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Cytotoxic and antimicrobial activities of some synthetic flavones

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Several flavones have been synthesized and their biocidal activity investigated along with their corresponding chalcones against some bacterial and fungal strains as well as brine shrimp nauplii. Compounds **13** and **14** show good antibacterial, antifungal and cytotoxic activity against some selected bacterial and fungal strains as well as brine shrimp nauplii. The synthesized compounds have been characterized using UV-Vis, IR, ^{1}H and ^{13}C NMR spectral data together with elemental analysis.

Keywords: Flavone, antibacterial activity, antifungal activity, cytotoxicity

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Flavones constitute a large segment of natural products. Synthesis of flavones and their derivatives has attracted considerable attention due to their significant biocidal[1-3], pharmaceutical[4-7], anti-oxidant[8-11], anti-anxiolytic[12], anti-cancer[13] and anti-inflammatory[14,15] effects. In the light of these results a number of flavone derivatives have been synthesised and their biological activities[16-21] studied. This paper reports the synthesis of four flavones **11-14** from their corresponding chalcones **7-10** by using DMSO/I_2, as an oxidizing agent. Flavones **11, 12** and **14** are synthetically new and flavone **13** (7,3′-dihydroxy-4′-methoxyflavone, farnisin) has been reported and isolated by Sahu *et al.*[22] from the seeds of *Acacia farnesiana*. The synthesis of **13** has not yet been reported elsewhere. *Acacia farnesiana* Wild is a thorny bush or small tree native to tropical America but extensively cultivated in France for production of the much valued Cassie perfume. It is naturalized in the South Asian region where it now has a widespread distribution. Different parts of the plant have been used for the treatment of various ailments[23]. The synthesized flavones and their corresponding chalcones were screened *in vitro* for their antibacterial and antifungal activity against four human pathogenic bacteria, viz., *Bacillus megaterium* (G^+), *Streptococcus-β-haemolyticus* (G^+), *Escherichia coli* (G^-), *Klebsiella sp.* (G^-) and two plants as well as molds fungi, *viz*., *Aspergillus niger* and *Aspergillus fumigatus*. The present study was carried out to investigate the cytotoxicity as well as antimicrobial properties of the above flavones and chalcones 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.

Results and Discussion

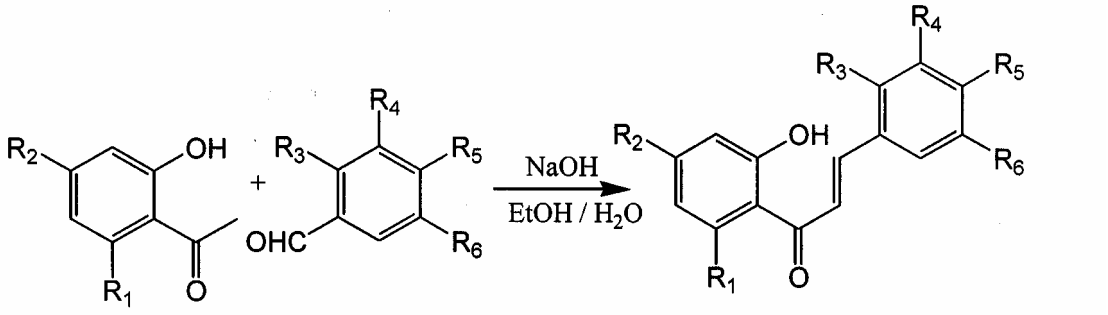
Synthesis of flavones

This paper presents the synthesis as well as the cytotoxic and antimicrobial activity of four flavones, *viz*., 5, 7, -dimethoxy-4′-methylflavone **11**, 2′,4′,5′-trimethoxyflavone **12**, 7,3′-dihydroxy-4′-methoxy-flavone **13**, and 4′-methylflavone **14**. The synthesis of the above flavones has been accomplished as shown in **Scheme I**.

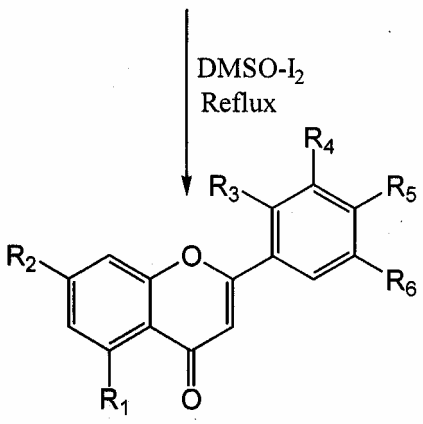
Alkaline condensation of 2-hydroxy-4,6-dimethoxyacetophenone **1** with 4-methylbenzalde-hyde **4** gave the corresponding chalcone **7** in moderate yield. It was purified by *n*-hexane-acetone (7:1) and purified by recrystallization from *n*-hexane-

