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Original Article

ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITIES OF SYNTHETIC 3-FORMYL, 7-FLAVONOL INTERMEDIATES OBTAINED BY MICROWAVE ASSISTED TECHNIQUE

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ABSTRACT

Objective: The synthesized compounds of 3-formyl, 7-flavonols*, after characterization, aimed to be tested for their anti-oxidant and anti-microbial activities.

Methods: i) anti-oxidant activities by hydrogen peroxide-nitric oxide-and by alkaline DMSO-methods and ii) anti-microbial activities against various gram-negative and gram-positive pathogens and against *candida albicans* by disc diffusion method.

Results: Findings 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 at a concentration of (1 µg/10 µl/disc) showed variable inhibitory activities against all bacteria with inhibition zone diameters ranging from 7-26 mm and a good antifungal activity against *Candida albicans* at the concentration of (1 µg/10 µl/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 derives have significant anti-oxidant and anti-microbial activities.

*Synthesis and characterization work of 3-formyl, 7-flavonols has already been accepted for publication by the journal Elseveir, Procedio Chemistry and is in process.

Keywords: 3-formyl, 7-flavonol, Anti-microbial, Disc diffusion method, Anti-oxidant, Hydrogen peroxide method, Nitric oxide method, Alkaline DMSO method.

INTRODUCTION

Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. Free radicals are reactive species like O^{2-}, OH^{-}, ROO^{-}, HO^{2-}, RO^{-}, NO^{-}, NO_2, N_2O_3 and ONO^{2-}etc., generated in the body during normal metabolic functions [1]. The role of free radicals has been implicated in the causation of several 100 diseases [2].

Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages. Most of the reactive oxygen species are scavenged by endogenous defense system [3]. But these systems may not be completely efficient which brings in the need for exogenous anti-oxidants. Thus, the importance of search for effective antioxidants has increased in the recent years and so many researchers focus the same.

Flavonols have been reported [4] to be free radical scavengers and antioxidants. Well known examples include catechin, quercetin, myricetin, rutin and kaempferol [5]. In the light of this, it was thought worthy to evaluate antioxidant activity of the 3-formyl, 7-flavonols and their derivatives which might have great potential in ameliorating the aforementioned disease processes. There are several methods available to assess antioxidant activity of compounds. The easy, rapid and sensitive methods for screening free radical scavenging activity are Hydrogen peroxide, Nitric oxide and alkaline DMSO methods.

In recent years, a number of researchers are working seriously to find out the substitutes for antibiotics as they caused side effect on the functioning of different body organ systems [6]. Chemical modification of these phyto-components for bioassay of the anti-bacterial activity has become of utmost importance because of the growing resistance of bacteria against penicillin-and cephalosporin-like compounds and the need for medicines with more specific antibacterial activity [7].

Flavonoids, classified under phenolic groups are known to possess antimicrobial activity [8], because of their biocompatibility and biological functions and consequently, potential application in the biomedical and pharmaceutical fields [9]. Flavonols constitute a large amount of natural products. Synthesis of flavonols and their derivatives has attracted considerable attention due to their significant biocidal, pharmaceutical, anti-oxidant, anti-cancer and anti-inflammatory effects. Whereas, the mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism.

The presence of a higher number of hydroxyl groups in flavonol derivatives makes them more hydrophilic, thus causing the penetration into the cell membrane of the bacteria to be more difficult, hence resulting in them less bioactive [10]. Therefore, the present synthetic compounds were designed to have one hydroxyl group only so as to make the synthesized compounds as lipophilic for better penetration into the cell membrane of the bacteria. Literature [11] has used the disk diffusion method to determine the antimicrobial activity of essential oil and methanol extracts using several microbial strains. The present work was planned to be investigating the antimicrobial activity of various compounds of 3-formyl, 7-flavonols against various pathogenic bacteria, both gram negative species *Escherichia coli*, *Pseudomonas aerogenosa, Klebsiella tribatta* and *Proteus vulgaris* and gram positive species *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Closteridium pefrigens* and fungi *Candida albicans.*

MATERIALS AND METHODS

Procedure for the synthesis of 3-formyl, 7-flavonols (Ia-i)

