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Studies of Antimicrobial Activity of two Synthetic 2′, 4′, 6′-trioxygenated Flavones

[1]Sayed Alam and Sohel Mostahar
[1]Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi-6204, Bangladesh
Department of Chemistry, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: Two 2′, 4′, 6′-trioxygenated flavones has been synthesized and tested for antibacterial and antifungal activities along with their corresponding chalcones against four human pathogenic bacteria and five plant as well as molds fungi. UV, IR and ^{1}H NMMR techniques together with elemental analysis elucidated the structures of the synthesized compounds. The antibacterial and antifungal screen was performed *in vitro* by the filter paper disc diffusion method and poisoned food technique. Compound 6 and 7 showed antibacterial activity but their corresponding chalcones didn't show any activity against tested bacteria. Compound 4 and its corresponding chalcone 6 didn't show any antifungal activity where as compound 5 and its flavone 7 showed moderate antifungal activity against *Penicillium* sp and *Colletorichum gloeosporioides.*

Key words: Aldol reaction, cyclization, oxidation, flavone, antibacterial, antifungal activity, inhibition zone

INTRODUCTION

2′,4′,6′-trioxygenated flavonoid compounds are widely occurring in natural plant pigments[1,2] and medicinal plants[3] 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, antiinflammatory, antimutagenic and antiallergic activities and inhibitory activities on several enzymes[4,5]. Here we describe the syntheses of two 2′,4′,6′-trioxygenated flavones (6 and 7) from their corresponding chalcones (4 and 5) by using differently DMSO/I_2, diphenyl sulphide and DDQ as an oxidizing agent. Both the flavones and their corresponding chalcones were screened *in vitro* for their antibacterial and antifungal activity against four human pathogenic bacteria, viz., *Sarcina lutea* (G^+), *Bacilus subtillis* (G^+), *Shigella dysenteriae* (G^-), *Pseudomonas aeruginosa* (G^-) and five plant as well as molds fungi, viz. *Colletorichum gloeosporioides* Penz., *Candida albicans*, *Aspergillus nigar*, *Aspergillus flavus* and *Penicillium* sp.

MATERIALS AND METHODS

General: Melting points were recorded on Gallenkamp apparatus and were uncorrected. IR spectra (KBr) were measured using a Shimadzu, DR-8001 spectrophotometer, ^{1}H NMR spectra ($CDCl_3$) on a Brucker WH 400 MHZ instrument with TMS as an internal standard and UV spectra (MeOH) on a LKB 4053 spectrophotometer. Purity of the compounds was checked by TLC.

Synthesis of 2′-hydroxy-2, 4, 6-trimethoxychalcone (4, $C_{18}H_{18}O_5$): A mixture of 2-hydroxyacetophenone (1, 10 mmol, 1.36 g) and 2,4,6-trimethoxybenzaldehyde (3, 1.1 eqv., 2.16 g) in ethanolic solution of KOH (5%, 15 mL) was kept at room temperature for about 75 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 evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with ether–acetone (8:1) and crystallized from benzene-acetone mixture as yellow crystals (2.14 g), yield 61.00%, mp.132-134°C, R_f 0.69 (benzene: acetone; 9:1).

Anal. Found: C, 68.43; H, 5.41%; Calc. for $C_{18}H_{18}O_5$; C, 68.78; H,5.77%

UV λ_{max}^{MeOH}: 238, 275 and 373 nm.

IR ν_{max}^{KBr}: 2893, 1623, 1605, 1575, 1548, 1488, 1450, 1419, 1368, 1313, 1298, 1269, 1215, 1198, 1155, 1116, 1063, 1027, 989, 976, 931, 860, 832, 812, 776, 750, 729, 690, 658, 637 cm^{-1}.

Corresponding Author: Mohammad Sayed Alam, Lecturer, Department of Chemistry,
Rajshahi University of Engineering and Technology, Rajshahi-6204, Bangladesh
Tel: 88 0172 534726 E-mail: alam@yahoo.com

^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H, -OCH_3), 2.57 (s, 6H, -OCH_3 X 2), 4.78 (s, 2H, C_3-H and C_5-H), 5.55 (m, 1H, C_5'-H), 6.08 (m, 1H, C_4'-H), 5.64 (dd, 1H, J= 2.6 and 9 Hz, C_3'-H), 6.54 (dd, 1H, J= 2.6 and 9 Hz, C_6'-H), 6.67 (d, 1H, J= 16 Hz, C_α-H), 7.03 (d, 1H, J= 16 Hz, C_β-H), 12.61 (s, 1H, C_2-OH).