acetone mixture to obtain light orange crystals with m.p. 61-3°C. The UV-Vis spectrum of **7** in MeOH (252 and 326 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 **7** has a free hydroxyl group and a band at 1620 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H NMR spectrum of **7** explained the presence of two aromatic methoxyl groups from the presence of two singlets at δ 3.79 ($C_{6'}$-OCH_3) and 3.84 ($C_{4'}$-OCH_3) as two singlets integrating for two protons respectively. A singlet at δ 2.39 (C_4-CH_3) indicated the presence of a methyl proton of B-ring integrating for three protons. Two *meta*-coupled doublets at δ 6.58 (J = 2.6 Hz) and 6.83 (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 **7** appeared as two doublets at δ 7.41 (J = 16 Hz) and 8.06 (J = 16 Hz) integrating for one proton each. Compound **7** also showed typical two double doublets integrating for two protons each respectively of B-ring at δ 6.91 (C_3-H and C_5-H; J= 2.5 and 7.6 Hz) and 7.43 (C_2- H and C_6-H; J= 2.5 and 7.6 Hz). A characteristic singlet at δ 13.18 indicated the presence of a chelated phenolic proton at $C_{2'}$-OH integrating for one proton.

Cyclization of chalcone **7** into the corresponding flavone **11** was carried out using DMSO/I_2 reagent[24,25]. It was purified by recrystallization from petroleum spirit and obtained as light brown needles with m.p. 105-07°C. The formation of **11** has been supported by spectral data and elemental analysis. The UV-Vis absorption spectrum of this flavone **11** in methanol with λ_{max} at 260 and 335 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



1 R_1, R_2 = -OCH_3
2 R_1, R_2 = -H
3 R_1 = -H, R_2 = -OH
4 R_3, R_4, R_6 = -H, R_5 = -CH_3
5 R_4 = -H, R_3, R_5, R_6 = -OCH_3
6 R_3, R_6 = -H, R_4 = -OH, R_5 = -OCH_3
7 R_1, R_2 = -OCH_3, R_3, R_4, R_6 = -H, R_5 = -CH_3
8 R_1, R_2, R_4 = -H, R_3, R_5, R_6 = -OCH_3
9 R_1, R_3, R_6 = -H, R_2, R_4 = -OH, R_5 = -OCH_3
10 R_1, R_2, R_3, R_4, R_6 = -H, R_5 = -CH_3



11 R_1, R_2 = -OCH_3, R_3, R_4, R_6 = -H, R_5 = -CH_3
12 R_1, R_2, R_4 = -H, R_3, R_5, R_6 = -OCH_3
13 R_1, R_3, R_6 = -H, R_2, R_4 = -OH, R_5 = -OCH_3
14 R_1, R_2, R_3, R_4, R_6 = -H, R_5 = -CH_3

Scheme I

confirmed the oxidation of chalcone **7** into flavone **11**. This was also supported by the ^{1}H and ^{13}C NMR spectrum of flavone **11** (see experimental section).

Similarly, the structure of the compounds **8-10** and **12-14** have been elucidated by using UV-Vis, IR, ^{1}H and ^{13}C NMR together with elemental analysis (see experimental).

Antibacterial activity

The antibacterial activity of compounds **7-14** have been assayed at concentrations of 100 and 200 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 **7-14** against these organisms are given in **Table I**.

The screening results indicate that compounds **13** and **14** showed good antibacterial activity against all tested bacteria. Compound **9** showed moderate antibacterial activity against *Bacillus megaterium*, *Streptococcus-β-haemolyticus* and *Klebsiella* sp. except against *Escherichia coli*. Compound **10** also showed moderate antibacterial activity against *Bacillus megaterium* and *Klebsiella* sp. and at high concentration showed antibacterial activity against *Streptococcus-β-haemolyticus* and *Escherichia coli*. Compounds **11** and **12** showed antibacterial activity at high concentration against all tested bacteria whereas compounds **7** and **8** did not show any antibacterial activity against tested bacteria. From the inhibition zone diameter data analysis the flavones **13** and **14** and their precursor chalcones **9** and **10** were identified as the more active compounds as compared to the flavones **11** and **12**. From the inhibition zone diameter data analysis it was also found that the flavones **11** and **12** showed antibacterial activity but their corresponding chalcone precursors **7** and **8** did not show any antibacterial activity. Activity might be lost due to the formation of flavone ring of chalcones **7** and **8**. This result is supported by the higher antibacterial activity of flavones **13** and **14** as compared to their corresponding chalcones **9** and **10**.

Minimum inhibitory activity

The minimum inhibitory concentration (MIC's, μg mL^{-1}) of compounds **7-14** in comparison to ciprofloxacin against antibiotic susceptible strains of both gram-positive and gram-negative bacteria *viz*. *Bacillus megaterium*, *Streptococcus-β-haemolyticus*, *Escherichia coli*, *Klebsiella sp*. were determined.

Table I—Antibacterial screening for the compds. **7-14***.