Freshly distilled dimethylformamide (0.16 mol) and phosphorus oxychloride (0.14 mol) were taken in a conical flask. A solution of 7-flavonols (0.12 mol) in dichloromethane (3 ml) was added to this and the resulted yellow solution was stirred for 30 min. The viscous solution was then irradiated for one minute and the syrup was discontinuously irradiated at half a minute interval. Crushed ice was added to that to get a cherry-red aqueous solution. An aqueous base, sodium hydroxide (12.4 g) in distilled water was added to above solution with vigorous stirring. The resulting suspension was irradiated rapidly and was placed in a refrigerator overnight. Most of the inorganic material dissolved on re suspension of the crude residue in distilled water. The pure product was obtained by re-crystallization from methanol. The products (IIa–i) were confirmed by Co-TLC technique. UV spectra of compounds (20 µg/ml) were recorded using spectral grade distilled water. The infrared spectral study was done by KBr disc method (0.5–1.0 mg). All the compounds were synthesized in high yields and sufficient purity and their characterization was done by HPTLC, NMR, Mass and CHN analyses.

Antioxidant activity

In all methods, the concentrations of the test samples ranging from 30-150 µg/ml were taken with the appropriate standard, ascorbic acid and checked for the antioxidant activity in different methods. Absorbance was measured against the blank by using Schimadzu UV spectrophotometer (Model No. UV-2400 PC). Percentage scavenging and IC50 values+S. D was calculated.

Hydrogen peroxide radical scavenging assay [17]

Glucose oxidase in the presence of glucose produces hydrogen peroxide. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS), pH 7.4. The various concentrations (30-150 µg/ml) of the test samples and standard, Ascorbic acid in methanol (each 1 ml) were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm in the UV spectrophotometer (Shimadzu, UV-2450) after 10 min of incubation at 37 °C against a blank solution. The percentage of scavenging of hydrogen peroxide was calculated using the formula, % 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 radical scavenging method [18]

Sodium nitroprusside (10 mM) in phosphate buffer saline (PBS), pH 7.4. To 1 ml of different concentrations of (30-150 µg/ml) of methanolic sample solutions and the standard, ascorbic acid, 0.3 ml of sodium nitroprusside solution was added. The aliquots were incubated at 25 °C for 3 h. The same reaction mixture without the sample/standard but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1 % sulphanamide, 2 % H_3PO_4 and 1 % N-napthyl ethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed during the diazotisation of the nitrile with sulphanilamide and the subsequent coupling with naphthaylene diamine dihydrochloride was measured at 546 nm. The experiment was performed in triplicate. The percentage of scavenging of nitric oxide was calculated using the formula, % 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 [19]

The method is based on generation of superoxide radical (O^{2-}) by auto oxidation of hydroxylamine hydrochloride in the presence of NBT, which gets reduced to nitrite. Nitrite in the presence of EDTA gives a color that can be measured at 560 nm. To the reaction mixture containing 1 ml of alkaline Dimethyl sulfoxide (1 ml of dimethyl sulfoxide containing 5 mM sodium hydroxide in 0.1 ml of distilled water) and 0.3 ml of various concentrations (30-150 µg/ml) of the test samples and standard, ascorbic acid in dimethyl sulfoxide, added 0.1 ml of Nitro blue tetrazolium (1 mg/ml) to give a final volume of 1.4 ml. The absorbance was measured at 560 nm. The percentage of scavenging was calculated using the formula, 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

Growth and maintenance of test microorganism for anti-microbial studies

Gram negative Bacterial cultures *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 culture *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 obtained from American Type Culture Collection centre through Boss clinical Laboratory, Madurai, India. The bacteria were maintained on Muller Hinton Agar medium (MHA) at conditions, 37 °C and fungus was maintained on Potato Dextrose Agar (PDA) at 28 °C [20].

Preparation of inoculum

The bacterial cultures were pre-cultured in nutrient broth, overnight in a rotary shaker at 37 °C, centrifuged at 10,000 rpm for 5 min. Pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm)[38]. The fungal inoculum *Candida albicans* was prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotyometer (A_{595} nm) to obtain a final concentration o f approximately $1\ 0^5$ s p o r e s/ml [21].

