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EVALUATION OF BIOLOGICAL ACTIVITY (*IN-VITRO*) OF SOME THIADIAZOLE DERIVATIVES

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ABSTRACT
Synthesized novel series of 1,3,4- thiadiazole derivatives have been explored for anti-microbial, anti-oxidant, anti-inflammatory activity. The synthesis and characterization of 1,3,4-thiadiazole has been accepted for publication in WJPR(World Journal of Pharmaceutical Research). The synthesized compounds were screened for their *in-vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus brasiliensis* by agar cup plate method. Anti-oxidant activity was evaluated by hydrogen peroxide scanvenging capacity method. Anti-inflammatory activity was done by human red blood cell (HRBC) membrane stabilization method.

KEYWORDS: 1,3,4-thiadiazole, anti-microbial, anti-oxidant, anti-inflammatory, HRBC.

INTRODUCTION

Antimicrobial is any substance of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host. Antimicrobial activity was evaluated by agar cup plate method.[1]

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or vitamin C, vitamin A and vitamin E obtained by ingestion. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer or coronary heart disease.[2]

Inflammation is a non specific, localized immune reaction of the organism, which tries to localize the pathogen agent. Many consider the syndrome a self-defense mechanism. It consists of vascular, metabolic, cellular changes, triggered by the entry of pathogenic agent in healthy tissues of the body.[3]

MATERIAL AND METHODS

Antibacterial activity by Agar Cup Plate Method

The newly synthesized compounds were screened for their antibacterial activity using agar cup plate method. The antibacterial activity of test compounds were evaluated against gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria, *Escherichia coli*. A test tube containing sterile melted top agar (1.5 %) previously cooled to room temperature with 0.2 mL suspension of the test culture, mixed methodically and poured in the petridish containing sterile base agar medium (autoclaved at 121°C for 15 min.) then allowed to solidify. With the help of sterile cup-borer, five and six cups in the agar-plate were marked and were injected with 0.1 mL of test solution, 0.1 mL of standard solution and 0.1 mL of DMSO solvent respectively. Then the plates were allowed to diffuse for 20 min. in refrigerator at 4-5°C. The plates were then incubated in upright position at 37°C for 24 hrs. After incubation, the relative susceptibility of the micro-organisms to the potential antimicrobial agent is demonstrated by a clear zone of growth inhibition around the cup. The inhibition zone caused by the various compounds on the micro-organisms was measured and the activity was rated on the basis of the size of the inhibition zone.[4]

Antifungal activity by Agar Cup Plate Method

The newly synthesized compounds were screened for their antifungal activity using agar cup plate method. The antifungal activities of test compounds were evaluated against *Aspergillus brasiliensis*. The plates were inoculated by dipping a sterile swab into inoculums. The inoculation was dried at room temperature in aseptic condition. The plates were bored; prepared test solutions added. These plates were placed in an incubator at 22°C within a few minutes of preparation. After 7 days of

incubation, the diameter of zone of inhibition was measured in mm.[5]

Anti-oxidant activity by Hydrogen Peroxide Scavenging Capacity method

The ability of the synthesized compound to scavenge hydrogen peroxide was determined (Ruch et al.,1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Synthesized compounds (100-300µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of this solution at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both synthesized compounds and standard compound were calculated.[6]

% Scavenged [H_2O_2] = [(AC – AS)/AC] x 100

Anti-inflammatory activity by Human Red Blood Cell (HRBC) Membrane Stabilization Method:

Fresh whole human blood (5 mL) was collected and transferred to the centrifuged tubes containing Heparin or EDTA or Sodium citrate to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

The reaction mixture consists of 1.0 mL of test sample of different concentrations (100µg – 300 µg) in normal saline and 0.5 mL of 10% HRBC suspension, 1 mL of 0.2 M phosphate buffer, 1 mL hyposaline were heated at 45 ˚C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes; the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared without drug. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula.[7]

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100]

RESULTS AND DISCUSSION

Antibacterial activity by Agar Cup Plate Method

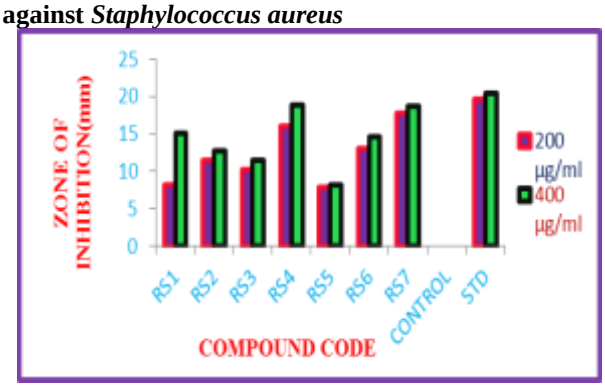
All the synthesized compounds were found to exihibit moderate to good *in-vitro* antibacterial activity. The comparative study on the zones of inhibition was done by the agar cup plate method. The **compounds RS4, RS6, RS7** showed potent activity, **RS2, RS3** shows moderate activity and **RS5, RS1** showed poor antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* when compared to standard gentamycin.