Compd	Concentration μg $disc^{-1}$	*Bacillus megaterium*	*Streptococcus-β-haemolyticus*	*Escherichia coli*	*Klebsiella sp.*
7	100	-	-	-	-
	200	-	-	-	-
8	100	-	-	-	-
	200	-	-	-	-
9	100	11	9	-	11
	200	19	15	11	16
10	100	12	-	-	10
	200	17	11	11	15
11	100	9	-	-	-
	200	18	16	12	12
12	100	-	-	-	-
	200	12	10	-	-
13	100	16	18	21	24
	200	24	26	26	29
14	100	20	18	19	21
	200	25	27	22	27
C-30**	30	29	31	27	28

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

Amongst all the compounds tested compounds **13** and **14**, showed lower MIC values against both the gram-positive and gram-negative bacteria strains. The MIC level of chalcones **7-10** and flavones **11-14** against these organisms are given in **Tables II** and **III** respectively. Antifungal activities

The antifungal activity of compounds **7-14** have been assayed *in vitro* at a concentration of 100 μg $disc^{-1}$ against *Aspergillus niger* and *Aspergillus fumigatus*. The inhibitory effects of compounds **7-14** against these organisms are given in **Table IV**. The screening results indicate that the compounds **9, 10, 13** and **14** exhibited antifungal activity against the tested fungi and the compounds **7, 8, 11** and **12** did not show any antifungal activity. It can also be noted that flavones **13** and **14** showed a greater inhibitory effect against both the fungi as compared to their corresponding chalcones **9** and **10**.

Table II—MIC level of compds **7-10**

Test organism	Minimum inhibitory concentration (μg mL^{-1}) of compd				
	7	8	9	10	Ciprofloxacin
B. megaterium	256	256	32	32	4
S-β-haemolyticus	256	128	32	128	4
E. coli	256	256	128	128	8
Klebsiella sp.	256	256	32	32	4

Table III—MIC level of compds **11-14**

Test organism	Minimum inhibitory concentration (μg mL^{-1}) of compd				
	11	12	13	14	Ciprofloxacin
B. megaterium	32	128	64	32	4
S-β-haemolyticus	128	128	32	64	4
E. coli	128	256	32	32	8
Klebsiella sp.	128	256	32	32	4

Table IV—Antifungal screening for the compds **7-14***

Compd	Concentration μg $disc^{-1}$	*Aspergillus niger*	*Aspergillus fumigatus*
7	100	-	-
8	100	-	-
9	100	8	6
10	100	10	11
11	100	-	-
12	100	-	-
13	100	12	10
14	100	1 3	15
Nystatin**	50	21	19

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

Cytotoxic activity

The LC_{50} values for the compounds **7-14** were found to be 4.59, 5.72, 1.65, 3.21, 3.22, 2.99, 1.53 and 2.19 μg mL^{-1}, respectively. The standard anticancer drug bleomycin gave an LC_{50} value of 0.41 μg mL^{-1}. The lowest LC_{50} value was found in case of the flavone **13** and chalcone **9** indicating its higher cytotoxicity as compared to the other compounds **7, 8, 10, 11, 12** and **14**. The flavonoid compounds **9-14** showed moderate biocidal activity against brine shrimp nauplii as compared to the control DMSO and gallic acid used as standard agents[26]. The cytotoxic effects of compounds **7-14** are given in **Table V**.

Experimental Section

Melting points were recorded on Gallenkamp apparatus and are uncorrected. IR spectra (KBr) were measured using a Shimadzu DR-8001 spectrophotometer. ^1H 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. Homogeneity of the compounds was checked by TLC.

Synthesis of (*E*)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(4-methylphenyl)-prop-2-en-1-one (2′-hydroxy-4′, 6′-dimethoxy-4-methylchalcone), 7. A mixture of 2-hydroxy-4,6-dimethoxyacetophenone[27] (**1**, 0.98 g, 5 mmoles) and 4-methylbenzaldehyde (**4**, 1.1 eq., 0.66 g, 5 mmoles) in ethanolic solution of KOH (5%, 15 mL) was kept at rt for about 84 hr. 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 (7:1) as developing solvent and the compound crystallized from *n*-hexane–acetone mixture as light orange crystals (0.92 g, 56%), m.p. 61-3°C, R_f 0.70 (*n*-hexane : acetone; 4:1).

Anal. Found: C, 72.01; H, 6.17; $C_{18}H_{18}O_4$ requires C, 72.47; H, 6.08%. UV-Vis (nm): 252 and 326; IR (KBr): 3421, 2974, 2924, 2345, 2291, 1620, 1594, 1508, 1461, 1423, 1373, 1342, 1257, 1218, 1157,

Table V — The results of cytotoxic effect of the compds 7-14 and standard bleomycin and gallic acid.

