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Synthesis and Antioxidant Activity of Some Chalcones and Flavanoids

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Abstract: In the present work we report the reaction of 2,4-dihydroxy acetophenone with different substituted aromatic aldehyde to form 2,4-dihydroxy chalcones. Various analogues of 7-hydroxy flavanoids were reported by oxidative cyclization of 2,4-dihydroxy chalcones. The structure of the newly synthesized chalcones (C1-C5) and flavanoids (F1-F5) were established by their elemental analysis and spectral data (IR, ^{1}H NMR). These newly synthesized compounds were also evaluated for *in-vitro* antioxidant activity by Diphenyl Picryl Hydrazine (DPPH) model. Out of 10 test compounds evaluated for their antioxidant activity, the test compounds C1 and F1 showed strong antioxidant activity (IC_{50}= 40.52 µg/mL and 42.90 µg/mL respectively) similar to that of the standard (ascorbic acid) (IC_{50}=31.48 µg/mL) used.
Keywords: Chalcones, Flavanoids, Antioxidant Activity, DPPH Model.

Introduction

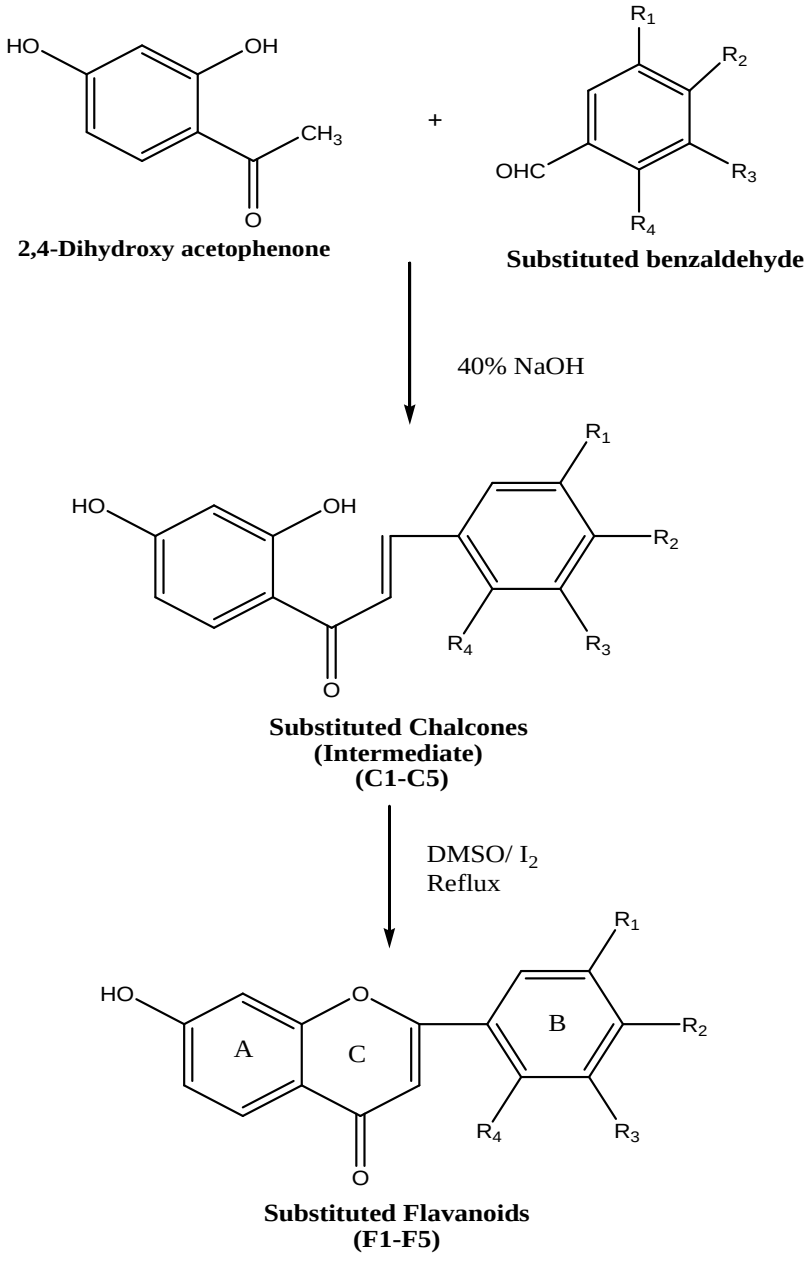
Flavanoids are a broad class of polyphenolic secondary metabolites abundant in plant and in a variety of common foods such as apples, onions, tea, and red wine. Flavonoids have their vital role in the synthetic chemistry as their major occurring class is flavone (2-phenyl chromen-4-one). Flavones and their synthetic derivatives signify very potent pharmacological activities including antioxidant[1,2], antibacterial[3-6], antifungal[7-9], anticancer[10-13], antiviral[14], anti HIV[15,16], antidiabetic[17], antihistaminics[18], antitubercular[19], cardioprotective[20], gastroprotective[21] and anti-inflammatory[22-24] properties.

