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# ANTI BACTERIAL ACTIVITY OF 3- (4- ARYL SUBSTITUTED- 3 - YL) - THIAZOLIDIN- 4 - ONE AND THEIR DERIVATIVES

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## ABSTRACT

This study involves synthesis of 3- [4- aryl substituted- 3 - yl] - thiazolidin- 4 – one - 5- acetic acid by microwave oven induced synthesis method. Synthesized compounds were screened for anti-microbial activity by MIC and zone inhibition tests against various gram positive organism and gram negative organisms respectively. The microbiological assay is based upon a comparison of the inhibition of growth of micro-organism by measured concentration of the antibiotics to be examined with that produced by known concentration of a standard preparation of the antibiotic having a known activity.

**INTRODUCTION** [1, 2, 4, 8]: In the last few years, microwave-induced organic reaction enhancement (MORE) chemistry has gained popularity as a non-conventional technique for rapid synthesis and many researchers have described accelerated organic reactions, with a large number of papers proving the synthetic utility of MORE chemistry in routine organic synthesis .The present study is related to the area of chemical synthesis. Thiazolidines are a perspective class of heterocycles for pharmacological research. Interest in these substances is caused by a variety of its biological activity. In the present study an attempt has been made to synthesize thiazolidinediones derivatives and to evaluate their antimicrobial activity.

**MATERIAL AND METHODS** [5, 6, 7]: All the microorganisms were obtained from the laboratory stocks, of the Department of Pharmaceutical Biotechnology, KMCH College of Pharmacy, and Coimbatore. (Table-1)

**Standard used:**

- Ciprofloxacin in the concentration of 5 mcg / disc
- Clotrimoxazole in the concentration of 5 mcg/ disc

**Preparation of Nutrient Broth for Bacteria:** The accurately weighed quantity of above ingredients were transferred to a conical flask, and dissolved in distilled water with the aid of heat with stirring and the pH was adjusted to 7.2 – 7.4 and plugged with non-absorbent cotton, covered by Aluminium foil and sterilized by autoclaving (121˚C at 15 lbs pressure for 15 min).

**Table 1: INGREDIENTS USED**

| INGREDIENTS | QUANTITY |
|---|---|
| Beef extract | 10 g. |
| Peptone | 10 g. |
| Sodium chloride | 5 g. |
| Distilled Water | 1000 ml. |

**Preparation and standardization of inoculum**

Each bacterial pure culture from the slant culture is picked up aseptically and was transferred into 100ml of nutrient broth. The inoculated broths were incubated at 37˚C for 24 hrs and growth was arrested by stored in the refrigerator (below 4˚C).After incubation inoculum were standardized to $10^6$-$10^8$ CFU/ml. for bacteria.

**Antimicrobial Activity by Disc Diffusion Method:**

**Sample Preparation:** 25 mg of each sample were dissolved in 2.5ml DMSO (Dimethyl Sulfoxide) to a final concentration of 1000µg/ml. The solvent control shows no antibacterial activity with all the test organisms used. The sterile disc (6 mm in diameter) were impregnated with 10µl of the sample and tested against microbial cultures.

**Media Used:** Muller – Hinton Agar Medium, Hi-media India (Pvt) Ltd

**Antibacterial activity** [2, 4]**:** Muller Hinton agar medium was prepared and transferred into sterile petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent

condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized bacterial inoculum was applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs were placed on the inoculated agar medium. All petriplates were incubated at 37˚C for 24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured and the details are tabulated in Table 2 and Fig. 1- 6.