^{13}C NMR ($CDCl_3$): δ 121.9 (C-1′), 157.8 (C-2′), 113.7 (C-3′), 134.9 (C-4′), 119.8 (C-5′), 133.1 (C-6′), 186.7 (>C=O), 123.8 (C-α), 143.3 (C-β), 108.5 (C-1), 159.9 (C-2 and C-6) 96.9 (C-3 and C-5), 162.4 (C-4), 56.8 (2 and 6-OMe), 56.3 (4-OMe).

Synthesis of 2′, 4′, 6′-trimethoxyflavone (6, $C_{18}H_{16}O_5$) using DMSO/I_2: The chalcone (4, 2 mmol, 628 mg) was suspended in dimethyl sulphoxide (DMSO, 25 mL) and a crystal of iodine[6] was added to it. The mixture was refluxed for 20 min. in a silicon oil bath and diluted with water. The solid obtained was filtered off, washed with 20% aq. sodium thiosulphate It was purified by preparative TLC over silica gel GF_{254} using benzene-acetone (12:1) as developing solvent and crystallized from ether as light needles (358 mg), yield 57.00%, mp. 142-143°C, R_f 0.67 (benzene-acetone; 10:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. Found : C,59.58; H, 5.30%; Calc. for $C_{18}H_{16}O_5$; C, 69.22; H, 5.16%

UV λ_{max}^{EtOH}: 231, 278 and 388 nm

IR ν_{max}^{KBr}: 2991, 2886, 1637, 1601, 1570, 1542, 1515, 1480, 1456, 1414, 1360, 1305, 1296, 1262, 1205, 1190, 1152, 1105, 1061, 1022, 991, 972, 929, 855, 828, 810, 771, 745, 720, 685, 651, 613 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 2.54 (s, 3H, -OCH_3), 2.59 (s, 6H, -OCH_3 X 2), 4.81 (s, 2H, C_3'-H and C_5'-H), 5.30 (s, 1H, C_3-H), 5.53 (dd, 1H, J= 2.5 and 8.6 Hz, C_8-H), 5.49- 5.55 (m, 1H, C_6-H), 6.56 (dd, 1H, J= 2.5 and 8.6 Hz, C_5-H), 6.84-6.87 (m, 1H, C_7-H).

^{13}C NMR ($CDCl_3$): δ 166.9 (C-2), 98.9 (C-3), 187.6 (C-4), 123.3 (C-4a), 129.3 (C-5), 121.9 (C-6), 135.7 (C-7), 116.4 (C-8), 157.6 (C-8a), 102.9 (C-1′), 161.5 (C-2′ and C-6′), 97.7 (C-3′ and C-5′), 163.9 (C-4′), 56.6 (2′ and 6′-OMe), 56.1 (4′-OMe).

Synthesis of 2′, 4′, 6′-trimethoxyflavone (6, $C_{18}H_{16}O_5$) using Ph-S-S-Ph: The chalcone (4, 2 mmol, 628 mg) was pasted with diphenyl sulphide[7] (145 mg) in a mortar and the mixture was transferred to a 100 mL three necked round bottom flask equipped with nitrogen inlet and outlet tubes. The central neck was closed by a glass stopper. The flask was then dipped into a silicon oil bath and heated at 265°C under nitrogen atmosphere until the distilling of the thiols formed through the other outlet tube ceased (2.5 h). The reaction mixture was then cooled at room temperature and 20 mL chloroform was added. The organic layer was washed with water several times. It was dried over anhydrous sodium sulphate and the solvent was removed by distillation. The product crystallized from ethanol as colorless needles (301 mg), yield 48.00%, mp. 142-143°C, R_f 0.67 (benzene-acetone; 10:1). 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 2′, 4′, 6′-trimethoxyflavone (6, $C_{18}H_{16}O_5$) using DDQ: The chalcone (4, 2 mmol, 628 mg) in dry dioxane (100 mL) was added DDQ (185 mg) and the solution refluxed for 3 h. The product purified by preparative TLC over silica gel using petroleum sprit-benzene (1:2) as developing solvent. It crystallized from ethanol colorless needles (424 mg), yield 67.50%, mp. 142-143°C, R_f 0.67 (benzene-acetone; 10:1). 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 and diphenyl sulphide method.

