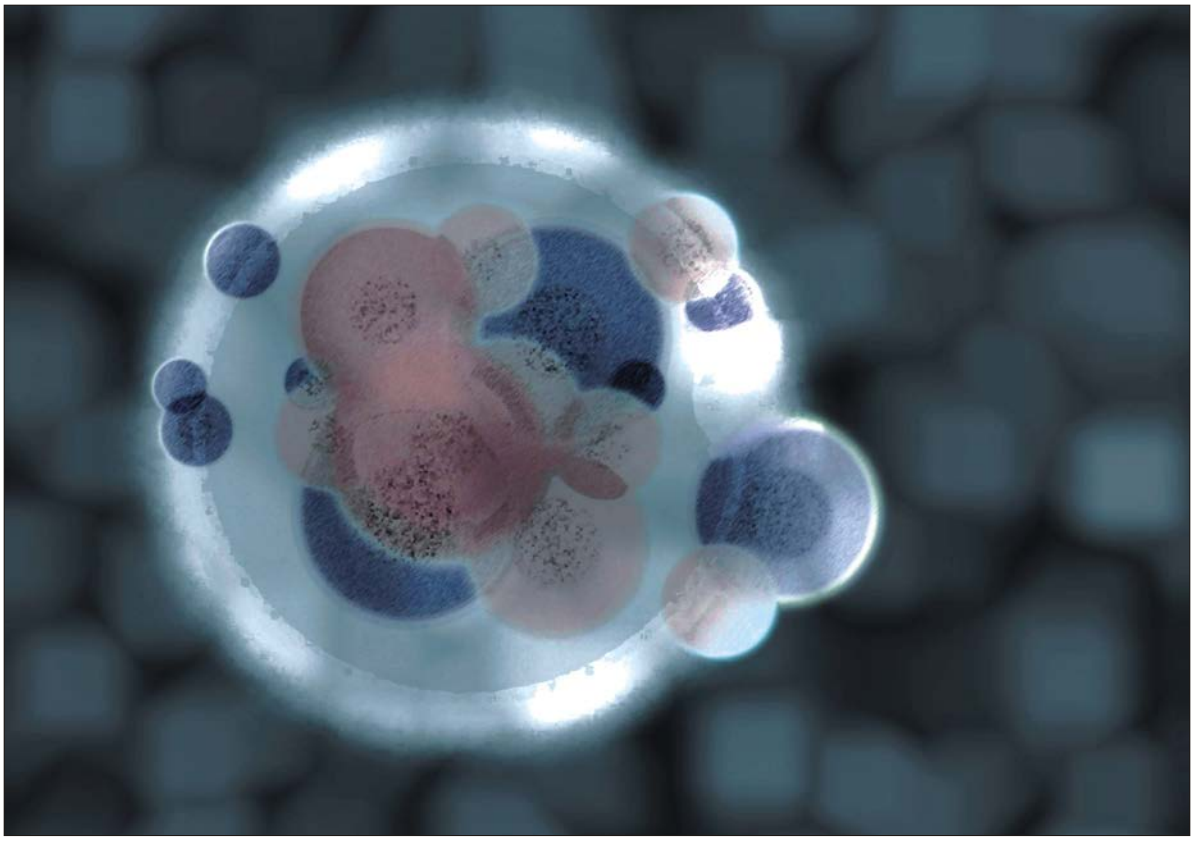

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Synthesis of Two Vanillin Ring Containing Flavones by Different Methods and Studies of Their Antibacterial and Antifungal Activities

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Abstract: Two 2′-oxygenated flavones have been synthesized from vanillin via chalcone precursor and their antibacterial and antifungal activity were investigated along with their corresponding chalcones against three human pathogenic bacterial and five molds fungal strains. Compounds 6 and 7 show good antibacterial and antifungal activity against the selected organisms. The structure of the synthetic compounds have been characterized using UV-Vis, IR, ^{1}H and ^{13}C NMR spectral data together with elemental analysis.