Compds	LC_{50} (ppm)	95% confidence limit (ppm)		Regression Equation	χ^2 (df)
		lower	upper		
7	4.59	2.77	7.58	Y = 3.80 + 1.81 X	0.25 (2)
8	5.72	3.14	10.45	Y = 3.80 + 1.59 X	0.26 (2)
9	1.65	0.90	2.99	Y = 3.19 + 1.48 X	0.41 (2)
10	3.21	1.74	5.95	Y = 4.20 + 1.56 X	5.61 (2)
11	3.22	1.79	5.80	Y = 2.62 + 1.57 X	5.62 (2)
12	2.99	1.46	6.10	Y = 4.39 + 1.28 X	0.40 (2)
13	1.53	.87	2.71	Y = 3.11 + 1.60 X	0.07 (2)
14	2.19	1.32	3.65	Y = 2.59 + 1.78 X	0.23 (2)
Standard Bleomycin	0.41	0.27	0.62	Y = 3.16 + 2.98 X	0.62 (2)
Gallic acid	4.53	3.33	6.15	Y = 3.93 + 1.62 X	1.25 (2)

1115, 1045, 991, 817 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.39 (3H, s, C_4-CH_3), 3.79 (3H, s, $C_{6'}$-OCH_3), 3.84 (3H, s, $C_{4'}$-OCH_3), 6.58 (1H, d, *J* = 2.6 Hz. $C_{3'}$-H), 6.83 (1H, d, *J* = 2.6 Hz, $C_{5'}$-H), 6.91 (2H, dd, *J* = 2.5 and 7.6 Hz, C_3-H and C_5-H), 7.43 (2H, dd, *J* = 2.5 and 7.6 Hz, C_2-H and C_6-H), 7.41 (1H, d, *J* = 16 Hz, C_α-H), 8.06 (1H, d, *J* = 16 Hz, C_β-H), 13.18 (1H, s, $C_{2'}$-OH); ^{13}C NMR (DMSO-d_6): δ 102.4 (C-1′), 159.5 (C-2′), 94.6 (C-3′), 170.2 (C-4′), 92.6 (C-5′), 164.6 (C-6′), 181.4 (>C=O), 123.9 (C-α), 143.5 (C-β), 128.5 (C-1), 126.7 (C-2 and C-6), 129.8 (C-3 and C-5), 137.4 (C-4), 59.7 ($C_{6'}$-OCH_3), 56.0 ($C_{4'}$-OCH_3), 21.9 (C_4-CH_3).

Synthesis of (*E*)-1-(2-hydroxyphenyl)-3-(2,4,5-trimethoxylphenyl)-prop-2-en-1-one (2′-hydroxy-2,4,5-trimethoxychalcone), 8. A mixture of 2-hydroxyacetophenone (**2**, 0.68 g, 5 mmoles) and 2,4,5-trimethoxybenzaldehyde (**5**, 1.1 eq., 1.08 g, 5 mmoles) in ethanolic solution of KOH (5%, 15 mL) was kept at rt for about 75 hr. and worked up using a previously described method. The reaction mixture was purified by preparative TLC over silica gel GF_{254} using *n*-hexane-acetone (9:2) as developing solvent and the compound purified by recrystallization from petroleum spirit and isolated as orange needles (1.26 g, 72%), m.p.114-16°C, R_f 0.69 (*n*-hexane : acetone; 4:1).

Anal. Found: C, 68.47; H, 5.89; $C_{18}H_{18}O_5$ requires C, 68.78; H, 5.77%. UV-Vis (nm): 259 and 328; IR (KBr): 3376, 2927, 2831, 2360, 1635, 1612, 1562, 1512, 1488, 1411, 1377, 1334, 1299, 1203, 1153, 1122, 1026, 979, 902, 840, 806, 759, 709 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.79 (3H, s, C_2-OCH_3), 3.81 (3H, s, C_4-OCH_3), 3.84 (3H, s, C_5-OCH_3), 6.12 (1H, s, C_3-H), 6.61(1H, s, C_6-H), 6.98 (1H, m, $C_{5'}$-H), 7.28 (1H, m, $C_{4'}$-H), 6.89 (1H, dd, *J* = 2.9 and 9 Hz $C_{3'}$-H), 7.54 (1H,dd, *J*= 2.6 and 9 Hz, $C_{6'}$-H), 7.39 (1H, d, *J*= 16 Hz, C_α-H), 8.03 (1H, d, *J* = 16 Hz, C_β-H), 12.61 (1H, s, $C_{2'}$-OH); ^{13}C NMR (DMSO-d_6): δ 122.4 (C-1′), 157.5 (C-2′), 112.6 (C-3′), 134.2 (C-4′), 119.6 (C-5′), 134.1 (C-6′), 185.4 (>C=O), 123.8 (C-α), 143.1 (C-β), 118.5 (C-1), 151.2 (C-2), 101.3 (C-3), 146.4 (C-4), 138.5 (C-5), 114.9 (C-6), 55.8 (C_2-OCH_3), 56.3 (C_4-OCH_3), 56.8 (C_5-OCH_3).

Synthesis of (*E*)-1-(2,4-dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (2′, 4′, 3-trihydroxy-4-methoxychalcone), 9. A mixture of 2,4-dihydroxyacetophenone (**3**, 0.76 g, 5 mmoles) and 3-hydroxy-4-methoxy benzaldehyde (**6**, 1.1 eq., 0.84 g, 5 mmoles) in ethanolic solution of KOH (5%, 15 mL) was kept at rt for about 96 hr. and worked up as previously described method. The reaction mixture was purified by preparative TLC over silica gel GF_{254} using acetone-*n*-hexane (1:10) and the compound purified by recrystallization from petroleum spirit as yellow crystals (1.12 g, 70%), m.p. 63-5°C, R_f 0.70 (*n*-hexane: acetone; 4:1).

