Anti-oxidant and Anti-microbial activities of 2'', 4''-thiazolidindione derivatives of 7-flavonols

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ABSTRACT:

Objective: The synthesized compounds of 2'',4''-thiazolidindione derivatives of 7-flavonols*, after characterization, aimed to be tested for their anti-oxidant and anti-microbial effects. **Methods**: i) Free radical scavenging actions tested by hydrogen peroxide- nitric oxide- and by alkaline DMSO- methods and ii) anti-microbial effects against various bacterial pathogens and against *candida albicans* by disc diffusion method. **Results**: Data were found to be dose dependent and IC_{50} value was 30-60 µg/ml and the results revealed that the dinitro-, trinitro- and acetyl, dinitro derivatives showed better and/or equipotent activity to that of the standard, ascorbic acid. The synthesized compounds exerted variable inhibitory activities at a concentration of 1µg /10µl /disc with inhibition zone ranging from 7-26 mm in diameter and a good antifungal activity against *Candida albicans* at the concentration of (1µg /10µl /disc) with inhibition of 10-24 mm. *Klebsiella tribatta* are more susceptible to the action of the formylated samples, giving high inhibition values comparing to the other organisms. Compounds Ie and Ih resulted to a higher activity index (AI>1); compounds Id, Ig and Ii showed an equal value (AI=1); whereas, Ia, Ib, Ic and If showed only a moderate activity (AI<1) compared to the standard, Amikacin. **Conclusion**: The findings confirmed that the synthetic compounds of 3-formyl, 7-flavonol derivatives have significant anti-oxidant and anti-microbial activities.

KEYWORDS: 2'',4''-thiazolidindione derivatives of 7-flavonols; Anti-microbial; Disc diffusion method; Anti-oxidant; Hydrogen peroxide method; Nitric oxide method; Alkaline DMSO method.

INTRODUCTION:

Flavonoids have extensive biological properties[1] elevating the health status in life, by preventing the risky, hazardous ill effects in them. When produced in excess, ROS can cause tissue injury[2], which becomes the reason for most of ailments[3] say bacterial and parasite infections, cancer, liver diseases, heart related problems, swelling, sugar complaints, kidney failure[4] and brain dysfunction, malaria, acquired immunodeficiency syndrome[5], stroke, hypertension, arteriosclerosis, pathophysiology of ischemia, aging and neoplastic diseases[6]. Body has itself antioxidant system, being produced as a complex process and should be capable of neutralizing free radicals[7] and repairing the damage[8]. Most of the reactive oxygen species are scavenged by endogenous defense system[9].

On the basis of above studies, it was thought useful to test the free radical scavenging activity of the 2'', 4''-thiazolidindione compounds of 7-flavonols which might have great potential in ameliorating the aforementioned disease processes. There are several methods[10] to assess radical species availability or vanishing effects of the synthesized substances. The simple, most repeated and sensitive methods for the same are Hydrogen peroxide, Nitric oxide and alkaline DMSO methods.

Drugs are less defending against microbial invasion, this because, moisture regaining composition provide room for their infestation[11]. In recent years, owing to their biocompatibility and biological functions, flavanoids are given potential importance in the biomedical[12] and pharmaceutical fields[13]. The effective synthesis of 2'', 4''-thiazolidindione compounds of 7-flavonols became a significant target based on the importance of flavanoids and other related biosynthesized substances[14]. Chemical modification[15] of these phyto-components for bioassay of the anti-bacterial activity has become a need of broad-spectrum resistance problems[16].

Demethoxylation helps in boosting up the cellular uptake of flavonoids and a free phenolic moiety usually facilitate its binding at endocytosis receptor[17]. Less activity may be by a greater number of hydroxyl groups, more hydrophilic and thus difficult in cell membrane penetration[18]. Therefore, the present synthetic compounds were designed to have one hydroxyl group only so as to make the synthesized compounds as lipophilic for better availability of drug inside the bacterial cell[19].