Key words: Flavone, antibacterial activity, antifungal activity, inhibition zone

INTRODUCTION

Vanillin ring containing flavonoid compounds are widely occurring in natural plant pigments (Martinez-Florez *et al*., 2002) and medicinal plants (Rao *et al*., 2002) as well. The flavonoid compounds are a group of natural products founds in fruits, vegetables, nuts, seeds and flowers as well as in teas and wines and are important constituent of human diet. They have been demonstrated to posses many biological and pharmacological activities such as antibacterial, antifungal, antiviral, antioxidant, antiinflanımatory, antimutagenic and antiallergic activities on several enzymes (Vender Berghe *et al*., 1993; Boss *et al.,* 1990). This study reports the synthesis of two flavones 6 and 7 from their corresponding chalcones 4 and 5 by using differently DMSO/I_2 and DDQ as an oxidizing agent. The synthesized flavones and their corresponding chalcones were screened *in vitro* for their antibacterial and antifungal activity against three human pathogenic bacteria, viz., *Proteus valguris* (G^+), *Sarcina lutea* (G^+) and *Escherichia coli* (G^-) and five plants as well as molds fungi, viz., *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp, *Biopolarise* sp. and *Tricoderma* sp.

MATERIALS AND METHODS

Melting points were recorded on Gallenkanıp apparatus and are uncorrected. IR spectra (KBr) were measured using a Shimadzu DR-8001 spectrophotometer. ^{1}H NMR spectra were recorded on a Brucker WH 250 MHZ and ^{13}C NMR spectra on a Brucker WH 62 MHZ instrument with TMS as an internal standard. UV-Vis spectra were recorded on a LKB 4053 spectrophotometer using MeOH as solvent. Column chromatography was packed with silica gel (Kiesel gel 60, 70-230 mesh, Merck). Completion of the reaction, purity and homogeneity of the compounds was checked by TLC. TLC was performed with silica gel GF_{254} (Fluka) and the spots were developed by spraying 2% $FeCl_3$ solution and 1% H_2SO_4 and heating the plates at 100°C until colouration took place.

Synthesis of (E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-one (2′,4 -dihydroxy-4′,6′,3-trimethoxychalcone, 4): A mixture of 2-hydroxy-4,6-dimethoxyacetophenone (Nagaranjan and Parmar, 1978) (1, 0.98 g, 5 m mole,) and 3-methoxy-4-hydroxylbenzaldehyde (2, 1.1 eq., 0.84 g, 5 m mole) in ethanolic solution of KOH (5%, 15 mL) was kept at room temperature for about 72 h. The reaction mixture was diluted with ice-cold water, acidified with cold dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and the solvent evaporated. The reaction mixture was purified by preparative TLC over silica gel GF_{254} using n-hexane-acetone (8:1) as developing solvent and the compound purified by recrystallized from petroleum spirit as brown crystals (1.12 g, 62%) with m.p. 44-46°C, R_f 0.72 (benzene: acetone; 5:1).

Anal. found: C, 66.98; H, 5.68; $C_{18}H_{18}O_6$ requires C, 67.24; H, 5.58%.

UV-Vis (nm): 252 and 322.

IR (KBr): 3421, 2974, 2924, 2345, 2291, 1620, 1594, 1508, 1461, 1423, 1373, 1342, 1257, 1218, 1157, 1115, 1045, 991, 817 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ 3.75 (3H, s, C_3-OCH_3), 3.79 (3H, s, $C_{6'}$-OCH_3), 3.84 (3H, s, $C_{4'}$-OCH_3), 6.38 (1H, d, J = 2.6 Hz.

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$C_{3'}$-H), 6.53 (1H, s, C_4-OH), 6.61 (1H, d, J = 2.6 Hz, $C_{5'}$-H), 6.76 (1H, d, J = 8.6 Hz, C_5-H), 7.03 (1H, s, C_2-H), 7.21 (1H, d, J = 8.6 Hz, C_6-H), 7.41 (1H, d, J = 16 Hz, C_α-H), 8.06 (1H, d, J = 16 Hz, C_β-H), 12.18 (1H, s, $C_{2'}$-OH).