Anal. Found : C, 66.91; H, 5.09; $C_{16}H_{14}O_5$ requires C, 67.13; H, 4.93%. UV-Vis (nm): 271 and 321 nm; IR (KBr): 3313, 2977, 2846, 2599, 1670, 1608, 1581, 1512, 1442, 1375, 1330, 1284, 1245, 1184, 1118, 1064, 1022, 987, 952, 867, 833, 794, 756, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.78 (3H, s, C_4-OCH_3), 6.41 (1H, d, *J* = 2.4 Hz, $C_{3'}$-H), 6.49 (1H s, $C_{4'}$-OH), 6.56

(1H, s, C_3-OH), 6.62 (1H, dd, *J* = 2.4 and 8.6 Hz, $C_{5'}$-H), 7.01 (1H, d, *J* = 2.6 Hz, C_2-H), 6.76 (1H, d, *J* = 8.4 Hz, C_5-H), 6.87 (1H, dd, *J* = 2.6 and 8.4 Hz, C_6-H), 7.21 (1H, d, *J* = 8.6 Hz, $C_{6'}$-H), 7.41 (1H, d, *J* = 16 Hz, C_α-H), 8.01 (1H, d, *J* = 16 Hz, C_β-H), 12.60 (1H, s, $C_{2'}$-OH); ^{13}C NMR (DMSO-d_6): δ 118.5 (C-1′), 158.9 (C-2′), 106.4 (C-3′), 161.5 (C-4′), 110.8 (C-5′), 133.9 (C-6′), 184.4 (>C=O), 124.1 (C-α), 141.9 (C-β), 126.6 (C-1), 117.4 (C-2), 145.1 (C-3), 149.4 (C-4), 119.5 (C-5), 121.3 (C-6), 56.4 (C_4-OCH_3).

Synthesis of (*E*)-1-(2-hydroxyyphenyl)-3-(4-methylphenyl)-prop-2-en-1-one (2′-hydroxy-4-methylchalcone), 10. A mixture of 2-hydroxyacetophenone (2, 0.68 g, 5 mmoles) and 4-methylbenzaldehyde (**4**, 1.1 eq., 0.66 g, 5 mmoles) in ethanolic solution of KOH (5%, 15 mL) was kept at rt for about 72 hr. and worked up using a previously described method. 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 recrystallization from petroleum spirit and isolated as yellow crystals (1.06 g, 79%), m.p. 39-40°C, R_f 0.78 (petroleum spirit : acetone; 10:1).

Anal. Found: C, 80.36; H, 6.08; $C_{16}H_{14}O_2$ requires C, 80.65; H, 5.92%. UV-Vis (nm): 216 and 308; IR (KBr): 3356, 2920,2858, 2634, 1640, 1604, 1577, 1515, 1461, 1415, 1365, 1303, 1269, 1222, 1153, 1114, 1064, 1026, 979, 906, 860, 840, 810, 759 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.41 (3H, s, C_4-CH_3), 6.91 (1H, dd, *J* = 2.6 and 8.9 Hz, $C_{3'}$-H), 6.99 (1H, m, $C_{5'}$-H), 7.11 (2H, dd, *J* = 2.6 and 7.8 Hz, C_3-H and C_5-H), 7.31 (1H, m, $C_{4'}$-H), 7.39 (1H, d, *J* = 16 Hz, C_α-H), 7.43 (2H, dd, *J* = 2.6 and 7.8 Hz, C_2-H and C_6-H), 7.54 (1H, dd, *J* = 2.6 and 8.9 Hz, $C_{6'}$-H), 8.03 (1H, d, *J* = 16 Hz, C_β-H), 12.63 (1H, s, $C_{2'}$-OH); ^{13}C NMR (DMSO-d_6): δ 121.9 (C-1′), 158.7 (C-2′), 117.2 (C-3′), 136.1 (C-4′), 119.6 (C-5′), 132.1 (C-6′), 186.4 (>C=O), 124.2 (C-α), 143.9 (C-β), 129.9 (C-1), 126.8 (C-2 and C-6), 130.1 (C-3 and C-5), 137.2 (C-4), 21.9 (C_4-CH_3).