Procedure for anti-bacterial activity

Minimum inhibitory concentration study by disc diffusion method [22]

Sterile Muller Hinton agar plates were prepared. The broth cultured as the plate using sterile cotton swabs. The filter paper discs 6 mm diameter (Whatmann's No.1 filter paper) were prepared and sterilized. The synthesized compounds to be tested, were prepared in distilled water and were added at different concentrations (100 µg/ml, 50 µg/ml and 25 µg/ml) to each disc of holding capacity (10 µl). The sterile impregnated disc with compounds was placed on the agar surface with flamed forceps and gently passed down to ensure complete contact of the disc with an agar surface. A loop full of the organisms previously diluted to 10^6 cfu/ml was also used to inoculate the plates which were incubated at 37 °C for 24 h. After incubation, the size (diameter) of the inhibition zones was measured.

Zone of inhibition study [23]

The discs have been prepared from Whatmann filter paper No. 1 with 4 mm diameter and sterilized at 160 ºC for 30 min. The test samples of flavonol derivatives were tested by the disc diffusion method. (100 µg/ml) concentration of the test sample was prepared by reconstituting with distilled water. The suspension of the test microorganisms 10 µl (10^6 cells/ml) was seeded into the respective medium by the spread plate method using sterilized cotton swab, with the 24 h cultures of bacteria growth in Muller Hinton agar media. After solidification, the filter paper discs were impregnated with the test samples and placed on test organism-seeded plates by sterilized forceps.

Compounds were screened against gram negative bacterial cultures and gram positive bacterial cultures. Amikacin (100 µg/ml) was used as a positive control and distilled water (100 µg/ml) used as negative control. The antibacterial assay plates were incubated at 37 °C for 24 h. The width of growth inhibition zones was measured with transparent ruler in millimeter and rounded off to the nearest whole numbers (mm) for analysis. These studies were performed in triplicate.

Procedure for antifungal activity

The antifungal activity was tested by antibacterial disc diffusion method. The potato dextrose agar plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were

allowed to solidify for 5 minutes and 0.1 % inoculum suspension of fungal culture (10 days old) was swabbed uniformly by point inoculation and the inoculum was allowed to dry for 5 min. The filter paper discs (4 mm in diameter) impregnated with 100 µg/ml concentrations of the test samples were placed on test organism-seeded plates. Blank disc impregnated with distilled water followed by drying off was used as negative control and Ketoconazole (100 µg/ml) used as positive control. The activity was determined after 72 h of incubation at 28 °C. The diameters of the inhibition zones were measured with the transparent ruler in millimeter and rounded off to the nearest whole numbers (mm) for analysis. These studies were performed in triplicate (table. 6).

Zones of inhibition, inhibited by the reference and test compounds were measured and relative activities were calculated as Activity index. The zone of inhibition is the diameter of the area in which microorganisms have been destroyed.

$$\text{Activity index (A. I)} = \frac{\text{Zone of inhibition exhibited by test compounds}}{\text{Zone of inhibition exhibited by reference compound}}$$

Statistical analysis

The data were subjected to the analysis of variance (one way ANOVA) to determine the significance of changes, Dunnett's multiple comparisons were made to analyze the significance of difference within the experimental groups between control and standard/test samples, P values of 0.05 or less were taken as significant. Results were expressed as mean S. E. M. Software: GraphPad Prism 5.01.

RESULTS

Anti-oxidant activity

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Andlauer). Phenolic compounds and flavanoids are major compounds of anti-oxidant property. Flavonols inhibit the ability of the free radicals to trigger negative charges within the body chemistry. It is well known that superoxide anions damage bio macromolecules directly or indirectly by forming H_2O_2, OH^-, peroxyl nitrite or singlet oxygen during pathophysiologic events. It can cause oxidation or reduction of solutes depending on their reduction potential [12].

Hydrogen peroxide method

Glucose oxidase injection in the paw leads to local generation of hydroxyl radical, OH* (unstable and more reactive) from hydrogen peroxide (H_2O_2), which in turn induce severe inflammatory changes. 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 lipid bi-layer, osmotic fragility, and aggregation of membrane protein and decrease activity of membrane bound enzymes.