^{13}C NMR (DMSO-d_6): δ 122.4 (C-1′), 159.5 (C-2′), 99.6 (C-3′), 165.2 (C-4′), 102.6 (C-5′), 154.6 (C-6′), 168.4 (>C = O), 126.9 (C-α), 147.5 (C-β), 128.5 (C-1), 116.7 (C-2), 144.8 (C-3), 147.4 (C-4), 112.6 (C-5), 124.6 (C-6), 59.7 ($C_{6'}$-OCH_3), 56.0 ($C_{4'}$-OCH_3), 54.9 ($C_{3'}$-OCH_3).

Synthesis of 3,8-dimethoxy-5-(4-hydroxy-3-methoxyphenyl)-benzo(b)pyran-7-one (4′-hydroxy-3′,5,7-trimethoxyflavone, 6) using DMSO/I_2: Cyclization of the chalcone 4 into the corresponding flavone 6 using DMSO/I_2 reagent was carried out (Doshi *et al*., 1986). The chalcone 4 (700 mg, 2 m mole) was refluxed with catalytic amount of iodine in dimethyl sulphoxide (Doshi *et al*., 1986) (DMSO, 10 m L) for 15 min. The flavone was purified by preparative TLC over silica gel GF_{254} using petroleum spirit-acetone (6:1) as developing solvent and the compound purified by recrystallization from ether as colourless needles (536 mg, 77%), mp 38-40°C, R_f 0.46 (petroleum spirit-acetone; 5:3). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. found: C, 72.71; H, 5.59 ; $C_{18}H_{16}O_6$ requires C, 72.96; H, 5.44%.

UV-Vis (nm): 260 and 333.

IR (KBr): 2314, 1647, 1604, 1512, 1469, 1384, 1311, 1288, 1157, 1053, 956, 906, 821, 771, 717 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ 3.81 (3H, s, $C_{3'}$-OCH_3), 3.86 (3H, s, C_7-OCH_3), 3.89 (3H, s, C_5-OCH_3), 6.48 (1H, s, C_6-H), 6.53 (1H, s, C_8-H), 6.56 (1H, s, C_3-H), 6.62 (1H, s, $C_{4'}$-OH), 6.79 (1H, d, J = 2.6 Hz, $C_{2'}$-H), 6.98 (1H, d, J = 8.6 Hz, C_5-H), 7.21 (1H, d, J = 8.6 Hz, $C_{6'}$-H).

^{13}C NMR (DMSO-d_6): δ 154.1 (C-2), 118.6 (C-3), 173.9 (C-4), 108.4 (C-4a), 162.3 (C-5), 94.9 (C-6), 161.5 (C-7), 90.2 (C-8), 163.4 (C-8a), 126.0 (C-1′), 115.8 (C-2′), 139.7 (C-3′), 145.1 (C-4′), 122.4 (C-5′), 124.9 (C-6′), 57.9 (C_5-OCH_3), 55.3 (C_7-OCH_3), 54.9 ($C_{3'}$-OCH_3).

Synthesis of 3,8-dimethoxy-5-(4-hydroxy-3-methoxyphenyl)-benzo(b)pyran-7-one 4-hydroxy-3′,5,7-trimethoxyflavone, 6) using DDQ: The chalcone 4 (350 mg, 1 m mole) was suspended in dry dioxane (50 m L) and then added to it DDQ (1 m mol). The mixture was refluxed for 3 h. The product was then purified by preparative TLC over silica gel GF_{254} using petroleum spirit-benzene (1:2) as developing solvent and the compound purified by recrystallization from ethanol as colourless needles (263 mg, 75%), mp 38-40°C, R_f 0.46 (petroleum spirit-acetone; 5:3). It gave blue fluorescence in UV light and positive Mg/HCl test. Spectral data of this flavone 6 was also similar to that prepared by DMSO/I_2 method.

Synthesis of (E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (2′,3 -dihydroxy-4′,6′,4-trimethoxychalcone, 5): The chalcone 5 was prepared by previously described method and purified by preparative TLC over silica gel GF_{254} using n-hexane-acetone (10:1) as developing solvent and the compound purified by recrystallized from petroleum spirit as orange needles (1.44 g, 79 %) with m.p. 164-166°C, R_f 0.81 (benzene: acetone; 5:1).

Anal. found: C, 68.47; H, 5.89; $C_{18}H_{18}O_6$ requires C, 68.78; H, 5.77%.

UV-Vis (nm): 271 and 328.