This project task was aimed to be investigating the antimicrobial activity of various compounds of 2'', 4''-thiazolidindiones of 7-flavonols against various pathogenic bacteria, both gram negative species *Escherichia coli*, *Pseudomonas aerogenosa, Klebsiella tribatta* and *Proteus vulgaris* and grampositive species *Staphylococcus aureus, Streptococcus pneumoniae* and *Closteridium pefrigens* and fungi *Candida albicans.*

MATERIALS AND METHODS:

2, 4- thiazolidindione derivatives of 7-flavonols

Table 1 The list of substituents in various derivatives of 2, 4- thiazolidindione of 7-flavonols

S. No.	Name of the compounds (Ia-i)	R^1	R^2	R^3	R^4	R^5	R^6
1.	2, 4- thiazolidindione (TZD) moiety attached 7-flavonols(Ia)	H	H	H	H	H	H
2.	5-nitro, TZD derivative of 7-flavonol (Ib)	$-NO_2$	H	H	$-NO_2$	H	H
3.	6-acetyl, TZD derivative of 7-flavonol (Ic)	$-COCH_3$	H	H	$-COCH_3$	H	H
4.	4'-nitro, TZD derivative of 7-flavonol (Id)	H	H	$-NO_2$	H	H	$-NO_2$
5.	4',5-dintro, TZD derivative of 7-flavonol (Ie)	$-NO_2$	H	$-NO_2$	$-NO_2$	H	$-NO_2$
6.	4'-nitro, 6-acetyl, TZD derivative of 7-flavonol (If)	$-COCH_3$	H	$-NO_2$	$-COCH_3$	H	$-NO_2$
7.	3'4'-dintro, TZD derivative of 7-flavonol (Ig)	H	$-NO_2$	$-NO_2$	H	$-NO_2$	$-NO_2$
8.	3'4'5,-trinitro TZD derivative of 7-flavonol (Ih)	$-NO_2$	NO_2	$-NO_2$	$-NO_2$	$-NO_2$	$-NO_2$
9.	3'4'-dinitro, 6-acetyl, TZD derivative of 7-flavonol (Ii)	$-COCH_3$	NO_2	$-NO_2$	$-COCH_3$	$-NO_2$	$-NO_2$

Antioxidant activity:
The concentration of test samples and standard for the study was 30-150µg/ml in all methods. Absorbance was measured against blank and a control was performed. Assessed the free radicals scavenging percentage and IC50 data $\pm$ S.D. Analytical grade chemicals, solvents were of in use, procured from E-Merck and Hi-media.

Hydrogen peroxide anti-oxidant method:
[20]Gulcin *et al* method was followed to determine the hydrogen peroxide scavenging activity of the synthesized 2, 4-thiazolidindiones of 7-flavonols. The solutions of test samples and standard at concentrations (30-150µg/ml) were prepared and added to 2ml of H_2O_2 (20mM) solutions in PBS (pH 7.4). The absorbance was measured at 230nm in UV spectrophotometer (Shimadzu, UV-2450) after 10 min of incubation at 37 °C against a blank.

% Scavenged $[H_2O_2] = [(A_0-A_1)/A_0]$ X100,

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample/standard

Nitric oxide anti-oxidant method:
The solutions of test samples and standard at concentrations (30-150µg/ml) were prepared in methanol and added in 1ml to 0.3ml of Sodium nitroprusside (10 mM) in phosphate buffer saline (PBS), pH7.4 and incubated at 25°C for 3 hrs. The same treatment was done for control. Then added, 0.5ml of Griess reagent (1% sulphanamide, 2% H_3PO_4 and 1% N-napthyl ethylene diamine dihydrochloride) was added. The absorbance was measured at 546nm and repeated for thrice.

% Scavenged [NO] = [(A_0-A_1)/A_0] X100,

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample/standard.

Scavenging of superoxide radical by alkaline DMSO method (Marcocci *et al*[21])
In this method, the color, obtained by EDTA in the presence of nitrite, being reduced from superoxide radical (O2-), which in turn by auto oxidation of hydroxylamine hydrochloride in NBT, is responsible for absorbance measurement at 560nm. To the reaction mixture containing 1ml of alkaline Dimethyl sulfoxide (1ml of dimethyl sulfoxide containing 5mM sodium hydroxide in 0.1ml of distilled water) and 0.3ml of various concentrations (30-150µg/ml) of the test samples and standard, ascorbic acid in dimethyl sulfoxide, added 0.1ml of Nitro blue tetrazolium (1mg/ml) to give a final volume of 1.4ml.