Synthesis of 2′-hydroxy-4′-benzyloxy-2, 4, 6-trimethoxychalcone (5, $C_{25}H_{24}O_6$): A mixture of 2-hydroxy-4-benzyloxyacetophenone (2, 10 mmol, 2.42 g) and 2, 4, 6-trimethoxybenzaldehyde (3, 1.1 eqv., 2.16 g) in ethanolic solution of KOH (5%, 15 mL) was kept at room temperature for about 75 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 evaporated to dryness. The reaction mixture was subjected to column chromatography over silica gel. The elution was done with ether–acetone (10:1) and crystallized from benzene-acetone mixture as yellow crystals (2.95 g), yield 64.50%, m.p.138-139°C, R_f 0.71 (benzene: acetone; 12:1).

Anal. Found : C, 71.53; H, 5.18%; Calc. for $C_{25}H_{24}O_6$; C, 71.41; H, 5.75%

UV λ_{max}^{MeOH}: 242, 285 and 385 nm.

IR ν_{max}^{KBr}: 3450, 2938, 2842, 1652, 1606, 1575, 1548, 1505, 1472, 1458, 1415, 1357, 1337, 1309, 1281, 1206, 1189, 1163, 1132, 1117, 1064, 1020, 1002, 972, 873, 856, 831, 817, 766, 739, 700, 648 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 2.61 (s, 3H, -OCH_3), 2.67 (s, 6H, -OCH_3 X 2), 3.85 (s, 2H, -O-$C\underline{H}_2$-C_6H_5), 4.89 (s, 2H, C_3-H and C_5-H), 5.26 (s, 1H, C_3'-H), 5.29 (d, 1H, J = 8.6 Hz, C_5'-H), 6.12

(s, 5H, -O-CH_2-$C_6\underline{H}_5$), 6.57 (d, 1H, J= 8.6 Hz, C_6'-H), 6.68 (d, 1H, J= 16 Hz, C_α-H), 7.09 (d, 1H, J= 16 Hz, C_β-H), 12.60 (s, 1H, C_2-OH).

^{13}C NMR ($CDCl_3$): δ 118.9 (C-1′), 158.7 (C-2′), 103.4 (C-3′), 170.9 (C-4′), 109.6 (C-5′), 132.8 (C-6′), 81.5 (-O-$\underline{C}H_2$-C_6H_5), 141.3 (C-1′′), 127.4 (C-2′′ and C-6′′), 128.8 (C-3′′ and C-5′′), 127.9 (C-4′′), 186.7 (>C=O), 123.6 (C-α), 142.9 (C-β), 103.5 (C-1), 160.8 (C-2 and C-6) 97.8 (C-3 and C-5), 162.9 (C-4), 56.3 (2 and 6-OMe), 55.9 (4-OMe).

Synthesis of 7-benzyloxy-2′, 4′, 6′-trimethoxyflavone (7, $C_{25}H_{22}O_6$) using DMSO/I_2: The chalcone (5, 1 mmol, 420 mg) was suspended in dimethyl sulphoxide (DMSO, 15 mL) and a crystal of iodine[6] was added to it. The mixture was refluxed for 15 min. in a silicon oil bath and diluted with water. The solid obtained was filtered off, washed with 20% aq. sodium thiosulphate. It was purified by preparative TLC over silica gel GF_{254} using benzene acetone (12:1) as developing solvent and crystallized from ethyl acetate as yellow needles (250 mg), yield 59.50%, mp. 148-149°C, R_f 0.63 (benzene-acetone; 10:1). It gave blue fluorescence in UV light and positive Mg/HCl test.

Anal. Found: C, 71.67; H, 5.41%; Calc. for $C_{25}H_{22}O_6$; C, 71.76; H, 5.30%

UV λ_{max}^{EtOH}: 225, 276 and 385 nm

IR ν_{max}^{KBr}: 2941, 2881, 1637, 1601, 1581, 1551, 1501, 1465, 1405, 1356, 1331, 1302, 1275, 1205, 1180, 1161, 1128, 1110, 1055, 1012, 1000, 975, 865, 840, 826, 805, 756, 731, 696, 641 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ 2.62 (s, 3H, -OCH_3), 2.68 (s, 6H, -OCH_3 X 2), 3.86 (s, 2H, -O-$C\underline{H}_2$-C_6H_5), 4.89 (s, 2H, C_3'-H and C_5'-H), 5.20 (d, 1H, J = 8.6 Hz, C_6-H), 5.27 (s, 1H, C_8-H), 5.61 (s, 1H, C_3-H), 6.13 (s, 5H, -O-CH_2-$C_6\underline{H}_5$), 6.58 (d, 1H, J= 8.6 Hz, C_5-H).