Synthesis of 3, 8-dimethoxy-5-(4-methylphenyl)-benzo(*b*)pyran-7-one (5, 7, -dimethoxy-4′-methyl-flavone), 11. Treatment of the chalcone **7** (298 mg, 1 mmole) with catalytic amount of iodine in dimethyl sulphoxide[26,27] (DMSO, 10 mL) gave the corresponding flavone **11**. The flavone was purified by preparative TLC over silica gel GF_{254} using petroleum spirit-acetone (4 : 1) as developing solvent and the compound purified by recrystallization from petroleum spirit as light brown needles(193 mg, 65%), m.p. 105-07°C, R_f 0.46 (petroleum spirit-acetone; 4:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. Found: C, 72.71; H, 5.59; $C_{18}H_{16}O_4$ requires C, 72.96; H, 5.44%. UV-Vis (nm): 260 and 335; IR (KBr): 3024, 2958, 2923, 2314, 1647, 1604, 1512, 1469, 1419, 1384, 1342, 1311, 1288, 1257, 1157, 1114, 1053, 1026, 956, 906, 821, 798, 771, 717 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.36 (3H, s, $C_{4'}$-CH_3), 3.81 (3H, s, C_5-OCH_3), 3.87 (3H, s, C_7-OCH_3), 5.41 (1H, s, C_3-H), 6.48 (1H, d, *J* = 2.3 Hz, C_6-H), 6.72 (2H, dd, *J* = 2.4 and 7.6 Hz, $C_{3'}$-H and $C_{5'}$-H), 6.78 (1H, d, *J* = 2.3 Hz, C_8-H), 7.36 (2H, dd, *J* = 2.4 and 7.6 Hz, $C_{2'}$-H and $C_{6'}$-H); ^{13}C NMR (DMSO-d_6): δ 166.4 (C-2), 102.4 (C-3), 185.8 (C-4), 106.1 (C-4a), 163.2 (C-5), 95.2 (C-6), 169.7 (C-7), 94.2 (C-8), 159.3 (C-8a), 129.4 (C-1′), 128.5 (C-2′ and C-6′), 132.1 (C-3′ and C-5′), 136.6 (C-4′), 56.1 (C_5-OCH_3), 55.9 (C_7-OCH_3), 21.1 ($C_{4'}$-CH_3).

Synthesis of 5-(2,4,5-trimethoxyphenyl)-benzo-(*b*)pyran-7-one (2′,4′,5′-trimethoxyflavone), 12. The flavone **12** was prepared by previously described methods and it was purified by preparative TLC over silica gel GF_{254} using petroleum sprit-acetone (2:9) as developing solvent and the compound purified by recrystallization from petroleum spirit as colorless needles (190 mg, 64%), m.p. 131-32°C, R_f 0.42 (petroleum ether-acetone; 5:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. Found: C, 69.01; H, 5.29; $C_{18}H_{16}O_5$ requires C, 69.22; H, 5.16%. UV-Vis (nm): 254 and 316; IR (KBr): 2935, 2962, 2356, 1643, 1612, 1580, 1469, 1331, 1292, 1276, 1222, 1149, 1022, 972, 894, 852, 813, 783, 756, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.78 (3H, s, $C_{2'}$-OCH_3), 3.81 (3H, s, $C_{4'}$-OCH_3), 3.83 (3H, s, $C_{5'}$-OCH_3), 6.21 (1H, s, $C_{3'}$-H), 6.61(1H, s, $C_{6'}$-H), 6.39 (1H, s, C_3-H), 6.99 (1H, dd, *J* = 2.5 and 8.6 Hz C_8-H), 7.01-7.06 (1H, m, C_6-H), 7.21 (1H, dd, *J* = 2.5 and 8.6 Hz, C_5-H), 7.29-7.34 (1H, m, C_7-H); ^{13}C NMR (DMSO-d_6): δ 167.5 (C-2), 100.9 (C-3), 186.6 (C-4), 119.1 (C-4a), 128.2 (C-5), 121.2 (C-6), 136.7 (C-7), 116.2 (C-8), 158.3 (8a), 112.9 (C-1′), 151.5 (C-2′) 102.7 (C-3′), 143.6 (C-4′), 138.8 (C-5′), 110.2 (C-6′), 56.6 ($C_{2'}$-OCH_3), 56.1 ($C_{4'}$-OCH_3), 55.9 ($C_{5'}$-OCH_3).

Synthesis of 3-hydroxy-5-(3-hydroxy-4-methoxy-phenyl)-benzo(b)pyran-7-one (7,3′-dihydroxy-4′-methoxyflavone), 13. The flavone **13** was prepared by previously described methods and it was purified by preparative TLC over silica gel GF_{254} using

chloroform-methanol (10 : 1) as developing solvent and obtained as pale yellow needles (210 mg, 71%), m.p. 263-65°C [Lit.[22] m.p. 264-65°C], R_f 0.58 (chloroform-methanol; 10:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. Found: 67.41; H, 4.47; $C_{16}H_{12}O_5$ requires C, 67.60; H, 4.25%. UV-Vis (nm): 245 and 337; IR (KBr): 2958, 2924, 2854, 2349, 1620, 1596, 1508, 1445, 1384, 1319, 1257, 1134, 1103, 1018, 952, 844, 806, 767 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 (3H, s, $C_{4'}$-OCH_3), 6.96 (1H, d, J = 2.4 Hz, $C_{2'}$-H), 6.89 (1H, dd, J = 2.5 and 8.6 Hz, C_6-H), 6.61 (1H, d, J = 2.5 Hz, C_8-H), 6.41 (1H, s, C_3-H), 6.51 (1H, s, C_7-OH), 6.54 (1H, s, $C_{3'}$-OH), 6.98 (1H, d, J = 8.4 Hz, $C_{5'}$-H), 7.11 (1H, dd, J = 2.4 and 8.4 Hz, $C_{6'}$-H), 7.29 (1H, d, J = 8.6 Hz, C_5-H); ^{13}C NMR (DMSO-d_6): δ 167.8 (C-2), 98.7 (C-3), 187.1 (C-4), 118.1 (C-4a), 130.2 (C-5), 111.9 (C-6), 161.5 (C-7), 106.7 (C-8), 159.8 (C-8a), 127.9 (C-1′), 116.5 (C-2′), 141.7 (C-3′), 146.6 (C-4′), 118.8 (C-5′), 121.2 (C-6′), 56.6 ($C_{2'}$-OCH_3).