Table 1: Anti-oxidant activity of 3-formyl, 7-flavonols by Hydrogen peroxide radical scavenging assay

Conc. (µg/ml)	Percentage of radical scavenging activity						
	Standard Ascorbic acid	Compound Id	Compound Ie	Compound If	Compound Ig	Compound Ih	Compound Ii
30	84.51+1.01	13.62+2.09	19.54+2.09	17.33+2.09	43.21+1.62	40.23+1.65	21.21+2.09
60	85.69+2.01	22.22+1.02	30.25+1.74	28.88+1.29	61.32+1.21	60.21+1.27	39.64+1.22
90	87.98+1.42	33.21+1.66	42.15+1.29	39.62+1.60	72.55+2.00	75.69+1.78	52.84+1.11
120	90.03+1.95	46.74+1.00	61.38+1.63	50.23+1.77	81.11+0.52	83.65+1.73	66.24+1.74
150	92.12+0.84	60.25+0.21	72.31+0.12	68.69+1.02	89.10+0.94	91.23+0.32	71.23+0.94

Id-3-formyl, 4'-nitro, 7 flavonol; Ie-4'6-dinitro, 3-formyl, 7 flavonol; If-6-acetyl, 3-formyl, 4'-nitro, 7 flavonol; Ig-3-formyl, 3'4'-dinitro, 7 flavonol; Ih-3-formyl, 3'4'6-trinitro, 7 flavonol; Ii-6-acetyl, 3-formyl, 3'4'-dinitro, 7 flavonol, 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.

The results of free radical scavenging activity of different concentrations (30-150 µg/ml) of 3-formyl, 7-flavonols (Ia-i) by hydrogen peroxide method were given in Table-1 and presented in Figure-1a. 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 IC_{50} values of the compounds Ih, Ig and ascorbic acid were found to be 60 µg/ml and 30 µg/ml respectively.

The maximum reducing ability at 150 µg/ml for the compounds of 3-formyl, 7-flavonols Ih (91.23 %), Ig (89.10 %), Ie (72.31 %) and ascorbic acid were found to be 90.82 %, 92.12 % and 91.65 % respectively. These compounds may thus act through two different processes: inhibition of the xanthine oxidase enzyme and ROS scavenging, as their antioxidant properties are challenging the standard, ascorbic acid's activity.

Nitric oxide free radical scavenging method

Nitric oxide radical scavenging procedure is based on the sodium nitro prusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrate ions that can be estimated by using Gries's reagent [13]. The results of free radical scavenging activity of different concentrations (30-150 µg/ml) of 3-formyl, 7-flavonols (**Ia-i**) by Nitric oxide method were given in Table-2 and presented in Figure-1b. In presence of antioxidants, nitric oxide is a very unstable species under aerobic condition. It reacts with O_2 to produce its stable product nitrate and nitrite through intermediates NO_2, $N_2O_4$and N_3O_4. It was estimated by using the Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid would decrease and could be measured at 546 nm.

Table 2: Anti-oxidant activity of 3-formyl, 7-flavonols by Nitric oxide radical scavenging assay

Conc. (µg/ml)	Percentage of radical scavenging activity						
	Standard Ascorbic acid	Compound Id	Compound Ie	Compound If	Compound Ig	Compound Ih	Compound Ii
30	42.35+1.62	11.26+2.01	28.72+1.99	12.25+1.22	22.36+2.11	44.44+1.45	24.25+1.22
60	53.23+1.12	20.36+1.66	31.77+1.25	22.36+1.44	31.98+1.74	54.66+1.76	32.32+1.77
90	61.21+1.11	25.62+1.19	39.55+1.13	29.54+1.66	41.21+1.00	54.66+1.76	48.55+0.88
120	65.36+1.00	26.35+1.77	39.69+1.66	33.62+1.22	54.44+1.66	64.58+0.99	54.64+1.09
150	72.55+0.11	30.23+0.11	48.25+0.12	41.15+0.52	61.25+0.44	67.25+0.52	59.62+0.52

Id-3-formyl, 4'-nitro, 7 flavonol; Ie-4'6-dinitro, 3-formyl, 7 flavonol; If-6-acetyl, 3-formyl, 4'-nitro, 7 flavonol; Ig-3-formyl, 3'4'-dinitro, 7 flavonol; Ih-3-formyl, 3'4'6-trinitro, 7 flavonol; Ii-6-acetyl, 3-formyl, 3'4'-dinitro, 7 flavonol, 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.