**TABLE 2: MICROBIAL ACTIVITY INDEX**

| MICRO ORGANISMS USED | ZONE OF INHIBITION IN DIAMETER (MM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | STD | I | II | III | IV | V | VI | SOLVENT |
| KLEBSIELLA AEROGENEOSA | 18 | 12 | 13 | 10 | 15 | 10 | 18 | 10 |
| ESCHERECHIA COLI | 33 | 15 | 8 | 7 | 6 | 6 | 22 | 6 |
| PROTEUS VULGARIS | 14 | 8 | 10 | 12 | 20 | 23 | 26 | 8 |
| SALMONELLA PARATYPHI | 7 | 8 | 11 | 8 | 8 | 17 | 26 | 8 |
| BACILLUS LINTUS | 13 | 22 | 19 | 13 | 9 | 11 | 21 | 11 |
| STAPHYLOCOCUS ALBUS | 7 | 12 | 13 | 10 | 17 | 22 | 26 | 10 |
| MICROCOCUS LUTEUS | 13 | 8 | 9 | 9 | 9 | 13 | 23 | 8 |
| BACILLUS CEREUS | 12 | 8 | 8 | 7 | 7 | 10 | 18 | 8 |
| CANDIDA ALBICANS (FUNGI) | 18 | 15 | 18 | 12 | 8 | 12 | 15 | 6 |

**Fig 1: MICROCOCUS LUTEUS**

**Fig. 3: BACILLUS LINTUS**

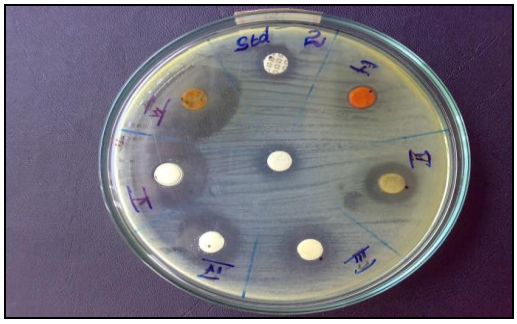

**Fig. 2: STAREPHAYLOCOCUS ALBUS**

**Fig. 4: PROTEUS VULGARIS**

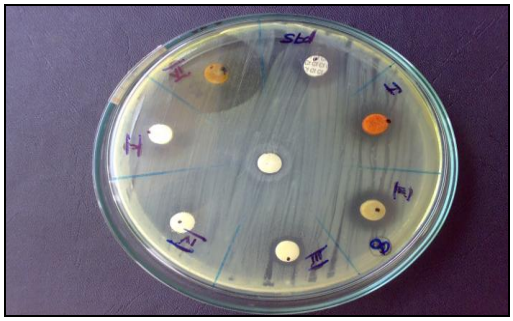

**Fig. 5: SALMONELLA PARATYPHI**

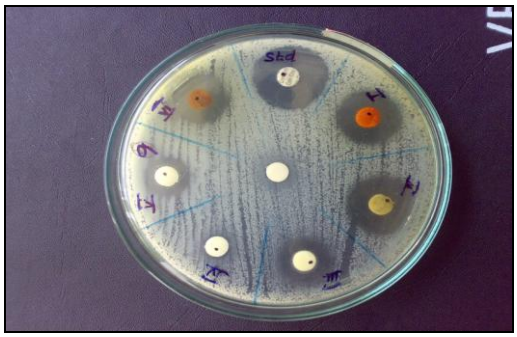

**Fig. 6: CANDIDA ALBICANS**

**Determination of Minimum Inhibitory Concentration for Synthesized Compounds (MIC) by Tube Assay Method:** The serial dilution of known concentration of compound solution are made from the stock (250 mg/ml) by using Muller Hinton broth using the method described below The tubes were labeled 1 to 8 and 1 ml of Muller Hinton broth were added to the first 5 tubes and 8$^{th}$ tube, then added 0.5 ml Muller Hinton broth to 6$^{th}$ and 7$^{th}$ tubes. One ml of different synthesized compounds was added to the 1$^{st}$ tube, mixed and transfer 1ml serially up to tube 5.From the 5$^{th}$ tube transfer 1ml to 6$^{th}$ tube. Mixed and transfer 0.5 ml to the 7$^{th}$ tube. Each tube, 1 to 7 contains 1ml diluted extract. The 8$^{th}$ tube is the control. With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 37 $^{0}$ c for 16 to 18hrs.The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC (Table 3-9).

**RESULTS AND DISCUSSION:** Based on the literature review an attempt was made to synthesize some thiazolidin-4-one compounds by microwave oven. The antibacterial screening of the synthesized compounds were performed in the concentration of 1000µg/ml in dimethyl formamide against against gram positive organisms such as Bacillus lintus, Bacillus cerus, Micrococcus luteus and Staphylococcus albus and gram negative organisms such as Escherichia coli, Klebsiella aerogenosa, Proteus vulgaris and Salmonella paratyphi respectively by using disc plate method in Muller Hinton agar medium and the anti bacterial activity was evaluated.