Synthesis of 5-(4-methylphenyl)-benzo(b)pyran-7-one (4′-methylflavone), 14. The flavone **14** was prepared by previously described methods and it was purified by preparative TLC over silica gel GF_{254} using ether-acetone (10:1) as developing solvent and purified by recrystallization from ether and isolated as light brown needles (164 mg, 69%), m.p. 75-6°C, R_f 0.35 (petroleum spirit-acetone; 24:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. Found: C, 81.11; H, 5.36; $C_{16}H_{12}O_2$ requires C, 81.34; H, 5.12%. UV-Vis (nm): 252 and 334; IR (KBr): 2966, 2920, 2866, 1639, 1569, 1508, 1465, 1411, 1373, 1311, 1286, 1226, 1188, 1122, 1041, 1007, 952, 906, 852, 817, 771, 752, 713 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.37 (3H, s, $C_{4'}$-CH_3), 6.41 (1H, s, C_3-H), 6.98 (2H, dd, J = 2.5 and 7.9 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.06 (1H, dd, J = 2.6 and 8.8 Hz, C_8-H), 7.11-7.16 (1H, m, C_6-H), 7.2 (2H, dd, J = 2.5 and 7.9 Hz, $C_{2'}$-H and $C_{6'}$-H), 7.41 (1H, dd, J = 2.6 and 8.8 Hz, C_5-H), 7.31-7.36 (1H, m, C_7-H); ^{13}C NMR (DMSO-d_6): δ 168.9 (C-2), 97.9 (C-3), 187.3 (C-4), 124.7 (C-4a), 129.5 (C-5), 123.1 (C-6), 135.9 (C-7), 117.8 (C-8), 158.2 (C-8a), 132.3 (C-1′), 127.9 (C-2′ and C-6′), 130.1 (C-3′ and C-5′), 137.8 (C-4′), 23.1 ($C_{4'}$-CH_3).

Antibacterial screening tests

The antibacterial activity of the synthesized compounds **7-14** were studied against four human pathogenic bacteria, *viz*., *Bacillus megaterium* (G^+), *Streptococcus-β-haemolyticus* (G^+), *Escherichia coli* (G^-), *Klebsiella sp*. (G^-). For detection of antibacterial activity the filter paper disc diffusion method[28,29] 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 of the 24 hr liquid cultures containing 10^7 microorganisms/mL. The diffusion time was 24 hr at 5°C and the incubation time was 12 hr 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"[30]. 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[31,32]. Serial dilution technique[33] was applied for the determination of minimum inhibitory concentration of the compounds against the four tested bacteria, *viz*., *Bacillus megaterium*, *Streptococcus-β-haemolyticus*, *Escherichia coli*, *Klebsiella sp*. 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 of nutrient broth medium. To each tube 100 μL of standardized suspension of the testing bacteria (10^7 cell/mL) were added and incubated at 30°C for 24 hr.

Antifungal screening tests

The antifungal activity of compounds **7-14** were evaluated towards two plant pathogenic and mold fungi, *viz*., *Aspergillus niger* and *Aspergillus fumigatus*. The antifungal activity was assessed by poisoned food technique[34] with some modification[35]. 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) ^{o}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^{o}C for 24 hr 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^{o}C for 72 hr. Dimethyl sulphoxide (DMSO) was used as a solvent to prepare desired solution (10 mg mL^{-1}) of the compounds initially.

Cytotoxic bioassay

Brine shrimp lethality bioassay[36-38] was carried out to investigate the cytotoxicity of the synthesized chalcones and flavones. Here *in vivo* lethality test were carried out using brine shrimp nauplii eggs (*Artemia salina* Lech.). Eggs were placed on one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. On the other side of the tank, a light source was placed in order to attract the nauplii. After two days of hatching period, the nauplii were ready for the experiment. Then 3 mg of each compound was accurately measured and dissolved in 0.6 mL (600 μL) of DMSO to get a concentration of 5 mg/mL. From the stock solutions 0.5, 1, 2, 5, 10, 20, 40 and 80 μL were placed in eight different vials making the volume upto 5 mL by NaCl solution. The final concentrations of the samples, in the vials became 0.5, 1, 2, 5, 10, 20, 40 and 80 μg/mL (ppm) respectively. Ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus seawater up to 5 mL was used. After 24 hr of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. The resulting data were transformed to the probit analysis[39] for the determination of LC_{50} values for the extracts.