IR (KBr): 3340, 3045, 2980, 2345, 1658, 1624, 1572, 1515, 1475, 1410, 1355, 1290, 1205, 1157, 1065, 978, 920, 860, 808, 749 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ 3.68 (3H, s, C_4-OCH_3), 3.74 (3H, s, $C_{6'}$-OCH_3), 3.79 (3H, s, $C_{4'}$-OCH_3), 6.46 (1H, d, J = 2.6 Hz. $C_{3'}$-H), 6.54 (1H, s, C_3-OH), 6.59 (1H, d, J = 2.6 Hz, $C_{5'}$-H), 6.83 (1H, d, J = 2.6 Hz, C_5-H), 7.12 (1H, d, J = 2.6 Hz, C_2-H), 7.23 (1H, d, J = 8.6 Hz, C_6-H), 7.33 (1H, d, J = 16 Hz, C_α-H), 7.98 (1H, d, J = 16 Hz, C_β-H), 12.78 (1H, s, $C_{2'}$-OH).

^{13}C NMR (DMSO-d_6): δ 119.9 (C-1′), 156.1 (C-2′), 99.6 (C-3′), 162.4 (C-4′), 112.7 (C-5′), 150.6 (C-6′), 164.8 (>C = O), 128.2 (C-α), 152.4 (C-β), 132.5 (C-1), 111.3 (C-2), 138.9 (C-3), 144.8 (C-4), 117.4 (C-5), 121.7 (C-6), 58.8 ($C_{6'}$-OCH_3), 56.9 ($C_{4'}$-OCH_3), 55.1 (C_4-OCH_3).

Synthesis of 3,8-dimethoxy-5-(3-hydroxy-4-methoxyphenyl)-benzo(b)pyran-7-one (3′-hydroxy-4′,5,7-trimethoxyflavone, 7) using DMSO/I_2: The flavone 7 was prepared by using DMSO/I_2 reagent as previously described method and it was purified by preparative TLC over silica gel GF_{254} using benzene-acetone (5:1) as developing solvent and the compound purified as semi-solid mass (198 mg, 57%), R_f 0.48 (benzene: ethyl acetate; 4:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. found: C, 58.13; H, 5.57; $C_{18}H_{16}O_6$ requires C, 58.48; H, 5.42%.

UV-Vis (nm): 260 and 335.

IR (KBr): 1640, 1603, 1584, 1458, 1374, 1335, 1254, 1170, 1147, 1112, 1083, 1035, 977, 922, 880, 817, 765, 745 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.86 (3H, s, $C_{4'}$-OCH_3), 3.88 (3H, s, C_7-OCH_3), 3.93 (3H, s, C_5-OCH_3), 6.34 (1H, s, C_6-H), 6.48 (1H, s, C_8-H), 6.55 (1H, s, C_3-H), 6.67 (1H, s, $C_{3'}$-OH), 6.83 (1H, d, J = 2.6 Hz, C_2-H), 6.94 (1H, d, J = 8.6 Hz, $C_{5'}$-H), 7.28 (1H, d, J = 8.6 Hz, $C_{6'}$-H).

^{13}C NMR (DMSO-d_6): δ 157.6 (C-2), 120.9 (C-3), 168.6 (C-4), 114.2 (C-4a), 161.8 (C-5), 98.3 (C-6), 166.7 (C-7), 96.5 (C-8), 160.8 (C-8a), 129.2 (C-1′), 112.5 (C-2′), 134.9 (C-3′), 143.6 (C-4′), 119.1 (C-5′), 124.6 (C-6′), 59.7 (C_5-OCH_3), 57.2 (C_7-OCH_3), 55.5 ($C_{4'}$-OCH_3).

Synthesis of 3,8-dimethoxy-5-(3-hydroxy-4-methoxyphenyl)-benzo(b)pyran-7-one (3′-hydroxy-4′,5,7-trimethoxyflavone, 7) using DDQ: The flavone 7 was prepared by using DDQ as previously described method and it was purified by preparative TLC over silica gel GF_{254} using petroleum spirit-benzene (1:2) as developing solvent and the compound purified as semi-solid mass (218 mg, 62%), R_f 0.48 (benzene: ethyl acetate; 4:1). It gave blue fluorescence in UV light and positive Mg/HCl test. Spectral data of this flavone 7 was also similar to that prepared by DMSO/I_2 method.