**Table 3: PROTOCOL FOR MIC**

| TUBE NO. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | CONTROL |
|---|---|---|---|---|---|---|---|---|
| Muller Hinton Broth (ml) | 1 | 1 | 1 | 1 | 1 | 0.5 | 0.5 | 1 |
| Diluted compound (ml) | 1 | 1 | 1 | 1 | 1 | 1 | 0.5 | 0 |
| Culture | Add 1 drop of approximately 0.01 ml. Mix gently incubate 16 to 18 hours at 37 $^{0}$ c. | | | | | | | |

**Table 4: COMPOUND I**

| ORGANISM | 1 500 µg | 2 250 µg | 3 125 µg | 4 62.5 µg | 5 31.2 µg | 6 15.6 µg | 7 7.8 µg | 8 CONTROL |
|---|---|---|---|---|---|---|---|---|
| MICROCOCUS ALBUS | - | - | - | - | + | + | + | + |
| STEPHAYLOCOCUS ALBUS | - | - | - | - | + | + | + | + |
| BACILLUS LINTUS | - | - | - | - | + | + | + | + |
| BACILLUS SCEREUS | - | - | - | + | + | + | + | + |
| ESCHERECHIA COLI | - | - | - | - | - | - | + | + |
| KLEBSIELLA AEROGONEASA | - | - | - | + | + | + | + | + |
| PROTEUS VULGARIS | - | - | - | - | + | + | + | + |
| SALMONELLA PARATYPHI | - | - | - | - | + | + | + | + |
| CANDIIDA ALBICANS | - | - | - | - | + | + | + | + |

( - ) INDICATES: NO GROWTH; (+) INDICATES: GROWTH

**Table 5: COMPOUND II**

| ORGANISM | 1 500 µg | 2 250 µg | 3 125 µg | 4 62.5 µg | 5 31.2 µg | 6 15.6 µg | 7 7.8 µg | 8 CONTROL |
|---|---|---|---|---|---|---|---|---|
| MICROCOCUS ALBUS | - | - | - | + | + | + | + | + |
| STEPHAYLOCOCUS ALBUS | - | - | - | + | + | + | + | + |
| BACILLUS LINTUS | - | - | - | + | + | + | + | + |
| BACILLUS SCEREUS | - | - | + | + | + | + | + | + |
| ESCHERECHIA COLI | - | - | - | + | + | + | + | + |
| KLEBSIELLA AEROGONEASA | - | - | - | + | + | + | + | + |
| PROTEUS VULGARIS | - | - | - | + | + | + | + | + |
| SALMONELLA PARATYPHI | - | - | - | + | + | + | + | + |
| CANDIIDA ALBICANS | - | - | - | - | + | + | + | + |

**Table 6: COMPOUND III**

| ORGANISM | 1 500 µg | 2 250 µg | 3 125 µg | 4 62.5 µg | 5 31.2 µg | 6 15.6 µg | 7 7.8 µg | 8 CONTROL |
|---|---|---|---|---|---|---|---|---|
| MICROCOCUS ALBUS | - | - | - | - | + | + | + | + |
| STEPHAYLOCOCUS ALBUS | - | - | - | - | + | + | + | + |
| BACILLUS LINTUS | - | - | - | - | + | + | + | + |
| BACILLUS SCEREUS | - | - | - | - | + | + | + | + |
| ESCHERECHIA COLI | - | - | - | - | + | + | + | + |
| KLEBSIELLA AEROGONEASA | - | - | - | - | + | + | + | + |
| PROTEUS VULGARIS | - | - | - | - | + | + | + | + |
| SALMONELLA PARATYPHI | - | - | - | - | + | + | + | + |
| CANDIIDA ALBICANS | - | - | - | - | + | + | + | + |