Table-1: Zone of inhibition of synthesized compounds against bacterial organism

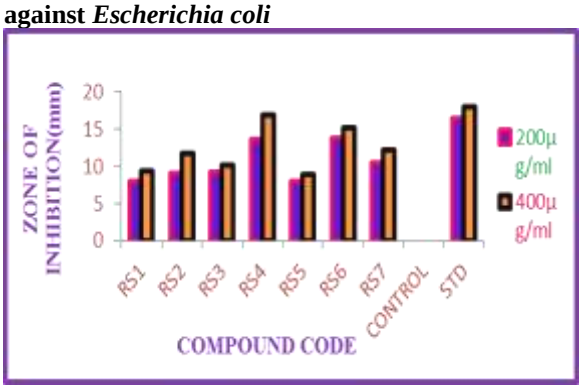
Sample Code	*Staphylococcus aureus*		*Escherichia coli*(E.Coli)	
	200µg/mL	400µg/mL	200µg/mL	400µg/ mL
RS1	8.17	15.11	7.98	9.39
RS2	11.45	12.67	9.09	11.69
RS3	10.28	11.47	9.27	10.09
RS4	16.01	18.85	13.65	16.84
RS5	7.90	8.24	7.96	8.93
RS6	13.01	14.67	13.67	15.12
RS7	17.73	18.65	10.51	12.18
CONTROL	R	R	R	R
STD	19.58	20.51	16.39	18.02

All measurement in mm R = Resistant Control=DMSO Std=Gentamycin

Antibacterial activity of synthesized compound against *Staphylococcus aureus*



Antibacterial activity of synthesized compound against *Escherichia coli*



Antifungal activity by Agar Cup Plate Method

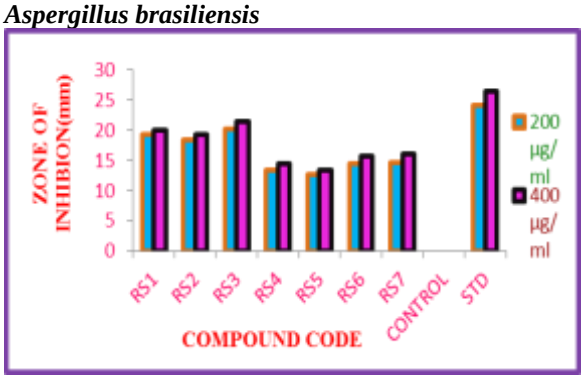
All the synthesized compounds were found to exihibit moderate to good *in-vitro* fungal activity. The comparative study on the zones of inhibition was done by the agar cup plate method. The **compounds RS1, RS2, RS3** showed more potent activity, **RS6, RS7** showed moderate activity and **RS4, RS5** showed poor antifungal activity against *Aspergillus brasiliensis* when compared to standard clotrimazole.

Table-2: zone of inhibition of synthesized compounds against fungal organism

Sample Code	*Aspergillus brasiliensis*	
	200µg/mL	400µg/mL
RS1	19.26	20.02
RS2	18.34	19.29
RS3	20.25	21.43
RS4	13.39	14.53
RS5	12.67	13.38
RS6	14.43	15.63
RS7	14.62	16.12
CONTROL	R	R
STD	24.12	26.49

All measurement in mm R = Resistant Control=DMSO Std= Clotrimazole

Antifungal activity of synthesized compound against *Aspergillus brasiliensis*



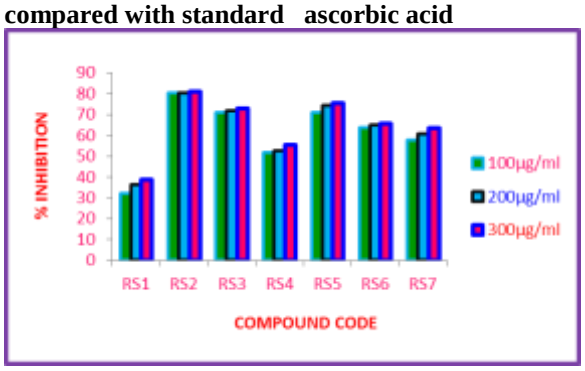
Anti-oxidant activity by Hydrogen Peroxide Scavenging Capacity method

All the synthesized compounds showed significant anti-oxidant property. The **compounds RS2, RS3, RS5** exhibited more potent activity, **RS4, RS6, RS7** showed moderate activity and **RS1** showed poor antioxidant activity when compared to standard ascorbic acid.