Inhibition (%) = [(A_0-A_1)/A_0] X100,

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample/standard

Anti-Microbial Study:
Details of Test Microorganism for Anti-microbial experiments:
Gram negative bacterial strains *Escherichia coli* (ATCC. No. 25922), *Pseudomonas aerogenosa* (ATCC. No. 25619), *Klebsiella tribatta* (ATCC. No. 27736) and *Proteus vulgaris* (ATCC. No. 33420) and gram-positive bacterial strains *Staphylococcus aureus* (ATCC. No. 51740), *Streptococcus pneumoniae* (ATCC. No. 27336), and *Closteridium pefrigens* (ATCC. No. 13124), and fungal cultures of *Candida albicans* (ATCC. No. 66027) were got from Boss clinical Laboratory, Madurai, India. The bacteria were maintained on Muller Hinton Agar medium (MHA) at room temperature and fungus on Potato Dextrose Agar (PDA) at 28°C. Analytical grade chemicals, solvents were of in use, procured from E-Merck and Hi-media.

Inoculum Preparation process:
The bacterial strains *Escherichia coli*, *Pseudomonas aerogenosa, Klebsiella tribatta, Proteus vulgaris, Staphylococcus aureus*, *Streptococcus pneumoniae* and *Closteridium pefrigens* maintained in nutrient broth, was centrifuged at 10,000rpm for 5 min and was standardized spectrophotometrically (A_{610}nm) to measure the cell density. 5 to 10 days old culture was used to prepare *Candida albicans* inoculum (spore density as $1\ 0^5$ s p o r e s /ml) and was grown on PDA medium.

Procedure for Anti-bacterial activity:
Minimum Inhibitory Concentration Study-Disc Diffusion Mechanism (Bauertal *et al*[22]**):**
Sterile Muller Hinton agar plates with Whatmann's Grade No.1 discs (6mm dia) were made and confirmed for microbes free. The title substances were prepared in distilled water and mixed at various strengths as 25 µg/ml, 50µg/ml, 100µg/ml) to each disc of holding capacity (10µl). A loop full of the organisms at 10^6 cfu/ml quantity, inoculated the incubated plates at 37°C. The size (diameter) of the inhibition zones were observed and calculated.

Zone of Inhibition study (Kirby *et al*[23]):
Test solution at 100µg/ml concentration, Whatmann No. 1 filter paper discs (4mm diameter, 160ºC for 30 min) and the suspension of the test microorganisms 10µl (10^6 cells/ml) were prepared and the latter one was smeared on to the individual medium by spreading method. After solidification, impregnated the filter paper discs with the analytes and kept by sterilized forceps on test organism-seeded plates. Compounds were screened for gram negative Bacterial cultures *Escherichia coli*, *Pseudomonas aerogenosa, Klebsiella tribatta* and *Proteus vulgaris* and gram-positive bacterial cultures *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Closteridium pefrigens*; Amikacin (100µg/ml) was used as the standard and distilled water (100µg/ml) as blank. The experimental plates were incubated at 37°C for one day and measured the width of growth inhibition zones. The studies were repeated thrice to get concordance in results.

Procedure for antifungal assay- Disc Diffusion Mechanism:
On sterile Petri plates, molten potato dextrose agar (15 ml) was poured, solidified and onto which, smeared the inoculum suspension (0.1%) of 10 days old fungal strains through point inoculation way. Test solutions (100µg/ml) dipped paper discs (4mm diameter) were then placed on test organism-seeded plates. Distilled water dipped disc was treated as blank and Ketoconazole (100µg/ml) used as the standard. Activity indices in terms of the diameters of the inhibition zone was observed after 72 hours of incubation. The studies were repeated thrice to get concordance in results.

$$\text{Activity} = \frac{\text{Zone of inhibition exhibited by test compounds}}{\text{Zone of inhibition exhibited by standard}}$$

index Zone of inhibition exhibited by reference compound

Statistical analysis:
The data were subjected to one-way ANOVA, Dunnett's multiple test and expressed as mean S.E.M. Software: Graph Pad Prism 5.01

RESULTS AND DISCUSSION:

Free radical scavenging substances like phenolic compounds and flavanoids may act as complexes initiator, reductants and scavengers of free radicals (Andlauer). From the literatures, the mechanisms of flavonoids eg., kaempferol, myricetin, naringenin, quercetin and rutin that are antimicrobial can be classified as the blockade of nucleic acid formation, integrity and action of cytoplasmic membrane and energy metabolism.

