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Synthesis and Biological Activity of 7-Benzyloxy and 7-Methoxy Flavone

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Abstract. Benzyl and methyl derivatives of 7-hydroxyflavone were synthesized from 2-hydroxy-4-benzyloxy-acetophenone and 2-hydroxy-4-methoxyacetophenone, respectively, via chalcone precursor and by the treatment with DMSO/I_2, diphenyl sulphide and dichloro dicyano quinone (DDQ). The antibacterial and antifungal activity of these flavones and their corresponding chalcones were found *in vitro* by the filter paper disc diffusion method and poisoned food technique.

Keywords: 7-hydroxyflavone, derivatives, antibacterial and antifungal activity

Introduction

The flavonoids are a group of natural products founds in fruits, vegetables, nuts, seeds and flowers as well as in tea and wine, and are important constituent of human diet. They have been demonstrated to posses many biological and pharmacological activities such as biocidal (Rao *et al.*, 1990), pharmaceutical (Akama *et al.*, 1997; Wolfman *et al.*, 1996; Wu *et al.*, 1992), antioxidant (Chan *et al.*, 2000; Rice-Evans *et al.*, 1996), anti-inflammatory (Dao *et al.*, 2004), antimutagenic, antiallergic activities, and inhibitory activities on several enzymes (Vender Berghe *et al.*, 1993; Bors *et al.*, 1990). In the light of these findings we have already synthesized a number of flavone derivatives and studied their biological activities (Alam *et al.*, 2005, 2004; Alam, 2004). 7-Hydroxyflavone (Roy and Pandey, 1994) has been isolated from the flowers of *Clerodendron phlomidis*. Here we describe the syntheses of benzyl and methyl derivatives of 7-hydroxyflavone from their corresponding chalcones 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

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

Synthesis of 2′-hydroxy-4′-benzyloxychalcone (3). A mixture of 2-hydroxy-4-benzyloxyacetophenone (**1**, 10 mmol, 2.42 g) and benzaldehyde (1.1 eqv., 1.10 g) in ethanol 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 dilute 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 collected and subjected to column chromatography over silica gel. The elution was done with ether: acetone (7:1). It was crystallized from benzene-petroleum spirit as yellow needles (2.62 g), yield 74.60%; mp. 122-23 °C; R_f 0.63 (benzene: acetone; 9:1); Analysis: found C, 76.35; H, 4.65%; calc. for $C_{22}H_{16}O_4$; C, 76.73; H, 4.68%; UV (MeOH): λ_{max} 241, 272, 368 nm; IR (KBr) : ν_{max} 3431 (OH), 1634 (conjugate > CO), 1585, 1471 cm^{-1} (aromatic ring); ^{1}H NMR ($CDCl_3$) : δ 5.14 (s, 2H, -O-C$\underline{H}_2$-C_6H_5), 6.52 (s, 1H, C_3′-H), 6.61 (d, 1H, J = 8.6 Hz, C_5′-H), 6.12 (s, 5H, -O-CH_2-$C_6\underline{H}_5$), 7.59 (d, 1H, J = 16 Hz, C_α-H), 7.16 (d, 1H, J = 8.6 Hz, C_6′-H), 7.29-7.34 (m, 5H, C_2-H, C_3-H, C_4-H, C_5-H and C_6-H), 8.18 (d, 1H, J = 16 Hz, C_β-H), 12.68 (s, 1H, C_2-OH).

Synthesis of 7-benzyloxyflavone (5) using DMSO/I_2. The chalcone (**3**, 2 mmol, 660 mg) was suspended in dimethyl sulphoxide (DMSO, 10 ml) and a crystal of iodine was added to it (Doshi *et al.*, 1986). 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% aqueous sodium thiosulphate. It was purified by preparative TLC over silica gel GF_{254} using benzene: acetone (10:1) as developing solvent and crys-