References

1 Rao K V, Chattopadhyay S K & Reddy G C, *J Agric Food Chem* 38, **1990**, 1427.

2 Weidenbörner M & Jha H C, *Pestic Sci* 38, **1993**, 347.

3 Silva A M, Weidenbörner M & Cavaleiro J A S, *Mycol Res* 102, **1998**, 638.

4 4 Wu E S C, Loch J T, Toder B H, Borrelli A R, Gawlak D, Radov L A & Gensmantel N P, *J Med Chem* 35, **1992**, 3519.

5 Wolfman C, Viola H, Marder M, Wasowski C, Ardenghi P, Izquierdo I, Paladini A C & Medina J H, *Eur J Pharmacol* 318**, 1996**, 23.

6 Akama T, Ishida H, Kimura U, Gomi K, Saito H, Fuse E, Kobayashi S, Yoda N & Kasai M, *J Med Chem* 40, **1997**, 1894.

7 Gee J M & Johnson I T, *Curr Med Chem* 8, **2001**, 1245.

8 Rice-Evans C A, Miller N J & Paganga G, *Free Rad Biol Med* 20, **1996**, 933.

9 Rice-Evans C A, *Curr Med Chem* 8, **2001**, 797.

10 Pietta P G, *J Nat P*rod 63, **2000**, 1035.

11 Chan E C H, Patchareewan P & Owen L W, *J Cardiovasc Pharmacol* 35, **2000**, 326.

12 Zonali P, Aallone R & Baraldi M, *Filtoterapia*, 71**, 2000**, 117.

13 Leu Y I, Ho D K, Cassady J M, Cook V M & Barid W M, *J Nat Prod* 55**, 1992**, 357.

14 Fishkin R J & Winslow J T, *Psychopharmacology (Berl*), 132,**1997**, 335.

15 15 Dao T T, Chi Y S, Kim J, Kim H P, Kim S & Park H, *Bioorg Med Chem Lett*, 14, **2004**, 1165.

16 Alam S, Sarkar Z & Islam A, *J Chem Sci* 116, **2004**, 29.

17 Alam S, Miah M A J & Islam A, *J Biol Sci* 4, **2004**, 527.

18 Alam S, *Acta Chim Slov* 51, **2004**, 447.

19 Alam S, *J Chem Sci* 116, **2004,** 325.

20 Alam S, & Mostahar S, *J Applied Sci* 5, **2005**, 327.

21 Alam S, Miah M A J & Islam A, *ACGC Chem Res Comm* 18, **2005**, 1.

22 Sahu N P, Achari B & Banerjee S, *Phytochemistry*, 49, **1998**, 1425.

23 23 Chadha Y R (ed.), The Wealth of India, Raw materials, Vol. IA (Revised edition 1985), CSIR, New Delhi, p-31.

24 Doshi A G, Soni P A & Ghiya B J, *Indian J Chem* 25B**, 1986**, 759.

25 Kosmeder J W, Moriarty R M, Pezzuto J M, Song L L & Moon R C, US Patent 6, 046, **2000**, 231.

26 Sarkar M K, Ergil D, Tamir A U & Sahin N, *Fitoterapia*, 69, **1988**, 457.

27 Nagaranjan G R & Parmar V S, *Indian J Chem* 16B, **1978**, 439.

28 Arima H, Ashida H & Danno G-I, *Biosci Biotechnol Biochem* 66, **2002**, 1014.

29 Jeongmok K, Maurice R M & Cheng I W, *J Agr Food Chem* 43, **1995**, 2834.

30 Carson C F, Hammer K A & Riley T V, *Microbios***,** 82, **1995**, 181.

31 Collins C H, *Antibiotics and Antibacterial Substances: Microbiological Methods*, (Butterworths London), **1964**, 296.

32 Davidson P M & Parish M E, *Food Technology*, 43, **1989**, 148.

33 Nishina C, Enoki N, Tawata S, Mori A, Kobayashi K & Fukushima M, *Agric Biol Chem*, 51, **1987**, 139.

34 Grover R K & Moore J D, *Phytopathology*, 52, **1962**, 876.
35 Miah M A T, Ahmed H U, Sharma N R, Ali A & Miah S A, *Bangladesh J Bot*, 19, **1990**, 5.
36 Mayer B N, Ferrigni N R, Putnam J E, Jacobsen L B, Nichols D E & McLaughlin J L, *Plant Medica*, 45, **1982**, 31.
37 McLaughlin J L & Andersen J E, *Brine shrimp and crown gall tumors: simple bioassay for the discovery of plant antitumour agents. Proceeding NIH Workshop. Bioassay for Discovery of Antitumour and Antiviral Agents from Natural Sources, Bethesda*, **1988**, p 22.
38 McLaughlin J L, *Bench Tops Bioassay for the Discovery of Bioactive Compounds in Higher Plants*, **1990**, Brenena, p 29.
39 Finney D J, *Probit Analysis*, 3rd edition (University Press, Cambridge, UK), **1971**, pp 18, 37, 77.