Anti-oxidant activity: Hydrogen Peroxide-Free Radical Scavenging Method:

Table 1: Anti-oxidant activity of 2'4'-thiazolidinedione derivatives of 7-flavonols by Hydrogen peroxide radical scavenging assay

Conc. (µg/ml)	Percentage of radical scavenging activity						
	Standard Ascorbic acid	**Sample I** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl)4'-nitro, 7-flavanol **(IIId)**	**Sample II** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,4'-dinitro, 7-flavanol **(IIIe)**	**Sample III** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6-acetyl, 4'-nitro, 7-flavanol **(IIIf)**	**Sample IV** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 3',4'- dinitro, 7-flavanol **(IIIg)**	**Sample V** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,3',4'-trinitro, 7-flavanol **(IIIh)**	**Sample VI** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6 –acetyl, 3',4'- dinitro, 7-flavanol **(IIIi)**
30	79.21±1.21	17.53±2.65	24.23±1.22	18.24±0.54	39.97±0.85	41.98±1.22	24.68±2.48
60	81.98±2.34	25.42±2.00	36.85±0.87	30.79±1.74	55.45±1.63	59.41±0.84	34.74±2.10
90	84.01±1.77	34.11±1.74	51.24±1.65	45.65±0.63	68.74±2.44	71.57±0.66	48.51±1.54
120	88.21±0.98	47.85±0.99	64.12±1.89	53.84±1.44	81.54±1.33	85.42±1.52	58.96±0.88
150	91.65±1.60	64.52±1.54	77.45±1.98	70.77±2.98	90.45±1.88	92.67±1.63	66.23±1.96

Values are expressed as mean± SD; Values are from triplicate readings; and are statistically significant at $p<0.05$*,$p<0.01$**, $p<0.001$***, when compared to the standard ascorbic acid.

Figure 1: Anti-oxidant activity of 2'4'-thiazolidinedione derivatives of 7-flavonols by Hydrogen peroxide radical scavenging assay

AA-Ascorbic acid; NF-6 nitro, 7-flavonol; DNF-4', 6-dinitro, 7-flavonol; DNF-3'4'-dinitro 7-flavonol; TNF-3'4'6-trinitro, 7-flavonol; ANF-6-acetyl, 4'-nitro, 7-flavonol; ADNF-6-acetyl, 3'4'-dintro, 7-flavonol.

Glucose oxidase (GO) is an enzyme which has been used as an oedemogen by local generation of hydroxyl radical (H_2O_2) and OH* (unstable and more reactive) from hydrogen peroxide (H_2O_2) to produce inflammatory paw oedema in mice. Glucose oxidase + Glucose --------- Gluconic acid + Hydrogen peroxide. H_2O_2 thus liberated by glucose oxidase cause direct oxidative attack on cell membrane leading to increase rigidity to lipidbilayer, osmotic fragility, and aggregation of membrane protein and decrease activity of membrane bound enzymes. Unsaturated radical effects, a rich supply of oxygen and presence of transitional metals, favour oxidative damage and erythrocytes meet the above condition.

The results of free radical scavenging activity of different concentrations (30-150µg/ml) of 2",4"-thiazolidinedione derivatives of 7-flavonols (Ia-i) by hydrogen peroxide method were given in Table 1 and presented in Figure 1. In presence of antioxidants, the hydrogen peroxide (H_2O_2) or reactive hydroxyl (OH^-), free radicals get reduced to water. A dose dependent radical scavenging activity was observed. The maximum reducing ability at 150µg/ml were found to be for the compounds of 2', 4'-thiazolidinedione derivatives of 7-flavonols categories Ih (92.67%), Ig (90.45%), Ie (77.45 %) and ascorbic acid 91.65 % respectively. These compounds may thus act through two different processes: inhibition of the xanthine oxidase enzyme and ROS scavenging (Cotelle), as their antioxidant properties are challenging the standard, ascorbic acid's activity. Whereas the other compounds of Id, Ifand Ii showed

a marked reducing ability in the range of 60-74%. The IC_{50} values of the compounds Ih and Ig and ascorbic acid were found to be 60µg/ml and 30µg/ml respectively. Whereas, the IC_{50} value of Ii was 90µg/ml and those of all the other compounds were found to be as 120µg/ml. It was inferred from the above data that the 6,3',4'-trinitro derivatives (h); 3',4'-dintro derivatives (g) were found to be effective in inhibiting the lyses of erythrocytes possibly by scavenging the hydrogen peroxides produced by the reaction of glucose and glucose oxidase than that of 3',4'-dintro,6-acetyl derivatives (i) and other mono substituted derivatives (a, b, c, d and e).