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tallized from ether as colourless needles (409 mg), yield 62%; mp. 94-95 °C; R_f 0.67 (benzene: acetone; 9:1); Analysis: found C, 77.54; H, 4.47%; calc. for $C_{19}H_{16}O_3$; C, 77.18; H, 4.12%. UV(EtOH) : λ_{max} 228, 280, 385 nm; IR (KBr) : ν_{max} 1635 (conjugated > CO), 1596, 1576, 1492, 1450 cm^{-1} (aromatic ring); ^1H NMR ($CDCl_3$) : δ 5.11 (s, 2H, -O-C<u>H</u>$_2$-C_6H_5), 6.48 (d, 1H, J = 2.5 Hz, C_8-H), 6.63 (d, 1H, J = 8.6 Hz, C_6-H), 5.43 (s, 1H, C_3-H), 6.22 (s, 5H, -O-CH_2-C_6<u>H</u>$_5$), 7.12 (d, 1H, J = 8.6 Hz, C_5-H), 7.30-7.35 (m, 5H, C_2'-H, C_3'-H, C_4'-H, C_5'-H and C_6'-H).

Synthesis of 7-benzyloxyflavone (5) using Ph-S-S-Ph. The chalcone (**3**, 2 mmol, 660 mg) was pasted with diphenyl sulphide (Hoshino *et al.*, 1986) (125 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 chloroform : hexane (4 : 1) as pale yellow needles (383 mg), yield 58 %; mp. 94-95 °C; R_f 0.67 (benzene: acetone; 9 : 1). Spectral data of this flavone (**5**) was also similar to that prepared by DMSO/I_2 method.

Synthesis of 7-benzyloxyflavone (5) using DDQ. The chalcone (**3**, 2 mmol, 660 mg) in dry dioxane (50 ml) was added DDQ (155 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 chloroform-hexane as pale yellow needles (602 mg), yield 69%; mp. 94-95 °C; R_f 0.67 (benzene : acetone; 9:1).

Spectral data of this flavone (**5**) was also similar to that prepared by DMSO/I_2 and diphenyl sulphide method.

Synthesis of 2′-hydroxy-4′-methoxychalcone (4). A mixture of 2-hydroxy-4-methoxyacetophenone (**2**, 10 mmol, 1.66 g) and benzaldehyde (1.1 eqv., 1.10 g) in ethanolic solution of KOH (5%, 15 ml) was kept at room temperature for about 75 h and workup as previously described method. It was crystallized from benzene-petroleum spirit as yellow needles (2.41 g), yield 87.5%; mp. 106-107 °C; R_f 0.56 (benzene : acetone; 4 : 1); Analysis: found C, 75.72; H, 5.25%; calc. for $C_{16}H_{14}O_3$; C, 75.57; H, 5.55%; UV (MeOH) : λ_{max} 244, 360 nm; IR (KBr) : ν_{max} 3450 (OH), 1625 (conjugated > CO), 1590, 1470 cm^{-1} (aromatic ring); ^{1}HNMR ($CDCl_3$). δ 3.81 (s, 1H, -OCH_3), 6.41 (s, 1H, C_3'-H), 6.56 (d, 1H, J = 8.6 Hz, C_5'-H), 6.97 (d, 1H, J = 8.6 Hz, C_6'-H), 7.22-7.27 (m, 5H, C_2-H, C_3-H, C_4-H, C_5-H and C_6-H), 7.61 (d, 1H, J = 16 Hz, C_α-H), 8.11 (d, 1H, J = 16 Hz C_β-H), 12.67 (s, 1H, C_2-OH).

Synthesis of 7-methoxyflavone (6) using DMSO/I_2. The flavone (**6**) was prepared by previously described method and it was crystallized from benzene as pale yellow needles (387 mg), yield 76.20%, mp. 108-109 °C; R_f 0.42 (benzene : acetone; 9:1); It gave a blue fluorescence in UV light. Analysis: found C, 76.48; H, 4.41%; calc. for $C_{16}H_{12}O_3$; C, 76.18; H, 4.79%; UV (EtOH) : λ_{max} 230, 285, 370 nm. IR (KBr) : ν_{max} 1653 (conjugated > CO), 1575, 1534, 1507, 1495, 1464 cm^{-1} (aromatic ring); ^{1}H NMR ($CDCl_3$) : δ 3.85 (s, 3H, -OCH_3), 5.42 (s, 1H, C_3-H), 6.45 (s, 1H, C_8-H), 6.78 (d, 1H, J = 8.4 Hz, C_6-H), 7.18 (d, 1H, J = 8.4 Hz, C_5-H), 7.33-7.35 (m, 5H, C_2'-H, C_3'-H, C_4'-H, C_5'-H and C_6'-H).