^{13}C NMR ($CDCl_3$): δ 167.6 (C-2), 97.8 (C-3), 187.3 (C-4), 121.3 (C-4a), 131.1 (C-5), 110.9 (C-6), 168.7 (C-7), 106.4 (C-8), 158.2 (C-8a), 80.3 (-O-$\underline{C}H_2$-C_6H_5), 141.6 (C-1′′), 127.7 (C-2′′ and C-6′′), 129.2 (C-3′′ and C-5′′), 128.6 (C-4′′), 102.8 (C-1′), 161.7 (C-2′ and C-6′), 98.1 (C-3′ and C-5′), 164.1 (C-4′), 56.5 (2′ and 6′-OMe), 56.1 (4′-OMe).

Synthesis of 7-benzyloxy-2′, 4′, 6′-trimethoxyflavone (7, $C_{25}H_{22}O_6$) using Ph-S-S-Ph: The chalcone (8, 1 mmol, 420 mg) was pasted with diphenyl sulphide[7] (135 mg) in a mortar and the mixture was transferred to a 100 mL three necked round bottom flask equipped with nitrogen inlet and outlet tubes. The central neck was closed by a glass stopper. The flask was then dipped into a silicon oil bath and heated at 265°C under nitrogen atmosphere until the distilling of the thiols formed through the other outlet tube ceased (2.5 h). The reaction mixture was then cooled at room temperature and 20 mL chloroform was added. The organic layer was washed with water several times. It was dried over anhydrous sodium sulphate and the solvent was removed by distillation. The product crystallized from ethyl acetate as pale yellow needles (256 mg), yield 61.00%, mp. 148-149°C, R_f 0.63 (benzene-acetone; 10:1). 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.

Synthesis of 7-benzyloxy-2′, 4′, 6′-trimethoxyflavone (7, $C_{25}H_{22}O_6$) using DDQ: The chalcone (5, 1 mmol, 420 mg) in dry dioxane (75 mL) was added DDQ (175 mg) and the solution refluxed for 3 h. The product purified by preparative TLC over silica gel using petroleum sprit-benzene (1:2) as developing solvent. It crystallized from ethyl acetate as pale yellow needles (248 mg), yield 59.00%, mp. 148-149°C, R_f 0.63 (benzene-acetone; 10:1). 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 and diphenyl sulphide method.

Antibacterial screening: The antibacterial activities of synthesized compounds 4, 5, 6 and 7 were studied against four human pathogenic bacteria, viz., *Shigella dysenteriae* (G^-), *Pseudomonas aeruginosa* (G^-), *Sarcina lutea* (G^+) and *Bacillus subtilis* (G^+). For the detection of antibacterial activities the filter paper disc diffusion method[8,9] was performed. Kanamycin was used as standard antibiotics for the antibacterial activities. Nutrient Agar (NA) was used as basal medium for test bacteria. These agar media were inoculated with 0.5 mL of the 24 h liquid cultures containing 10^7 microorganisms mL^{-1}. The diffusion time was 24 h at 5°C for bacteria. The incubation time was 12 h at 37°C for bacteria. Discs with only DMSO were used as control. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones.

Determination of the Minimum Inhibitory Concentration (MIC): Minimal inhibitory concentration is defined as the lowest concentration that inhibits bacterial growth. To determine of the Minimum Inhibitory Concentration (MIC) the serial dilution tecnique[10] was followed using nutrient broth medium. The MIC value of the compound 6 and 7 were determined against *Pseudomonas aeruginosa* (G^-) and *Bacillus subtilis* (G^+).