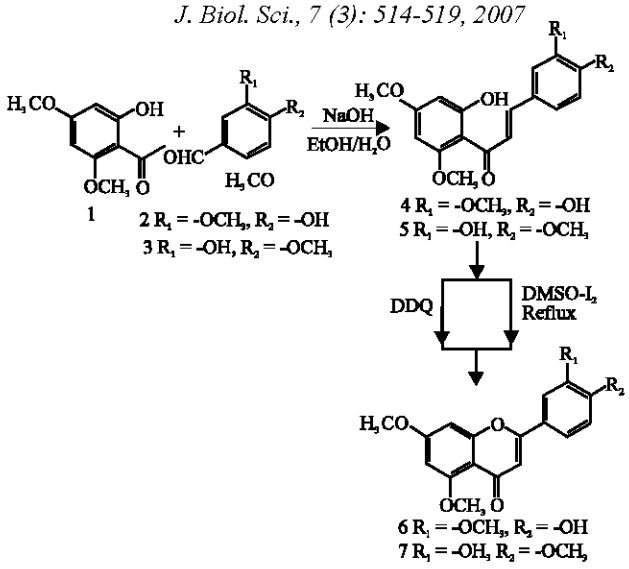
Antibacterial screening tests: The antibacterial activity of the synthesized compounds 4, 5, 6 and 7 were studied against three human pathogenic bacteria, viz., *Proteus valguris* (G^+), *Sarcina lutea* (G^+) and *Escherichia coli* (G^-). For detection of antibacterial activity the filter paper disc diffusion method (Arima *et al*., 2002; Jeongmok *et al*., 1995) was employed. Ciprofloxacin was used as standard antibiotic for the antibacterial test. Nutrient Agar (NA) was used as the basal medium for test bacteria. These agar media were inoculated with 0.5 mL^{-1} of the 24 h liquid cultures containing 10^7 microorganisms mL^{-1}. The diffusion time was 24 h at 5°C and the incubation time was 12 h at 37°C for bacteria. Discs with only DMSO were used as control. The diameter (in mm) of the observed inhibition zones were taken as a measure of inhibitory activity.

Determination of the Minimum Inhibitory Concentration (MIC): A current definition of the minimum inhibitory concentration, MIC, is the lowest concentration which resulted in maintenance or reduction of inoculum viability (Colle *et al*., 1989). The determination of the MIC involves a semi quantitative test procedure, which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. The method displays tubes of growth broth containing a test level of preservatives into which an inoculum of microbes was added. The end result of the test was the minimum concentration of antimicrobial (test materials), which gave a clear solution, i.e., no visual growth (Collins, 1964). Serial dilution technique (Nishina *et al*., 1987) was applied for the determination of minimum inhibitory concentration of the compounds against the four tested bacteria, viz., *Proteus valguris*, *Sarcina lutea* and *Escherichia coli*. The media used in this respect were nutrient broth (DIFCO). Dilution series were setup with 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 μg mL^{-1} of nutrient broth medium. To each tube 100 μL of standardized suspension of the testing bacteria (10^7 cell mL^{-1}) were added and incubated at 30°C for 24 h.

Antifungal screening tests: The antifungal activity of compounds 4, 5, 6 and 7 were evaluated towards five plants pathogenic and mold fungi, viz., *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Biopolarise* sp. and *Tricoderma* sp. The antifungal activity was assessed by poisoned food technique (Grover and Moore, 1962) with some modification (Miah *et al*., 1990). Nystatin (50 μg $disc^{-1}$) was used as standard fungicide for the antifungal test. Potato Dextrose Agar (PDA) was used as basal medium for test fungi. Glass petri dishes were sterilized and 15 mL of sterilized melted PDA medium (~45°C) was poured into each petri dish (90 mm). After solidification of the medium small portions of mycelium of each fungus were spread carefully over the center of each PDA plate with the help of sterilized needles. Thus, each fungus was transferred to a number of PDA plates. The PDA plates were then incubated at 25±2°C and after five days of incubation they were ready for use. The prepared discs of test samples were placed gently on the solidified agar plates, freshly seeded with the test organisms with sterile forceps. Control discs were also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvents, respectively. The plates were then kept in a refrigerator at 4°C for 24 h in order that the materials had sufficient time to diffuse to a considerable area of the plates. Afterwards the plates were incubated at 37.5°C for 72 h. Dimethyl sulphoxide (DMSO) was used as a solvent to prepare desired solution (10 mg mL^{-1}) of the compounds initially.