Synthesis of 7-methoxyflavone (6) using Ph-S-S-Ph. The flavone (**6**) was prepared by previously described method using Ph-S-S-Ph and it was crystallized from chloroform-hexane (4:1) as pale yellow needles (350 mg), yield 69%, mp. 108-109 °C, R_f 0.42 (benzene : acetone; 9:1). Spectral data of this flavone (**6**) was also similar to that prepared by DMSO/I_2 method.

Synthesis of 7-methoxyflavone (6) using DDQ. The chalcone (**4**, 2 mmol, 508 mg) in dry dioxane (50 ml) was added DDQ (155 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 chloroform-hexane as pale yellow needles (298 mg), yield 58.60%, mp. 108-109 °C, R_f 0.42 (benzene-acetone; 9 : 1). Spectral data of this flavone (**6**) was also similar to that prepared by DMSO/I_2 and diphenyl sulphide method.

Antibacterial screening. The antibacterial activity of synthesized compounds (**3**), (**4**), (**5**) and (**6**) was 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 activity the filter paper disc diffusion method (Arima *et al.*, 2002; Jeongmok *et al.*, 1995) 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. 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 technique (Nishina *et al.*, 1987) was followed using

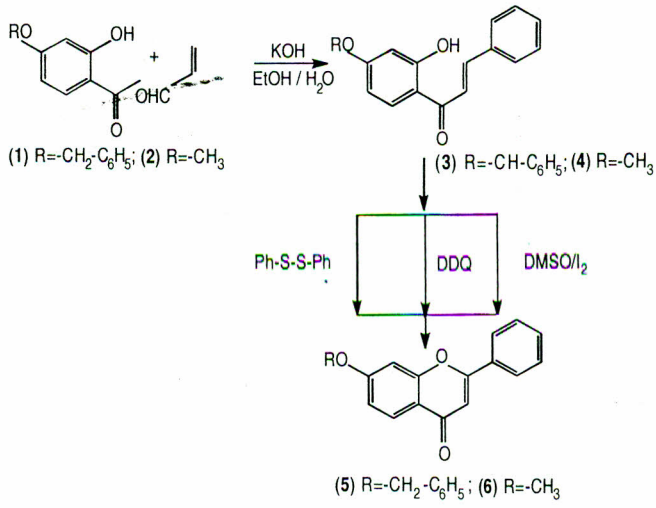
nutrient broth medium. The MIC value of the compound (**3**) and (**9**) were determined against *Pseudomonas aeruginosa* (G^-) and *Bacillus subtilis* (G^+).

Antifungal screening. The antifungal activity of compound (**2**), (**3**), (**8**) and (**9**) was 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 (Grover *et al.*, 1962) in some modified condition (Miah *et al.*, 1990). Fluconazole (200 µg/disc) was used as standard fungicide for the anti-fungal 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 DMSO.

Results and Discussion

The syntheses of 7-benzyloxyflavone and 7-methoxyflavone were accomplished starting from 2-hydroxy-4-benzyloxy-acetophenone (**1**) (Aneja *et al.*, 2003) and 2-hydroxy-4-meth-oxyacetophenone (**2**), respectively as shown in scheme-1.