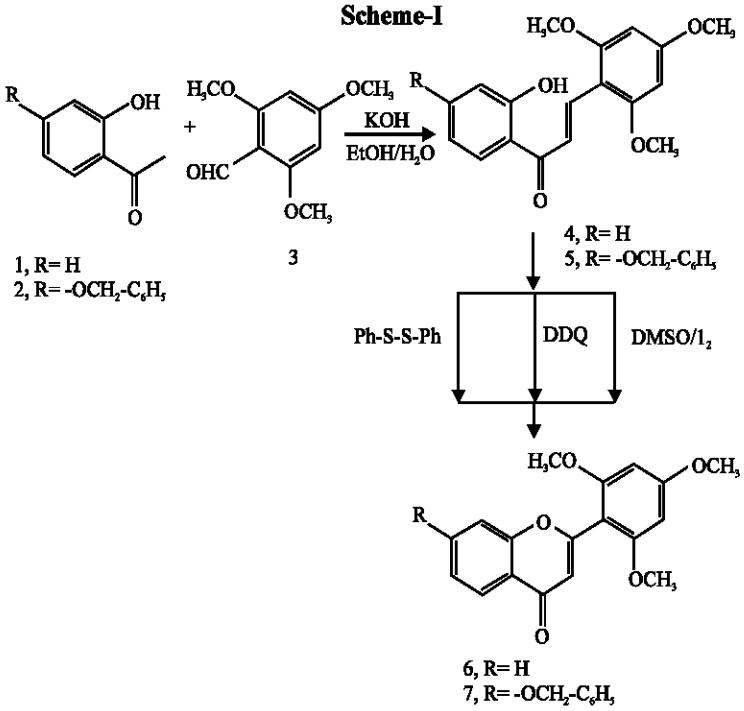
Antifungal screening: The antifungal activities of compound 4, 5, 6 and 7 were studied towards five plant pathogenic and molds fungi, viz., *Colletorichum gloeosporioides* Penz. (Plant pathogen), *Candida albicans* (Human pathogen), *Aspergillus nigar* (Molds), *Aspergillus flavus* (Molds) and *Penicillium* sp. (Blue molds). The antifungal activity was assessed by poisoned food technique[11], in some modified condition [12]. Fluconazole (200 μg/disc) was used as standard fungicide for the antifungal activity. Potato Dextrose Agar (PDA) was used as basal medium for test fungi. Glass petridishes were sterilized and sterilized melted PDA medium (~45°C) was poured at the rate of 15 mL in each petridish (90 mm). After solidification of the medium the small portions of mycelium of each fungus were spreaded 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 samples were placed gently on the solidified agar plates, freshly seeded with the test organisms with sterile forceps. Control disc was 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. After this, 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) of the compounds initially. Proper control was maintained with Dimethyl sulphoxide (DMSO).

RESULTS AND DISCUSSION

The syntheses of 2′, 4′, 6′-trimethoxyflavone and 7-benzyloxy-2′, 4′, 6′-trimethoxyflavone were accomplished starting from 2-hydroxyacetophenone (1) and 2-hydroxy-4-benzyloxyacetophenone[13] (2), respectively as shown in scheme-I.

Aldol condensation of 2-hydroxyacetophenone (1) and 2, 4, 6-trimethoxybenzaldehyde (3) produced 2′-hydroxy-2, 4, 6-trimethoxychalcone (4). It was obtained as yellow crystals, mp. 132-134°C. The structure of this chalcone 4 has been confirmed by spectral data and elemental analysis. The UV absorption band of 4 (λ_{max} 238, 275 and 373 nm) suggested the presence of a chalcone skeleton. It gave brown color with alcoholic ferric chloride solution but no IR absorption band for hydroxy group (-OH)[13]. This was supported by (I) a relatively weaker IR band at 1623 cm^{-1} (chelated >C=O) and (ii) an appropriately dishielded phenolic proton signal at δ 12.61 (1H, s), exchangeable with D_2O. The ^1H NMR spectrum explained the presence of three methoxyl groups in the B ring at δ 2.50 and 2.57 as two singlets, integrating for three and six protons, respectively. The two aromatic protons

Scheme-I



of the B ring, which appeared as a singlet at δ 4.78 assigned to C_3-H and C_5-H, integrating for two protons. The C_α-H and C_β-H protons of 4 appeared as two doublets at δ 6.67 (J = 16 Hz) and 7.03 (J = 16 Hz) integrating for one proton each. The four aromatic protons of the A ring appeared as two double doublets and two multiplets at δ 5.64 (dd, 1H, J= 2.6 and 9 Hz), 6.54 (dd, 1H, J= 2.6 and 9 Hz), 6.08 (m, 1H) and 5.55 (m, 1H) assigned to C_3', C_6', C_4' and C_5' protons, respectively.