RESULTS AND DISCUSSION

Synthesis of flavones: This study presents the synthesis as well as the antimicrobial activity of two flavones, viz., 3,8-dimethoxy-5-(4-hydroxy-3-methoxyphenyl)-benzo



Scheme 1

(b)pyran-7-one (4′-hydroxy-3′,5,7-trimethoxy flavone) 6 and 3,8-dimethoxy-5-(3-hydroxy-4-methoxyphenyl)-benzo(b)pyran-7-one (3′-hydroxy-4′,5,7-trimethoxyflavone) 7. The synthesis of the above flavones were accomplished starting from 2-hydroxy-4,6-dimethoxyacetophenone (1) as shown in Scheme 1.

Alkaline condensation of 2-hydroxy-4, 6-dimethoxyacetophenone 1 with 4-methylbenzaldehyde 2 gave the corresponding chalcone 4 in moderate yield. It was purified by *n*-hexane–acetone(8:1) and purified by recrystallization from petroleum spirit as brown crystals with m.p. 44-46°C. The UV-Vis spectrum of 4 in MeOH (252 and 322 nm) suggested a chalcone structure and the IR absorption band at 3421 cm^{-1} indicated the presence of hydroxyl group. A positive ferric chloride test also indicated that compound 4 has a free hydroxyl group and a band at 1620 cm^{-1} showed the presence of a conjugated carbonyl group (>C = O). The ^1H NMR spectrum of 4 explained the presence of three aromatic methoxyl groups from the presence of three singlets at δ 3.75 (C_3-OCH_3), 3.79 ($C_{6'}$-OCH_3) and 3.84 ($C_{4'}$-OCH_3) integrating for three protons, respectively. A singlet at δ 6.53 (C_4-OH) indicated the presence of a hydroxyl proton of B-ring integrating for one proton. Two *meta*-coupled doublets at δ 6.38 (J = 2.6 Hz) and 6.71 (J = 2.6 Hz) each integrating for one aromatic proton of A-ring were assigned to $C_{3'}$-H and $C_{5'}$-H, respectively. The C_α-H and C_β-H protons of 4 appeared as two doublets at δ 7.41 (J = 16 Hz) and 8.06 (J = 16 Hz) integrating for one proton each. A singlet at δ 7.03 (C_2-H) indicated the presence of a hydroxyl proton of B-ring integrating for one proton. Compound 4 also showed typical two doublets integrating for two protons each respectively of B-ring at δ 6.76 (C_5-H J= 8.6) and 7.21 (C_6-H; J= 8.6 Hz). A characteristic singlet at δ 12.18 indicated the presence of a chelated phenolic proton at $C_{2'}$-OH integrating for one proton.

Cyclization of chalcone 4 into the corresponding flavone 6 was carried out using DMSO/I_2 reagent and DDQ reagent. It was purified by recrystallization from ether as colourless needles (536 mg, 77%), mp 38-40°C. The formation of 6 has been supported by spectral data and elemental analysis. The UV-Vis absorption spectrum of this flavone 6 in methanol with λ_{max} at 260 and 333 nm suggested the presence of a flavone nucleus. The IR absorption at 1647 cm^{-1} showed the presence of a carbonyl group (>C = O) and the absence of a hydroxyl group band confirmed the oxidation of chalcone 4 into flavone 6. This was supported by the ^1H and ^{13}C NMR spectrum of flavone 6.

Similarly, the structure of the compounds 5 and 7 have been elucidated by using UV-Vis, IR, ^1H and ^{13}C NMR together with elemental analysis.