The maximum reducing ability at 150 µg/ml for the compounds of 3-formyl, 7-flavonols Ih (67.25 %), Ig (61.25 %), Ii (59.62 %) and ascorbic acid were found to be 79.90 %, 72.55 % and 72.58 % respectively. The IC_{50} values of the compounds Ih and ascorbic acid were found to be 90 µg/ml and 60 µg/ml respectively.

Free radical scavenging activity by alkaline DMSO method

The results of free radical scavenging activity of different concentrations (30-150 µg/ml) of 3-formyl, 7-flavonols (Ia-i) by alkaline DMSO method were given in Table-3 and presented in fig. 1c. Alkaline DMSO, used as a superoxide generating system reacts with NBT to give colored diformazan. In the presence of scavenger the reduction of NBT can be measured at 560 nm. The maximum reducing ability at 150 µg/ml for the compounds of 3-formyl, 7-flavonols Ig (86.23 %), Ih (83.33 %), Ii (78.22 %) and Ie (74.22 %) and ascorbic acid were found to be 88.34 %, 84.26 % and 89.99 % respectively. The IC_{50} values of the compounds Ig, Ig and ascorbic acid were found to be 30 µg/ml. The reason is that the presence of a hydroxyl group at C-7 appeared to be necessary for xanthine oxidase inhibition and thereby showing a strong superoxide radical scavenging action.

Flavonols have the proton donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The results were found to be dose dependent i.e. higher the concentration, more was the scavenging activity. 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 un-substituted derivatives of the same. The IC_{50} value of hydrogen peroxide scavenging activity of the synthesized compounds was found to be 60 µg/ml which was higher than that of the standard, ascorbic acid (30 µg/ml). A dose dependent radical scavenging activity was observed. Based on the above results indicated, 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.

Table 3: Scavenging of superoxide radical activity of the synthesized 3-formyl, 7-flavonols by alkaline DMSO method

Conc. (µg/ml)	Percentage of radical scavenging activity						
	Standard Ascorbic acid	Compound Id	Compound Ie	Compound If	Compound Ig	Compound Ih	Compound Ii
30	71.32+1.54	38.65+2.15	44.23+1.45	26.31+1.52	50.23+1.55	45.66+1.64	41.25+0.63
60	77.77+1.41	41.42+1.26	59.66+1.11	32.64+0.36	66.11+1.86	50.00+1.26	51.24+1.25
90	80.23+1.06	48.51+1.74	66.35+1.22	46.32+1.74	72.36+1.88	61.23+1.78	56.23+1.55
120	81.26+1.74	50.31+1.25	70.33+0.66	52.36+0.24	81.23+1.72	74.24+1.00	69.33+1.63
150	84.26+0.24	58.24+0.64	74.22+0.22	61.23+0.20	86.23+1.25	83.33+0.77	78.22+1.52

Id-3-formyl, 4'-nitro, 7 flavonol; Ie-4'6-dinitro, 3-formyl, 7 flavonol; If-6-acetyl, 3-formyl, 4'-nitro, 7 flavonol; Ig-3-formyl, 3'4'-dinitro, 7 flavonol; Ih-3-formyl, 3'4'6-trinitro, 7 flavonol; Ii-6-acetyl, 3-formyl, 3'4'-dinitro, 7 flavonol, 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.

Derivatives (**i**) and other mono substituted derivatives. The scavenging activity of ascorbic acid was greater than that of the test samples. 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 3-formyl, 7-flavonol in comparison to the standard, Amikacin (10 µg/ml) against antibiotic susceptible strains of bacteria *Pseudomonas aerogenosa, Klebsiella tribatta* and *Proteus vulgaris* were determined. The MIC levels of the newly synthesized compounds against these organisms were given in table-4.