Oxidation of chalcone 4 into the corresponding flavone 6 was carried out differently by using DMSO/I_2, diphenyl sulphide and DDQ reagent. Compound 6 was obtained as light yellow, mp. 142-143°C. The structure of this flavone 6 has been confirmed by spectral data and elemental analysis. The UV absorption band of 6 (λ_{max} 231, 278 and 388 nm) suggested the presence of a flavone skeleton. The IR absorption frequency at υ 1637 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. The ^{1}H NMR spectrum of flavone 6 indicated the presence of three methoxyl groups by two singlets at δ 2.54 and 2.59, integrating for three and six protons, respectively. The aromatic protons of both the A and B ring appeared in a similar pattern as appeared for the chalcone 4. The flavone 6 also gave a characteristics singlet at δ 5.38 for C_3-H proton.

Claisen condensation of 2-hydroxy-4-benzyloxyacetophenone (2) and 2, 4, 6-trimethoxybenzaldehyde (3) gave 2′-hydroxy-4′-benzyloxy-2, 4, 6-trimethoxychalcone (5). The chalcone 5 was obtained as yellow crystals, mp. 138-139°C. The structure of this chalcone 5 has been confirmed by spectral data and elemental analysis. The UV absorption band of 5 (λ_{max} 242, 285 and 385 nm) suggested the presence of a chalcone skeleton. The IR absorption frequency at υ 3450 cm^{-1} indicated the presence of hydroxyl group and υ 1652 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H NMR spectrum explained the presence of a benzyloxy group by two singlets at δ 3.85 (2H, -O-C$\underline{H_2}$-C_6H_5) and 6.12 (2H, -O-CH_2-$C_6\underline{H_5}$) integrating for two and five protons, respectively. The two aromatic protons of the B ring, which appeared as a singlet at δ 4.89 assigned to C_3-H and C_5-H, integrating for two protons. The ^{1}H NMR spectrum of 5 also explained the presence of three methoxyl groups in the B ring at δ 2.61 and 2.67 as two singlets, integrating for three and six protons, respectively. The C_α-H and C_β-H protons of 5 appeared as two doublets at δ 6.68 (J = 16 Hz) and 7.09 (J = 16 Hz) integrating for one proton each. The three aromatic protons of A ring which appeared as an ABC system at δ 5.26 (s, 1H, C_3'-H), 5.29 (d, 1H, J = 8.6 Hz, C_5'-H) and 6.57 (d, 1H, J = 8.6 Hz, C_6'-H) integrating for one proton each. A singlet at δ 12.60 were indicated the presence of a chelated phenolic proton at C-2, integrating for one proton.

Cyclization of chalcone 5 into the corresponding flavone 7 were done differently by using DMSO/I_2, diphenyl sulphide and DDQ reagent. The flavone 7 was obtained as yellow needles, mp. 148-149°C. The structure of this flavone 7 has been supported by spectral data and elemental analysis. The UV spectrum of this flavone 7 (λ_{max} 225, 276 and 385 nm) suggested the presence of a flavone nucleus. The IR absorption frequency at υ 1637 cm^{-1} showed the presence of a carbonyl group (>C=O) and the absence of a hydroxyl group band, confirmed the oxidation of chalcone 5 into flavone 7 and it was also supported by the ^{1}H NMR spectrum of flavone 7. The ^{1}H NMR spectrum of 7 explained the presence of three methoxyl groups in the B ring at δ 2.62 and 2.68 as two singlets, integrating for three and six protons, respectively. The aromatic and methylene protons of O-benzyl group of 7 appeared in the usual way. The two aromatic protons of the B ring, which appeared as a singlet at δ 4.89, were assigned to C_3'-H and C_5'-H protons, integrating for two protons. The three aromatic protons of A ring which appeared as an ABC system at δ 5.26 (s, 1H, C_8-H), 5.28 (d, 1H, J = 8.6 Hz, C_6-H) and 6.58 (d, 1H, J = 8.6 Hz, C_5-H) integrating for one proton each. The flavone 7 also gave a characteristic singlet at δ 5.61 assigned to C_3-H proton.