Flavones have been a potential source in the search for lead compounds and biologically active components and have been the focus of many researchers. Their interesting pharmacological activities prompted us to design a novel series of chalcones and flavanoids in attempt to get compound with remarkable antioxidant activity. The aim of the study was to synthesize some substituted 2,4-dihydroxy chalcones and 7-hydroxy flavanoids as well as evaluate them for their antioxidant activity. To investigate the positional effects of different groups on the B ring on bioactivity, compounds C1-C5 & F1-F5 were prepared using a straightforward chemical approach, as shown in scheme 1.

Experimental

All the chemicals used for the synthesis of the compounds were obtained from Merck and SD Fine chemicals. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin Elmer) using KBr disc method. ^{1}HNMR spectra were recorded on ^{1}H NMR (Brucker AMX 300 MHz) spectrometer in $CDCl_3$. The compounds were analyzed for elemental analysis. Physical data of the compounds are recorded in Table-1 and the spectral data are recorded in Table-2.

Scheme 1



Where;

Chalcones	Flavanoids	R_1	R_2	R_3	R_4
C1	**F1**	H	OH	H	H
C2	**F2**	H	OCH_3	H	H
C3	**F3**	H	Cl	H	H
C4	**F4**	H	H	NO_2	H
C5	**F5**	OCH_3	H	H	OCH_3

General procedure for the synthesis of chalcone (C1-C5)

A mixture of 2,4-dihydroxy acetophenone (0.01 mol, 1.52 g) and aryl aldehydes (0.01 mol) was taken in ethanol (30 mL) and stirred at 10-15 ºC. To this solution an aqueous solution of sodiumm hydroxide (40 %) (5.0 mL) was added drop wise with continuous stirring. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with dilute hydrochloric acid. The chalcone precipitated as solid. The precipitated chalcone was collected and recrystallized from ethanol. Purity of the synthesized compound was checked by TLC analysis.

General procedure for the synthesis of flavanoids from chalcones (F1-F5)

A solution of substituted chalcone (0.001 mol) and iodine (0.1 mmol) in DMSO (5.0 mL) was refluxed for 45 min. The mixture was cooled, diluted with water and filtered. The filtrate was washed with sodium thiosulphate solution (20 %) to remove iodine and subsequently washed with water. The product obtained was recrystallized from ethanol to afford title compounds. Purity of the synthesized compound was checked by TLC analysis.

Table 1: Physical properties of synthesized compounds

S. No.	Code No.	Molecular Formula (Molecular Weight)	% Yield	M.p. (ºC)	R_f Value*	Elemental Analysis (%) Calcd. (Found)		
						C	H	N/Cl
1	C1	$C_{15}H_{12}O_4$ (256.25)	73	168-169	0.68	70.31 (70.29)	4.72 (4.73)	-
2	C2	$C_{16}H_{14}O_4$ (270.28)	76	175-176	0.66	71.10 (71.09)	5.22 (5.21)	-
3	C3	$C_{15}H_{11}ClO_3$ (274.7)	83	197-198	0.73	65.58 (65.56)	4.04 (4.05)	12.91 (12.92)
4	C4	$C_{15}H_{11}NO_5$ (285.25)	65	210-212	0.82	63.16 (63.15)	3.89 (3.87)	4.91 (4.92)
5	C5	$C_{17}H_{16}O_5$ (300.31)	68	186-187	0.77	67.99 (67.97)	5.37 (5.38)	-
6	F1	$C_{15}H_{10}O_4$ (254.24)	66	202-203	0.76	70.86 (70.88)	3.96 (3.95)	-
7	F2	$C_{16}H_{12}O_4$ (268.26)	70	248-249	0.71	71.64 (71.65)	4.51 (4.52)	-
8	F3	$C_{15}H_9ClO_3$ (272.68)	67	263-264	0.78	66.07 (66.06)	3.33 (3.33)	13.00 (13.02)
9	F4	$C_{15}H_9NO_5$ (283.24)	65	285-286	0.80	63.61 (63.60)	3.20 (3.22)	4.95 (4.95)
10	F5	$C_{17}H_{14}O_5$ (298.29)	61	255-257	0.63	68.45 (68.46)	4.73 (4.72)	-