Cross aldol-condensation of 2-hydroxy-4-benzyloxyaceto-phenone (**1**) and benzaldehyde furnished 2′-hydroxy-4′-benzyloxychalcone (**3**). It was obtained as yellow needles, mp. 122-123 °C. The structure of this chalcone (**3**) has been confirmed by spectral data and elemental analysis. The UV absorption band of (**3**) (λ_{max} 241, 272 and 368 nm) suggested the presence of a chalcone skeleton. The IR absorption frequency at υ 3431 cm^{-1} indicated the presence of hydroxyl group and at υ 1634 cm^{-1} showed the presence of a conjugated



Scheme - 1

carbonyl group (> C = O). The ^{1}H NMR spectrum explained the presence of a benzyloxy group by two singlets at δ 5.14 (2H, -O-C<u>H</u>$_2$-C$_6$H$_5$) and 6.12 (2H, -O-CH$_2$-C$_6$<u>H</u>$_5$) integrating for two and five protons, respectively. The five aromatic protons of the unsubstituted B ring was appeared as multiplet at δ 7.29-7.34 integrating for five protons and the other three aromatic protons of A ring were appeared as an ABC system at δ 6.52 (s, 1H, C$_3$′-H), 6.61 (d, 1H, J = 8.6 Hz, C$_5$′-H) and 7.16 (d, 1H, J = 8.6 Hz, C$_6$′-H). The C$_\alpha$-H and C$_\beta$-H protons of (**3**) appeared as two doublets at δ 7.59 (J = 16 Hz) and 8.18 (J = 16 Hz) integrating for one proton each. The chelated C$_2$-OH proton was appeared as singlet at δ 12.68 integrating for one proton.

Cyclization of chalcone (**3**) into the corresponding flavone (**5**) were done differently by using DMSO/I$_2$, diphenyl sulphide and DDQ reagent. The flavone (**5**) was obtained as colourless needles, mp. 94-95 °C. The formation of (**5**) has been sup-ported by spectral data and elemental analysis. The UV spec-trum of this flavone (**5**) (λ_{max} 241, 272 and 368 nm) suggested the presence of a flavone nucleus. The IR absorption frequency at υ 1635 cm^{-1} showed the presence of a carbonyl group (> C = O) and the absence of a hydroxyl group band, con-firmed the oxidation of chalcone (**3**) into flavone (**5**) and it was also supported by the ^{1}H NMR spectrum of flavone (**5**). The five aromatic protons of the unsubstituted B ring was appeared as multiplet at δ 7.30-7.35 integrating for five protons. The other three aromatic protons of A ring were was appeared as an ABC system at δ 6.48 (s, 1H, C$_8$-H), 6.63 (d, 1H, J = 8.6 Hz, C$_6$-H) and 7.12 (d, 1H, J = 8.6 Hz, C$_5$-H)

integrating for one proton each. The C_3-H proton of the flavone nucleus appeared as singlet at δ 5.43 integrating for one proton.

Claisen condensation of 2-hydroxy-4-methoxyacetophenone (**2**) and benzaldehyde gave 2′-hydroxy-4′-methoxychalcone (**4**). The chalcone (**4**) was obtained as yellow needles, mp. 106-107 °C. The UV absorption band of chalcone (**4**) was appeared at 244 and 360 nm. It showed IR absorption frequency at υ 3450 cm^{-1} and 1625 cm^{-1} indicating the presence of a hydroxyl and a carbonyl group, respectively. The ^1H NMR spectrum of chalcone (**4**) was very much similar with the chalcone (**3**), except the presence of O-methyl group instead of O-benzyl group at position 4 of the A ring.

Oxidation of chalcone (**4**) into the corresponding flavone (**6**) using differently DMSO/I_2, diphenyl sulphide and DDQ reagent was carried out. The flavone (**6**) was obtained as pale yellow needles, mp. 108-109 °C. The UV spectrum of this flavone (**6**) (λ_{max} 230, 285 and 370 nm) suggested the presence of a flavone nucleus. The IR absorption frequency at υ 1653 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 flavone (**6**) gave the ^1H NMR spectrum in similar fashion of flavone (**5**), except the presence of O-methyl group instead of O-benzyl group at position 7 of the A ring.