Table 2: Anti-oxidant activity of 2'4'-thiazolidinedione derivatives of 7-flavonols by Nitric oxide radical scavenging assay

Conc. (µg/ml)	Percentage of radical scavenging activity						
	Standard Ascorbic acid	**Sample I** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl)4'-nitro, 7-flavanol **(IIId)**	**Sample II** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,4'-dinitro, 7-flavanol **(IIIe)**	**Sample III** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6-acetyl, 4'-nitro, 7-flavanol **(IIIf)**	**Sample IV** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 3',4'- dinitro, 7-flavanol **(IIIg)**	**Sample V** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,3',4'- trinitro, 7-flavanol **(IIIh)**	**Sample VI** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6 –acetyl, 3',4'-dinitro, 7-flavanol **(IIIi)**
30	37.63±0.62	14.21±1.41	21.52±0.33	14.62±1.53	39.40±0.41	42.12±1.22	25.96±1.33
60	46.41±1.42	22.52±1.77	34.11±1.02	21.52±0.65	41.12±1.85	49.62±1.48	31.96±0.46
90	52.85±1.85	29.95±0.88	40.23±1.57	28.69±1.52	49.65±0.96	52.41±0.65	46.00±0.84
120	64.52±0.98	37.41±0.91	44.12±1.96	34.74±1.63	56.84±0.77	61.85±0.94	54.77±0.27
150	72.58±2.10	39.12±0.42	55.23±0.87	41.95±1.87	64.11±0.65	69.11±1.44	63.94±1.31

Values are expressed as mean± SD; Values are from triplicate readings; and are statistically significant at p<0.05*,p<0.01**, p<0.001***, when compared to the standard ascorbic acid.

Figure 2: Anti-oxidant activity of 2'4'-thiazolidinedione derivatives of 7-flavonols by Nitric oxide radical scavenging assay

AA-Ascorbic acid; NF-6 nitro, 7-flavonol; DNF-4', 6-dinitro, 7-flavonol; DNF-3'4'-dinitro 7-flavonol; TNF-3'4'6-trinitro, 7-flavonol; ANF-6-acetyl, 4'-nitro, 7-flavonol; ADNF-6-acetyl, 3'4'-dintro, 7-flavonol.

The results of free radical scavenging activity of different concentrations (30-150 µg/ml) of 2",4"-thiazolidinedione derivatives of 7-flavonols (Ia-i) by Nitric oxide assay were tabulated in Table 2 and charted in Figure 2., The stable products of nitrates and nitrites are being produced when nitric oxide reacts with O_2 and there will be a reduction in nitrous acid concentration which could be measured at 546 nm. The maximum reducing ability at 150 µg/ml were found to be for the compounds of 2', 4'-thiazolidinedione derivatives of 7-flavonols categories Ih (69.11 %), Ig (64.11 %), Ii (63.94 %) and ascorbic acid 72.58 % respectively. Whereas the other compounds of Id, Ie and If showed an average reducing ability in the range of 30-55 %. The IC_{50} values of Ih and ascorbic acid were 60 µg/ml and 90 µg/ml respectively. Whereas, the IC_{50} value of Ii were 90 µg/ml and those of all the other compounds were found to be as 150 µg/ml. It was observed from the resulted findings that the 6,3',4'-trinitro derivatives (h); 6-acetyl, 3',4'-dintro derivatives (i) were found to be most effective, in inhibiting the nitric oxide free radical activity by scavenging them, than that of 3',4'-dintro derivatives (g) and other mono substituted derivatives (a,b,c,d, e).

Table 3: Scavenging of superoxide radical activity of the synthesized 2'4'-thiazolidinedione derivatives of 7-flavonols by alkaline DMSO method

Conc. (µg/ml)	Percentage of radical scavenging activity						
	Standard Ascorbic acid	**Sample I** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl)4'-nitro, 7-flavanol **(IIId)**	**Sample II** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,4'-dinitro, 7-flavanol **(IIIe)**	**Sample III** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6-acetyl, 4'-nitro, 7-flavanol **(IIIf)**	**Sample IV** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 3',4'- dinitro, 7-flavanol **(IIIg)**	**Sample V** (3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,3',4'- trinitro, 7-flavanol **(IIIh)**	**Sample VI** 3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6 –acetyl, 3',4'-dinitro, 7-flavanol **(IIIi)**