Amongst all the compounds of 3-formyl, 7-flavonols except compound Ie (50 µg/ml), all the other If, Ih and Ii showed antibacterial activity at the lowest concentration, 25 µg/ml. Here, *Klebsiella tribatta* organism was resistant to the tested samples comparing to the remaining organisms. These formylated 7-flavonols brought out a remarkable inhibitory action against pathogens, the inhibition diameter in mm for test samples have been increased greatly when compared to that of standard, Amikacin.

Table 4: Details of minimum inhibitory concentration of 3-formyl, 7-flavonols by disc diffusion method

Name of the compounds	Microorganisms	Inhibition zone (mm)			
		</</= 25 µg/ml ml	50 mcg /ml	100 µg/ml mcg/ml	Amikacin 10 µg/ml
Compound Ie	*Proteus vulgaris is*	-	20	-	19 9
	Klebsiella tribatta	-	26	-	23
	Pseudomonas aurogenosa	-	-	-	16
Compound If	*Proteus vulgaris*	-	-	-	19
	Klebsiella tribatta	-	12	-	23
	Pseudomonas aurogenosa	23	-	-	16
Compound Ih	*Proteus vulgaris*	22	-	-	19
	Klebsiella tribatta	-	25	-	23
	Pseudomonas aurogenosa	18	-	-	16
Compound Ii	*Proteus vulgaris*	18	-	-	19
	Klebsiella tribatta	17	-	-	23
	Pseudomonas aurogenosa	20	-	-	16

3-formyl, 4',6-dinitro, 7-flavonol (Ie); 3-formyl, 4'-nitro,6-acetyl, 7-flavonol (If); 3-formyl, 3'4'6,-trinitro, 7-flavonol (Ih); 3-formyl, 3'4'-dinitro, 6-acetyl, 7-flavonol (Ii)

The synthesized serial of compounds of 3-formyl, 7-flavonol derivatives were all screened for their anti microbial activities against various pathogenic bacteria, both gram negative species *Escherichia coli*, *Pseudomonas aerogenosa, Klebsiella tribatta* and *Proteus vulgaris* and gram positive species *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Closteridium pefrigens* and fungi *Candida albicans.* The results have been compared with those for the reference compounds Amikacin and Ketoconazole for evaluating antibacterial and antifungal activities respectively.

Table 5: Anti-bacterial activity of 3-formyl, 7-flavonols (1 µg/10 µl/disc), Amikacin (1 µg/10 µl/disc), against gram negative bacterial species tested by disc diffusion method

S. No.	Name of the compounds (Ia-i)	Zone of inhibition (mm)			
		E. coli	*P. vulgaris*	*K. tribatta*	*P. aurogenosa*
1.	3-formyl, 7-flavonol (Ia)	20+1.54	1+0.13	8+0.12	6+0.05
2.	3-formyl, 6-nitro, 7-flavonol (Ib)	16+0.33	2+0.05	2+0.00	7+0.07
3.	6-acetyl, 3-formyl, 7-flavonol (Ic)	21+0.88	1+0.63	7+0.22	7+0.41
4.	3-formyl, 4'-nitro, 7-flavonol (Id)	19+0.99	14+1.00	26+1.33	13+1.52
5.	3-formyl, 4',6-dinitro, 7-flavonol (Ie)	18+1.11	20+0.66	20+1.54	17+1.89
6.	3-formyl, 4'-nitro,6-acetyl7-flavonol (If)	15+1.10	17+1.33	12+1.00	23+1.63
7.	3-formyl, 3'4',-dintro, 7-flavonol (Ig)	17+0.87	13+0.87	17+1.23	13+0.84
8.	3-formyl, 3'4'6,-trinitro, 7-flavonol (Ih)	17+0.85	22+1.22	25+2.01	18+1.77
9.	3-formyl,3'4'-dinitro,6-acetyl, 7-flavonol (Ii)	18+1.60	18+1.65	17+1.22	20+0.54
10.	Amikacin	17+1.21	19+0.99	23+1.77	16+0.98

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

The results obtained from antimicrobial assay for 3-formyl, 7-flavonols were presented in Tables-(5 & 6) at a concentration of (1 µg/10 µl/disc). The synthesized compounds showed variable inhibitory activities against all bacteria with inhibition zone diameters ranging from 7-26 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. In this study, it was shown that all the synthesized compounds could give higher inhibition to gram-positive bacteria compared to gram negative bacteria. These results are in agreement [14] that Gram negative microorganisms are typically more resistant to antimicrobial agents than gram positive bacteria. This has long been explained by the presence of an outer membrane permeability barrier in gram negative bacteria, which limits access of the antimicrobial agents to their targets in the bacterial cells.