Antibacterial activity. The antibacterial activity of compounds **3, 4, 5** and **6** 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 were gram-negative. The inhibitory effects of compounds (**3**), (**4**), (**5**) and (**6**) against these organisms are given in Table 1.

The screening results indicate that compound (**3**), (**4**) and (**5**) did not show any marked antibacterial activity to the tested bacteria. Compound (**6**) showed moderate antibacterial activity against the tested bacteria at the high concentration in comparison with the standard antibiotic, Kanamycin-30.

Minimum inhibitory activity. The minimum inhibitory concentration of the compound (**5**) and (**6**) were determined against *Bacilus subtillis* and *Pseudomonas aeruginosa* by serial dilution method. The MIC level of the compound (**5**) was found to be 256 μg/ml against *P. aeruginosa* whereas it did not show any MIC level against *B. subtillis* and for (**6**) was found to be 64 μg/ml against *B. subtillis* and *P. aeruginosa*.

Antifungal activity. The antifungal activity of compounds (**3**), (**4**), (**5**) and (**6**) assayed at the concentration of 100, 200 and 300 μg/disc against five plant pathogenic and molds fungi. The inhibitory effects of compounds (**3**) (**4**), (**5**) and (**6**) against these organisms are given in Table 2.

Table 1. Antibacterial screening for the compounds (**3**), (**4**), (**5**) and (**6**)

Compound	Conc. μg/disc	Diameter of the zone of inhibition (mm)			
		Shigella dysenteriae	*Pseudomonas aeruginosa*	*Sarcina lutea*	*Bacilus subtillis*
(**3**)	100	--	--	--	--
	200	--	--	--	--
	300	12	--	--	--
(**4**)	100	--	--	--	--
	200	--	--	--	--
	300	10	--	5	--
(**5**)	100	--	--	--	--
	200	--	--	--	--
	300	9	11	17	--
(**6**)	100	--	10	--	8
	200	10	13	9	12
	300	13	18	10	14
*K-30	30	26	28	34	30

* = Kanamycin-30

Table 2. Antifungal screening for the compounds (**3**), (**4**), (**5**) and (**6**)

Compound	Conc. μg/disc	Diameter of the zone of inhibition (mm)				
		Penicil-lium sp.	*Asper-gillus niger*	*Asper-gills flavus*	*Candida albicans*	*Colletorichum gloeosporioides*
(**3**)	100	--	--	--	--	--
	200	–	–	–	–	–
	300	--	--	--	6	--
(**4**)	100	–	–	–	–	–
	200	6	--	--	7	--
	300	11	--	--	--	--
(**5**)	100	–	–	–	–	–
	200	5	--	--	--	--
	300	9	--	--	7	--
(**6**)	100	6	--	--	--	--
	200	8	--	--	5	--
	300	13	--	--	9	--
Fluco-nazole	200	--	--	10	--	--

The screening results indicate that the compound (**3**) did not show any antifungal activity against *Penicillium* sp., *Aspergillus niger, Aspergillus flavus* and *Colletorichum gloeosporioides* except *Candida albicans*. Whereas, compound (**4**), (**5**) and (**6**) showed good antifungal activities against *Penicillium* sp. and *C. albicans* in comparison with standard fungicides and did not show antifungal activities against *A. niger, A. flavus* and *C. gloeosporioides*.

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References

Akama, T., Ishida, H., Kimura, U., Gomi, K., Saito, H., Fuse, E., Kobayashi, S., Yoda, N., Kasai, M. 1997. Design and synthesis of potents antitumor 5,4′-diaminoflavone derivatives based on metabolic considerations. *J. Med. Chem.* **40:** 1894-1900.

Alam, S., Miah, M. A.J., Islam, A. 2005. Synthesis and studies of antimicrobial activity of two benzylated flavones. *J. Bang. Chem. Soc.* **18:** 30-40.

Alam, S., Sarkar, Z., Islam, A. 2004. Synthesis and studies of antibacterial activity of pongaglabol. *J. Chem. Sci.* **116:** 29-32.