30	76.66±0.36	32.52±1.96	37.42±1.61	25.12±1.94	62.15±0.84	51.02±0.36	41.96±1.64
60	79.69±0.98	37.98±1.48	48.21±0.77	36.23±0.63	67.81±0.94	59.62±1.11	49.26±1.94
90	82.34±1.52	49.12±1.91	51.23±1.52	44.44±1.48	70.12±0.91	66.36±1.36	53.67±1.63
120	87.98±1.96	50.17±1.52	59.62±0.84	52.78±1.94	76.59±1.22	74.25±1.01	64.18±1.02
150	89.99±0.48	57.26±0.86	71.32±0.94	60.71±1.64	84.11±1.97	86.23±0.96	70.63±0.86

Values are expressed as mean± SD; Values are from triplicate readings; and are statistically significant at p<0.05*,p<0.01**, p<0.001***, when compared to the standard ascorbic acid.

Figure 3: Scavenging of superoxide radical activity of 2'4'-thiazolidinedione derivatives 7-flavanols by alkaline DMSO method

AA-Ascorbic acid; NF-6 nitro, 7-flavonol; DNF-4', 6-dinitro, 7-flavonol; DNF-3'4'-dinitro 7-flavonol; TNF-3'4'6-trinitro, 7-flavonol; ANF-6-acetyl, 4'-nitro, 7-flavonol; ADNF-6-acetyl, 3'4'-dintro, 7-flavonol.

The results of free radical scavenging activity of different concentrations (30-150µg/ml) of 2",4"-thiazolidinedione derivatives of 7-flavonols (Ia-i) by alkaline DMSO method were given in Table 3 and presented in Figure 3. Diformazan, a product from alkaline DMSO and NBT, is responsible for the absorbance measurement at 560nm. The maximum reducing ability at 150µg/ml were found to be for the substances of 2",4"-thiazolidinedione derivatives of 7-flavonols categories Ih (86.23%), Ig (84.11 %), Ie (71.32 %) and Ii (70.63%) and ascorbic acid 89.99% respectively. Whereas the other compounds of Id, Ie, If showed an average reducing ability in the range of 57-69 %. The IC_{50} values of Ig, Ih and ascorbic acid were 30 µg/ml. Whereas, the IC_{50} value of Ie and Ii were 60 µg/ml and those of all the other compounds were as 90 µg/ml and above. This because the hydroxyl group at C-7 is required for inhibition of xanthine oxidase and thus a strong superoxide anti-oxidant activity. It was inferred as the 6,3',4'-trinitro derivatives (h); 6-acetyl, 3',4'-dintro derivatives (i) and 3',4'-dintro derivatives (g) were more effective in scavenging action on superoxide anion than that of other derivatives (a, b, c, d and e). A dose dependent anti-oxidant effect was observed and that of the standard was greater than the test samples. Overall, the scavenging potential of 2",4"-thiazolidinedione derivatives of 7-flavonols were greater towards appreciable antioxidant activity. Comparing to all the three assays, Hydrogen peroxide method was easy and reliable for results comparing to the other methods.

Anti-Microbial Activity:

The minimum inhibitory concentration (MIC) of 2",4"-thiazolidinedione derivatives of 7-flavonols in comparison of the standard, Amikacin (10 µg/ml) against antibiotic susceptible strains of bacteria *Pseudomonas aeruginosa, Klebsiella tribatta* and *Proteus vulgaris* was determined. Amongst all the compounds of 2",4"-thiazolidinedione derivatives of 7-flavonols categories, except compound Ie (50µg/ml), all the other If, Ih and Ii showed antibacterial activity at lowest concentration, 25µg/ml. Here, *Klebsiella tribatta* organism was resistant to the tested samples comparing to the remaining organisms. These compounds brought out a remarkable inhibitory action against pathogens.

The synthesized serial of compounds of 2",4"-thiazolidinedione derivatives of 7-flavonols were all estimated for their anti-microbial effects on many pathogenic bacteria, both gram negative strains *Escherichia coli*, *Pseudomonas aerogenosa*, *Klebsiella tribatta* and *Proteus vulgaris* and gram-positive strains *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Closteridium pefrigens* and *Candida albicans.* The results, MIC levels (Table 4) and Zone of inhibition values given tables (5 and 6) and Figures (4-9) of the newly synthesized compounds against these organisms have been compared with those for the reference compounds Amikacin and Ketoconazole for evaluating antibacterial and antifungal activities respectively.