Table-3: Percentage inhibition of synthesized compounds compared with standard ascorbic Acid

S.no	Compound Code	Percentage Inhibition		
		100µg/mL	200µg/mL	300µg/ mL
1	RS1	32.10	36.35	38.85
2	RS2	80.30	80.32	80.88
3	RS3	71.03	71.89	72.98
4	RS4	51.92	52.50	55.18
5	RS5	70.74	74.25	75.58
6	RS6	63.69	64.89	65.60
7	RS7	57.75	60.84	63.66

Percentage inhibition of synthesized compounds compared with standard ascorbic acid



Anti-inflammatory activity by Human Red Blood Cell (HRBC) Membrane Stabilization Method

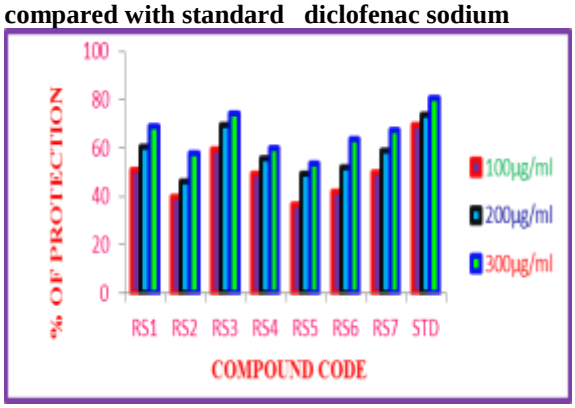
All the synthesized compounds showed significant anti-inflammatory property. The **compounds RS1, RS3, RS4, RS7** showed more potent activity where as **RS6** showed moderate activity and **RS2, RS5** showed poor anti-inflammatory activity when compared to standard diclofenac sodium.

Table-4: Percentage of inhibition of synthesized compounds compared with standard diclofenac sodium

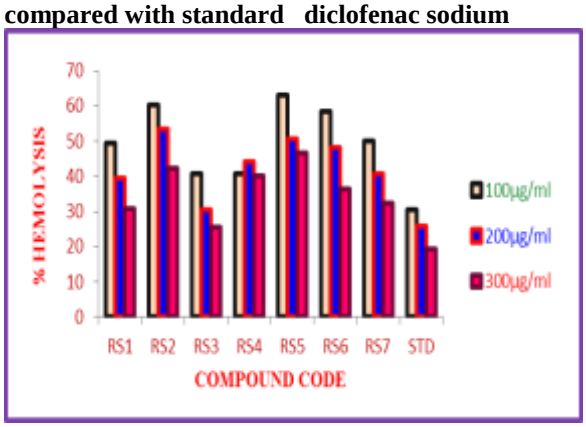
S.no	Compound Code	% of Inhibition			% of Hemolysis		
		100µg/mL	200µg/mL	300µg/mL	100µg/mL	200µg/mL	300µg/mL
1	RS1	50.75	60.52	69.17	49.25	39.48	30.83
2	RS2	39.66	46.42	57.70	60.34	53.58	42.30
3	RS3	59.39	69.54	74.43	40.61	30.46	25.57
4	RS4	49.24	55.82	59.96	50.76	44.18	40.04
5	RS5	36.84	49.24	53.38	63.16	50.76	46.62
6	RS6	41.72	51.87	63.72	58.28	48.13	36.28
7	RS7	50.00	59.21	67.66	50.00	40.79	32.34
8	STD	69.54	74.06	80.82	30.46	25.94	19.18

STD – Standard – Diclofenac Sodium

Percentage inhibition of synthesized compounds compared with standard diclofenac sodium



Percentage hemolysis of synthesized compounds compared with standard diclofenac sodium



CONCLUSION

In conclusion, present study revealed the *in-vitro* anti-microbial, anti-oxidant and anti-inflammatory activity of some 1,3,4-thiadiazole derivatives. The **compounds RS4, RS6, RS7** exhibited significant anti-bacterial activity, The **compounds RS1, RS2, RS3** exhibited more potent anti-fungal activity, The **compounds RS2, RS3, RS5** showed more significant anti-oxidant activity, The **compounds RS1, RS3, RS4, RS7** exhibited more significant anti-inflammatory activity. Toxicological studies and exploration of in-vivo pharmacological activity will be the further scope of study.

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