Alam, S. 2004. Synthesis and studies of antimicrobial activity of lanceolatin B. *Acta Chim. Slov.* **51:** 447-452.

Aneja, R., Khanna, R.N., Sheshadri, T.R. 1963. 6-Methoxy-furoflavone, a new component of the seeds of *Pongmia glabra*. *J. Chem. Soc.* 163-168.

Arima, H., Ashida, H., Danno, G.I. 2002. Rutinenhanched antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. *Biosci. Biotechnol. Biochem.* **66:** 1014-1015.

Bors, W., Heller, W., Michel, C., Saran, M. 1990. In: *Methods in Enzymology*, L. Packer, A.N. Glazer (eds.), vol. **186**, pp. 343-355, Academic Press, New York, USA.

Chan, E.C.H., Patchareewan, P., Owen, L.W. 2000. Relaxation of flavones and flavonoids in rat isolated thoracic aorta: mechanism of action and structure-activity relationships. *J. Cardiovasc. Pharmacol.* **35:** 326-333.

Dao, T.T., Chi, Y.S., Kim, J., Kim, H.P., Kim S., Park, H. 2004. Synthesis and inhibitory activity against COX-2 catalyzed prostaglandin production of chrysin derivatives. *Bioorg. Med. Chem. Lett.* **14:** 1165-1167.

Doshi, A.G., Soni, P.A., Ghiya, B.J. 1986. Oxidation of 2′-hydroxychalcones. *Indian J. Chem.* **258:** 759.

Grover, R.K., Moore, J.D. 1962. Toximetric studies of fungicides against the brown rot organisms, *Sclerotinia fructicola* and *S. laxa*. *Phytopathology* **52:** 876.

Hoshino, Y., Oohinata, T., Takeno, N. 1986. The direct preparation of flavones from 2′-hydroxychalcones using-disulphides. *Bull. Chem. Soc. Japan.* **59:** 2351.

Jeongmok, K., Maurice, R.M., Cheng, I.W. 1995. Antibacterial activity of some essential oil components against five food-borne pathogens. *J. Agric. Food Chem.* **43:** 2834.

Miah, M.A.T., Ahmed, H.U., Sharma, N.R., Ali, A., Miah, S.A. 1990. Antifungal activity of some plant extracts. *Bangladesh J. Bot.* **19:** 5.

Nishina, C., Enoki, N., Tawata, S., Mori, A., Kobayashi, K., Fukushima, M. 1987. Antibacterial activity of flavonoids against *Staphylococcus epidermidis*, a skin bacterium. *Agri. Biol. Chem.* **51:** 139.

Rao, K.V., Chattopadhyay, S.K., Reddy, G.C. 1990. Flavonoids with mosquito larval toxicity. *J. Agric. Food. Chem.* **38:** 1427-1430.

Rice-Evans, C.A., Miller N.J., Paganga, G. 1996. Structur-Antioxidant activity relationships of flavonoids and phenolic acids. *Free Rad. Biol. Med.* **20:** 933-956.

Roy, R., Pandey, V.B. 1994. A chalcone glycoside from clerodendron phlomidis. *Phytochemistry* **37:** 1775-1776.

Vender Berghe, D.A.R., Haemers, A., Vlieunek, A.J. 1993. *Bioactive natural products; detection and structural determination*, S.M.Colegate, K.J.Molyneux (eds.), pp. 405-440, CRC Press, London, UK.

Wolfman, C., Viola, H., Marder, M., Wasowski, C., Ardenghi, P., Izquierdo, I., Paladini, A.C., Medina, J.H. 1996. Anxioselective properties of 6,3′-dinitroflavone, a high-affinity benzodiazepine receptor ligand. *Eur. J. Pharmacol.* **318:** 23-30.

Wu, E.S.C., Loch, J.T., Toder, B.H., Borrelli, A.R., Gawlak, D. Radov L.A., Gensmantel, N.P. 1992. Synthesis, biological activities and conformational analysis of isoflavone derivatives and related compounds. *J. Med. Chem.* **35:** 3519-3525.