Table 1: Antibacterial screening for the compounds 4, 5, 6 and 7*

Compd	Concentration μg $disc^{-1}$	*Escherichia coli*	*Proteus valguris*	*Sarcina lutea*
4	50	-	-	7
	100	7	8	17
5	50	6	14	-
	100	10	18	12
6	50	14	15	-
	100	21	24	14
7	50	12	12	17
	100	17	23	28
C-30**	30	27	30	29

*Inhibitory activity is expressed as the diameter (in mm) of the observed inhibition zone.**Ciprofloxacin-30

Table 2: MIC level of compounds 4, 5, 6 and 7

	Minimum inhibitory concentration (μg mL^{-1}) of compd.				
Test organism	Comp.4	Comp.5	Comp.6	Comp.7	Ciprofloxacin
E. coli	32	32	64	32	4
Proteus valguris	64	32	32	32	4
Sarcina lutea	64	128	64	32	8

Table 3: Antifungal screening for the compounds 4, 5, 6 and 7

Comp.	Conc. μg disc^{-1}	*Aspergillus* sp.	*Penicillum*sp.	*Fusarium* sp.	*Biopolarise* sp.	*Tricoderma* sp.
4	50	11	9	-	-	7
	100	17	12	-	-	13
5	50	-	-	8	-	-
	100	-	10	12	-	11
6	50	13	18	10	10	15
	100	21	26	14	17	21
7	50	-	12	16	14	10
	100	10	18	22	22	15
Nystatin**	50	21	19	17	22	17

*Inhibitory activity is expressed as the diameter (in mm) of the observed inhibition zone, ** Nystatin-50

Antibacterial activity : The antibacterial activity of compounds 4, 5, 6 and 7 have been assayed at concentrations of 50 and 100 μg disc^{-1} against strains of both, gram-positive and gram-negative pathogenic bacteria. Initially, susceptibility testing was carried out by measuring the inhibitory zone diameters on Nutrient Agar (NA), with conventional paper disc method and the inhibitory zone diameters were read and rounded off to the nearest whole numbers (mm) for analysis. The inhibitory effects of compounds 4, 5, 6 and 7 against these organisms are given in Table 1.

The screening results indicate that compound 5, 6 and 7 showed moderate antibacterial activity to the tested bacteria except, Compound 4 did not show antibacterial activity at the concentration of 50 μg disc^{-1} against *Escherichia coli* and *Proteus valguris*. From the flavones 5 and 6 were identified as the most active compound in comparison with their corresponding chalcones 4 and 5. Both the flavone 6 and 7 showed higher antibacterial activity at the concentration of 100 μg disc^{-1}.

From the result it can be concluded that flavone ring system is responsible for the shown of high antibacterial activity of compounds 6 and 7.

Minimum inhibitory activity: The minimum inhibitory concentration (MIC's, μg mL^{-1}) of compounds 4, 5, 6 and 7 in comparison to ciprofloxacin against antibiotic susceptible strains of both gram-positive and gram-negative bacteria viz. *Escherichia coli* (G^-), *Proteus valguris* (G^+), *Sarcina lutea* (G^+) were determined. Amongst all the compounds tested compound 7 showed moderate MIC values against both the gram-positive and gram-negative bacteria strains. The MIC level of chalcones 4, 5, 6 and 7 against these organisms are given in Table 2.

Antifungal activities: The antifungal activity of compounds 4, 5, 6 and 7 have been assayed *in vitro* at a concentration of 50 and 100 μg disc^{-1} against five plants pathogenic and mold fungi, viz., *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Biopolarise* sp. and *Tricoderma* sp. The inhibitory effects of compounds 4, 5, 6 and 7 against these organisms are given in Table 3.

The screening results indicate that all compounds exhibited antifungal activity against the tested fungi at the concentration of 100 μg disc^{-1}. It can also be noted that flavones 6 showed a greater inhibitory effect against all the fungi as compared to their corresponding chalcones 4 as well as chalcone 5 and flavone 7. Compound 6 and 7 did not show antifungal activity at the low concentration (50 μg disc^{-1}) against the *Fusarium* sp. and *Aspergillus* sp., respectively. From the result, It can be see that flavone 6 showed higher fungicidal effect compared to the flavone 7 as well as chalcone 4 showed higher fungicidal effect compared to the chalcone 5. From the result it can be concluded that the flavone ring system is responsible for the antifungal effects.

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