**Table 7: COMPOUND IV**

| ORGANISM | 1<br>500 µg | 2<br>250 µg | 3<br>125 µg | 4<br>62.5 µg | 5<br>31.2 µg | 6<br>15.6 µg | 7<br>7.8 µg | 8<br>CONTROL |
|---|---|---|---|---|---|---|---|---|
| MICROCOCUS ALBUS | - | - | - | + | + | + | + | + |
| STEPHAYLOCOCUS ALBUS | - | - | - | + | + | + | + | + |
| BACILLUS LINTUS | - | - | - | + | + | + | + | + |
| BACILLUS SCEREUS | - | - | + | + | + | + | + | + |
| ESCHERECHIA COLI | - | - | - | + | + | + | + | + |
| KLEBSIELLA AEROGONEASA | - | - | - | + | + | + | + | + |
| PROTEUS VULGARIS | - | - | - | + | + | + | + | + |
| SALMONELLA PARATYPHI | - | - | - | + | + | + | + | + |
| CANDIIDA ALBICANS | - | - | - | - | + | + | + | + |

**Table 8: COMPOUND V**

| ORGANISM | 1<br>500 µg | 2<br>250 µg | 3<br>125 µg | 4<br>62.5 µg | 5<br>31.2 µg | 6<br>15.6 µg | 7<br>7.8 µg | 8<br>CONTROL |
|---|---|---|---|---|---|---|---|---|
| MICROCOCUS ALBUS | - | - | - | - | + | + | + | + |
| STEPHAYLOCOCUS ALBUS | - | - | - | - | + | + | + | + |
| BACILLUS LINTUS | - | - | - | - | + | + | + | + |
| BACILLUS SCEREUS | - | - | - | - | + | + | + | + |
| ESCHERECHIA COLI | - | - | - | - | + | + | + | + |
| KLEBSIELLA AEROGONEASA | - | - | - | - | + | + | + | + |
| PROTEUS VULGARIS | - | - | - | - | + | + | + | + |
| SALMONELLA PARATYPHI | - | - | - | - | + | + | + | + |
| CANDIIDA ALBICANS | - | - | - | - | + | + | + | + |

( - ) Indicates: No Growth; (+) Indicates: Growth

**Table 9: COMPOUND V**

| ORGANISM | 1<br>500 µg | 2<br>250 µg | 3<br>125 µg | 4<br>62.5 µg | 5<br>31.2 µg | 6<br>15.6 µg | 7<br>7.8 µg | 8<br>CONTROL |
|---|---|---|---|---|---|---|---|---|
| MICROCOCUS ALBUS | - | - | - | + | + | + | + | + |
| STEPHAYLOCOCUS ALBUS | - | - | - | + | + | + | + | + |
| BACILLUS LINTUS | - | - | - | + | + | + | + | + |
| BACILLUS SCEREUS | - | - | - | + | + | + | + | + |
| ESCHERECHIA COLI | - | - | - | + | + | + | + | + |
| KLEBSIELLA AEROGONEASA | - | - | + | + | + | + | + | + |
| PROTEUS VULGARIS | - | - | - | + | + | + | + | + |
| SALMONELLA PARATYPHI | - | - | - | + | + | + | + | + |
| CANDIIDA ALBICANS | - | - | - | + | + | + | + | + |

The anti fungal activity of the synthesized compounds was performed against Candida albicans in same concentration and anti fungal activity was evaluated. The results are shown in the table no: 6.Further, all the compounds have been subjected to perform MIC at concentration of 500 µg/ml. The result shows that the compound V and VI has activity at 62.5µg/ml. But compound II, III, and IV shows MIC at 125µg/ml. The compound I only shows activity at a concentration of 15.6µg/ml against E. coli. Based on these findings, the compound I, V, and VI shows greater activity against respective organism and other compounds II, III and IV shows moderate action.

**CONCLUSION:** In this study new thiazolidinone derivatives have been synthesized and purified. The synthesized compounds have been subjected to anti-microbial activity. Result shows that compound I, V, and VI shows greater activity against respective organism and other compounds II, III, and IV shows moderate action. Hence the continuation of this work can be done by same parameters using microwave oven. Furthermore biological activities such as anti-inflammatory, antidiabetic, analgesic, antiepileptic activities etc. can be done for the synthesized compounds in future.

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