Table 4: Details of Minimum Inhibitory Concentration of 2", 4"-thiazolidinedione derivatives of 7-flavonols by Disc Diffusion Method

Name of the compounds	**Microorganisms**	**Inhibition zone (mm)**			
		</< / = 25 µg/ml	**50 µg/ml**	**100 µg/ml/**	**Amikacin 10 µg/ml**
3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,4'-dinitro, 7-flavonol **(IIIe)**	*Proteus vulgaris*	-	-	12	19
	Klebsiella tribatta	-	15	-	23
	Pseudomonas aeruginosa	-	-	6	16
3-(5''-(thiazolidine-2'',4''-dionyl)methyl)	*Proteus vulgaris*	-	-	12	19

6-acetyl, 4'- nitro, 7-flavonol **(IIIf)**	*Klebsiella tribatta*	-	-	12	23
	Pseudomonas aeruginosa	-	-	7	16
3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6,3',4'- trinitro, 7-flavonol **(IIIh)**	*Proteus vulgaris*	-	13	-	19
	Klebsiella tribatta	-	-	10	23
	Pseudomonas aeruginosa	-	-	7	16
3-(5''-(thiazolidine-2'',4''-dionyl)methyl) 6 –acetyl, 3',4'- dinitro, 7-flavonol **(IIIi)**	*Proteus vulgaris*	-	-	11	19
	Klebsiella tribatta	-	11	-	23
	Pseudomonas aeruginosa	-	11	-	16

Values represent the mean± SD; number of readings in each group = 3

Figure 4: Anti-bacterial activity of TZD derivatives of 7-flavonols against gram negative (*Eschersia Coli and Proteus vulgari*), standard at center

Figure5: Anti bacterial activity of TZD derivatives of 7-flavonols against gram negative (*Klebsiella tribatta and Pseudomonas aerogenusa*)

Table 5: Anti-bacterial activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols (1µg /10µl /disc), Amikacin (1µg /10µl /disc), against gram negative bacterial species tested by Disc Diffusion Method

Sl. No.	Name of the compounds (IIIa-i)	Zone of Inhibition (mm)			
		E. coli	*P. vulgaris*	*K. tribatta*	*P. aeruginosa*
1.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)7-flavonol **(IIIa)**	11±1.01	7±0.06	14±0.87	12±1.01
2.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-nitro, 7-flavonol **(IIIb)**	13±0.87	11±0.42	11±0.76	11±0.97
3.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-acetyl, 7-flavonol **(IIIc)**	19±1.31	16±1.44	12±0.65	9±0.87
4.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)4'-nitro, 7-flavonol **(IIId)**	12±0.63	9±0.77	11±0.94	13±0.27
5.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6,4'-dinitro,7-flavonol **(IIIe)**	13±0.84	10±0.98	14±0.91	10±0.67
6.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-acetyl,4'-nitro,7-flavonol **(IIIf)**	20±1.57	12±0.52	12±0.96	8±0.15
7.	3-(5"-(thiazolidine-2",4"-dionyl)methyl) 3'4'- dinitro,7-flavonol **(IIIg)**	16±1.18	7±0.46	8±0.09	11±0.88
8.	3-(5"-(thiazolidine-2",4"-dionyl)methyl) 63'4'- trinitro,7-flavonol **(IIIh)**	15±1.00	13±1.02	10±0.65	13±0.85
9.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-acetyl, 3'4'- dinitro,7-flavonol **(IIIi)**	18±0.33	15±0.97	17±0.41	14±1.11
10.	Amikacin	17±0.62	19±1.41	23±1.42	16±1.22

Values represent the mean± SD; number of readings in each group = 3

Figure 6: Anti-bacterial activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols (1µg /10µl /disc), Amikacin (1µg /10µl /disc) against gram negative bacterial species tested by Disc Diffusion Method

Figure 7: Anti-microbial activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols (1µg /10µl /disc), Amikacin (1µg /10µl /disc), against gram positive bacterial species tested by Disc Diffusion Method

Figure 8: Anti-bacterial activity of TZD derivatives of 7-flavonols against gram positive (*Staphylococcus aureus*) and against Candida albicans, standard at center

Table 6: Anti-microbial activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols (1µg /10µl /disc), Amikacin (1µg /10µl /disc) against gram positive bacterial species and Ketoconazole (1µg /10µ/well) against *Candida albicans* tested by Disc Diffusion Method