Table 6: Anti-microbial activity of 3-formyl, 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

S. No.	Name of the compounds (Ia-i)	Zone of inhibition (mm)			
		S. aureus	*S. pneumoniae*	*C. pefrigens*	*Candida albicans*
1.	3-formyl, 7-flavonol (Ia)	8+0.11	6+0.01	1+0.00	19+0.31
2.	3-formyl, 6-nitro, 7-flavonol (Ib)	11+0.44	7+0.52	7+0.17	14+0.11
3.	6-acetyl, 3-formyl, 7-flavonol (Ic)	18+0.19	14+0.41	12+0.12	23+0.52
4.	3-formyl, 4'-nitro, 7-flavonol (Id)	20+1.09	18+1.00	13+0.99	20+1.66
5.	3-formyl, 4',6-dinitro, 7-flavonol (Ie)	18+1.77	15+0.56	11+0.22	17+1.00
6.	3-formyl, 4'-nitro,6-acetyl7-flavonol (If)	14+1.11	13+1.19	10+1.13	14+0.98
7.	3-formyl, 3'4',-dintro, 7-flavonol (Ig)	18+0.99	14+0.77	11+0.88	23+1.76
8.	3-formyl, 3'4'6,-trinitro, 7-flavonol (Ih)	20+1.74	17+1.44	16+1.25	22+1.66
9.	3-formyl, 3'4'-dinitro, 6-acetyl, 7-F (Ii)	22+1.45	20+1.22	17+1.12	23+1.22
10.	Amikacin	22+1.62	20+2.01	19+1.99	-
11.	Ketoconazole	-	-	-	18+2.11

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

DISCUSSION

Based on the results summarized, the antifungal activity 3-formyl, 7-flavonol derivatives showed a good antifungal activity against *Candida albicans* at the concentration of (1 µg/10 µl/disc) with inhibition of 10-24 mm. This inhibition was compared to the standard Ketoconazole (1 µg/10 µl/disc). Formulated 7-flavonols showed a very good antifungal, almost all compounds, here gave Activity index value greater than 1 when compared to the standard, Ketoconazole.

The results showed that the synthesized 7-flavonols and their derivatives inhibit the growth rate of bacteria and fungi. The results of the present study are quite encouraging as almost all of the synthesized 7-flavonols and their derivatives exhibited antimicrobial activity against most of the pathogens.

The well-known antioxidant and antiradical flavones are flavon-3-ols: morin, quercetin, myricetin etc., Though the role of these substituents in antioxidant activities has been debated [15], the present work has demonstrated that flavones devoided of this C-3 hydroxyl group can also be efficient radical scavengers and antioxidants *in vitro* [16]. The presence of a hydroxyl group in the C-7 position seems to be a common feature favoring this property. Nevertheless, the significance of these results on isolated enzymes must be verified *in vivo* to establish whether these new synthetic compounds are better candidates for anti-oxidant interventions than other well known flavonoids.

Since our synthesized 7-flavonols too have a maximum structural similarity to that of quercetin and kaempferol, the antimicrobial activity of the same might be due to one of the mechanisms of action of inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolism. In the light of these results, the newly synthesized 3-formyl, 7-flavonol derivatives were verified against a broad spectrum of food-borne pathogens in the present study. The study confirmed that 3-formyl, 7-flavonols are potent

antimicrobial agents. Compounds were relatively more effective towards Gram-positive bacteria than Gram-negative bacteria, which could be the reason of their differences in cell membrane constituents and structures. In some cases, antimicrobial activities of the synthesized compounds were found to be enhanced as compared to the standard drug, Amikacin and Ketoconazole.

CONCLUSION

The present work has been carried out with the hope of adding new and potent chemotherapeutic agents to the arsenal of weapons used against resistant organisms as well as other highly infectious lethal diseases. Based on the discussion above, the 3-formyl, 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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CONFLICT OF INTERESTS

The authors declare no conflict of interests

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