivatives exhibited their MIC to be in range of 250-125

µg/ml., 2,3-dihydroflavan-3-ol derivatives exhibited their MIC to be in range of 1000- 500 µg/ml. The chalcone derivatives exhibited their MIC to be at 250 µg/ml.

Table 2: Zone of inhibition

| Compound code | Zone of inhibition (<i>Streptococcus mutans</i> 1000µg/ml) |
|---------------|----------------------------------------------------------------|
| J1 | 13mm |
| J2 | 10 mm |
| J3 | 12mm |
| J4 | 9mm |
| J5 | NA |
| J6 | 15mm |
| V1 | 6mm |
| V2 | 12mm |
| V3 | 7mm |
| V4 | 6mm |
| V5 | 10mm |
| V6 | NA |
| R1 | 10mm |
| R2 | 12mm |
| R3 | 13mm |
| R4 | 10mm |
| R5 | 11mm |
| R6 | 9mm |

Table 3: MIC of flavonoid derivatives with *Streptococcus mutans*

| Compound code | % Transmittance | | | |
|---------------|-----------------|-----------|-----------|-----------|
| | 1000 µg/ml | 500 µg/ml | 250 µg/ml | 125 µg/ml |
| J1 | 37 | 34 | 30 | 17 |
| J2 | 24 | 26 | 29 | 16 |
| J3 | 18 | 27 | 17 | 19 |
| J4 | 33 | 35 | 28 | 19 |
| J5 | 30 | 31 | 29 | 26 |
| J6 | 31 | 39 | 34 | 27 |
| V1 | 30 | 37 | 41 | 25 |
| V2 | 27 | 33 | 30 | 27 |
| V3 | 32 | 34 | 36 | 12 |
| V4 | 33 | 36 | 25 | 15 |
| V5 | 33 | 35 | 28 | 19 |
| V6 | 32 | 33 | 30 | 28 |
| R1 | 30 | 39 | 35 | 27 |
| R2 | 47 | 24 | 21 | 25 |
| R3 | 34 | 15 | 18 | 15 |
| R4 | 58 | 43 | 23 | 27 |
| R5 | 44 | 48 | 32 | 30 |
| R6 | 53 | 16 | 13 | 18 |

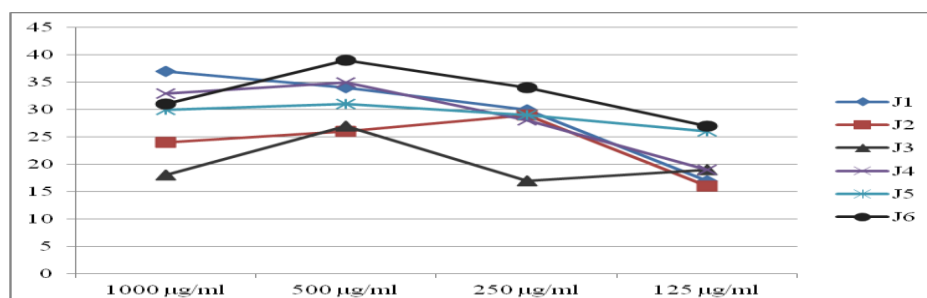


Figure 1 MIC of 2-hydroxy chalcone derivatives

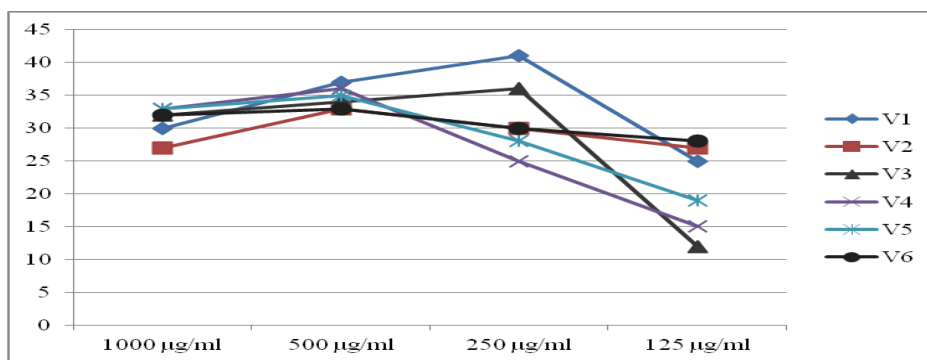


Figure 2 MIC of 3-hydroxy Flavone derivatives

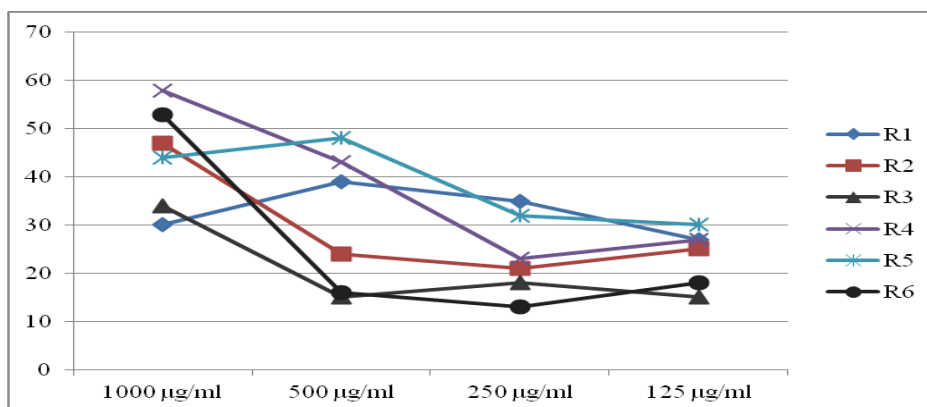


Figure 3 MIC of 2, 3-dihydroxy Flav-3-ol derivatives

The screening results have shown the contribution of electron withdrawing group to antimicrobial activity. As electron withdrawing ability increases

CONCLUSION:

The 2'-hydroxychalcone derivatives, 3-hydroxyflavone derivatives and 2,3-dihydroflavan 3-ol derivatives were synthesized and were confirmed by physicochemical and spectral analysis. The result of study indicated that C5 [1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is only inactive against *Streptococcus mutans*. All 3-hydroxyflavone derivatives exhibited their MIC to be in range of 250-125 µg/ml, 2,3-dihydroflavan-3-ol derivatives exhibited their MIC to be in range of 1000- 500 µg/ml. The chalcone derivatives exhibited their MIC to be at 250 µg/ml.

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