Sl. No	Name of the compounds	Zone of Inhibition (mm)			
		S. aureus	*S. pneumoniae*	*C. pefrigens*	*C. albicans*
1.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)7-flavonol **(IIIa)**	10±0.78	8±0.67	4±0.44	10±0.97
2.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-nitro, 7-flavonol **(IIIb)**	14±0.20	13±0.56	11±0.77	10±101
3.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-acetyl, 7-flavonol **(IIIc)**	18±1.23	17±0.24	14±0.64	14±0.22
4.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)4'-nitro, 7-flavonol **(IIId)**	14±0.66	11±0.24	9±0.72	17±1.00
5.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6,4'-dinitro,7-flavonol **(IIIe)**	18±1.63	16±0.55	15±1.12	19±1.25
6.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-acetyl,4'-nitro,7-flavonol **(IIIf)**	14±0.74	11±0.97	10±0.74	15±1.12
7.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)3'4'- dinitro,7-flavonol **(IIIg)**	20±1.46	18±1.26	15±1.25	10±1.06
8.	3-(5"-(thiazolidine-2''2",4"-dionyl)methyl)63'4'- trinitro,7-flavonol **(IIIh)**	18±1.26	16±1.11	14±0.67	20±1.36

9.	3-(5"-(thiazolidine-2",4"-dionyl)methyl)6-acetyl,3'4'-dinitro,7-flavonol**(IIIi)**	18±1.41	17±0.63	16±1.55	18±1.52
10.	Amikacin	22±1.52	20±1.62	19±1.45	-
11.	Ketoconazole	-	-	-	18±1.15

Values represent the mean± SD; number of readings in each group = 3

Figure 9: Anti-microbial activity of 2", 4"-thiazolidinedione derivatives of 7-flavonols (1µg /10µl /disc), Ketoconazole (1µg /10µ/well) against *Candida albicans* tested by Disc Diffusion Method

The results obtained from antimicrobial assay for 2',4'-thiazolidinedione derivatives of 7-flavonols are presented in Tables-(at a concentration of (1µg/10µl /disc). The synthesized compounds exerted variable inhibitory activities at a concentration of 1µg/10µl/disc with inhibition zone ranging from 7-26mm in diameter. Of all the compounds of 2'',4''-thiazolidinedione derivatives of 7-flavonols, Ie, If, Ih and Ii could exhibit the antibacterial activity only at 50µg/ml and 100µg/ml. Also, the inhibition values were comparatively lesser than the compared standard that might be owing to the reason of masked formyl group and of a derivatized thiazole moiety. Though less in inhibitory action, but made all the tested organisms so susceptible to their actions.

The tested TZD derivatives of 7-flavonols were potent antibacterial only against *Escherchia coli* and *Staphlococcus aureus* (AI >1), whereas against other strains their activity index was less (AI<1) compared to the standard, Amikacin. Compounds Ie, Ih and Ii had a wide spectrum of antibacterial actions. In this study, it was studied that the synthesized ones were confirming higher inhibition rate for gram-positive bacteria in comparison to gram negative bacteria owing to the theory of outer membrane permeability barrier (Othman). As tabulated in Table 6, the antifungal actions of 3-formyl, 7-flavonol derivatives showed a good antifungal activity against *Candida albicans* at the concentration of (1µg/10µl /disc) with inhibition of 10-24 mm. This inhibition was compared to the standard Ketoconazole (1µg/10µl/disc). Thiazolidinedione derivatives gave significant activity index value and shows equal action to that of the standard, Ketoconazole.

CONCLUSION:

Results revealed that the dinitro-, trinitro- and acetyl, dinitro derivatives of thiazolidinedione attached 7- flavonols showed better and/or equipotent activity to that of the standard, ascorbic acid when compared to the mono nitro or unsubstituted derivatives of the same. The IC_{50} value of hydrogen peroxide scavenging activity of thiazolidinedione derivatives of 7-flavonols was found to exhibit an equal IC_{50} value i.e. 30µg/ml as that of the standard, ascorbic acid and the results were found to be dose dependent. Based on the discussion above, the thiazolidinedione derivatives of 7-flavonols can be utilized as a potent candidate for prolonging the shelf-life of food products by controlling micro-organisms spoilage processes and could be very well applied in cosmetic, nutritional and pharmaceutical products.

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CONFLICTS OF INTEREST:

Nil.

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