Antibacterial activities: The antibacterial activities of compounds 4, 5, 6 and 7 have been assayed at the concentration of 100, 200 and 300 μg/disc against four human pathogenic bacteria. Among them, two were gram-positive and the rest two were gram-negative. The inhibitory effects of compounds 4, 5, 6 and 7 against these organisms are given in Table 1 and 2.

The screening results indicate that compound 4 and 5 did not show any antibacterial activity to the tested bacteria. Compound 6 showed low antibacterial activity at a concentration of 300 μg/disc where as compound 7 showed moderate activity. From the above result it can be concluded that flavone ring system is responsible for the antibacterial activity of compound 6 and 7.

Minimum inhibitory activity: The minimum inhibitory concentration of the compound 6 and 7 were determined *Bacilus subtillis* and *Pseudomonas aeruginosa* by serial dilution method. The MIC level of the compound 6 was found 256 μg mL^{-1} against *Bacilus subtillis* and *Pseudomonas aeruginosa* and for 7 was found 64μg mL^{-1} against *Bacilus subtillis* and *Pseudomonas aeruginosa*.

Table 1: Antibacterial screening for the compound 4 and 6

Comp.	Concentration µg/disc	*Shigella dysenteriae*	*Pseudomonas aeruginosa*	*Sarcina lutea*	*Bacilus subtilis*
4	100	-	-	-	-
	200	-	-	-	-
	300	-	-	-	-
6	100	-	-	-	-
	200	-	-	-	-
	300	7	9	6	10
K-30*	30	26	28	34	30

Table 2: Antibacterial screening for the compound 5 and 7

Comp.	Concentration µg/disc	*Shigella dysenteriae*	*Pseudomonas aeruginosa*	*Sarcina lutea*	*Bacilus subtilis*
5	100	-	-	-	-
	200	-	-	-	-
	300	-		-	
7	100	-	9	-	8
	200	9	13	8	14
	300	12	15	10	19
K-30*	30	26	28	34	30

*Kanamycin-30

Table 3: Antifungal screening for the compound 4

Name of the organisms	Diameter of the zone of inhibition (mm) 100 µg/disc	200 µg/disc	300 µg/disc	Fluconazole 200 µg/disc
Penicillium sp.	-	-	-	-
Aspergillus nigar	-	-	-	8
Aspergillus flavus	-	-	-	10
Candida albicans	-	-	-	9
Colletorichum gloeosporioides.	-	-	-	-

Table 4: Antifungal screening for the compound 5

Name of the organisms	Diameter of the zone of inhibition (mm) 100 µg/disc	200 µg/disc	300 µg/disc	Fluconazole 200 µg/disc
Penicillium sp.	-	6	8	-
Aspergillus nigar	-	-	-	8
Aspergillus flavus	-	-	-	10
Candida albicans	-	-	-	9
Colletorichum gloeosporioides	-	7	10	-

Table 5: Antifungal screening for the compound 6

Name of the organisms	Diameter of the zone of inhibition (mm) 100 µg/disc	200 µg/disc	300 µg/disc	Fluconazole 200 µg/disc
Penicillium sp	-	-	-	-
Aspergillus nigar	-	-	-	8
Aspergillus flavus	-	-	-	10
Candida albicans	-	-	-	9
Colletorichum gloeosporioides				

Table 6: Antifungal screening for the compound 7

Name of the organisms	Diameter of the zone of inhibition (mm) 100 µg/disc	200 µg/disc	300 µg/disc	Fluconazole 200 µg/disc
/disc				
Penicillium sp.	-	5	6	-
Aspergillus nigar	-	-	-	8
Aspergillus flavus	-	-	-	10
Candida albicans	-	-	-	9
Colletorichum gloeosporioides	-	8	12	-

Antifungal activities: The antifungal activities of compounds 4, 5, 6 and 7 have been assayed at the concentration of 100, 200 and 300 µg/disc against five plants pathogenic and molds fungi. The inhibitory effects of compounds 4, 5, 6 and 7 against these organisms are given in Table 3-6, respectively.

The screening results indicate that the compound 4 and 6 did not show any antifungal activities to the tested fungi. Where as compound 5 and 7 showed moderate antifungal activity against *Penicillium* sp and *Colletorichum gloeosporioides* in comparison with standard fungicides. The above results reveals that compound 5 and 7 showed antifungal activity due to the presence of benzyloxy group ($-OCH_2-C_6H_5$).

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