*****Solvent system: Pet. ether: ethylacetate (2:3)

Table 2: Spectral data of synthesized compounds

S. No.	Code No.	IR (KBr) ν (cm-1)	^{1}H NMR ($CDCl_3$)/ δ in ppm
1	C1	3261 (O-H str.), 3015 (Ar=C-H str.), 1674 (C=O str.), 1590 (C-C str.), 1554, 1461, 1429 (Ar C=C str.)	6.92-7.92 (m, 7H, Ar-H), 7.67 (d, 1H, *α*-H), 7.95 (d, 1H, *β*-H), 12.81 (s, 3H, 3 x OH)
2	C2	3310 (O-H str.), 3037 (Ar=C-H str.), 2842 (C-H str.), 1675 (C=O str.), 1592 (C-C str.), 1552, 1448, 1412 (Ar C=C str.), 1120 (C-O str.)	3.86 (s, 3H, OCH_3), 6.91-7.90 (m, 7H, Ar-H), 7.64 (d, 1H, *α*-H), 7.93 (d, 1H, *β*-H), 12.94 (s, 2H, 2 x OH)
3	C3	3326 (O-H str.), 3032 (Ar=C-H str.), 1680 (C=O str.), 1594 (C-C str.), 1563, 1507, 1465 (Ar C=C str.), 668 (C-Cl str.)	6.94-8.00 (m, 7H, Ar-H), 7.64 (d, 1H, *α*-H), 7.91 (d, 1H, *β*-H), 12.75 (s, 2H, 2 x OH)
4	C4	3206 (O-H str.), 3094 (Ar=C-H str.), 1680 (C=O str.), 1593 (C-C str.), 1550, 1477, 1415 (Ar C=C str.), 1490 (N-O asym. str.), 1366 (N-O sym. str.)	7.26-7.64 (m, 7H, Ar-H), 7.59 (d, 1H, *α*-H), 7.90 (d, 1H, *β*-H), 11.94 (s, 2H, 2 x OH)
5	C5	3260 (O-H str.), 3035 (Ar=C-H str.), 2844 (C-H str.), 1683 (C=O str.), 1580 (C-C str.), 1557, 1476, 1454 (Ar C=C str.), 1212 (C-O str.)	3.83 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 6.93-7.51 (m, 6H, Ar-H), 7.55 (d, 1H, *α*-H), 7.87 (d, 1H, *β*-H), 11.90 (s, 2H, 2 x OH)
6	F1	3258 (O-H str.), 3010 (Ar=C-H str.), 1686 (C=O str.), 1589 (C-C str.), 1516, 1462, 1419 (Ar C=C str.), 1190 (C-O-C str.)	7.13-8.53 (m, 7H, Ar-H), 6.83 (s, 1H, *α*-H), 12.38 (s, 2H, 2 x OH)
7	F2	3324 (O-H str.), 3065 (Ar=C-H str.), 2840 (C-H str.), 1658 (C=O str.), 1600 (C-C str.), 1559, 1458, 1420 (Ar C=C str.), 1196 (C-O-C str.), 1124 (C-O str.)	3.80 (s, 3H, OCH_3), 7.29-6.89 (m, 7H, Ar-H), 6.86 (s, 1H, *α*-H), 11.05 (s, 1H, OH)
8	F3	3341 (O-H str.), 3012 (Ar=C-H str.), 1667 (C=O str.), 1598 (C-C str.), 1567, 1485, 1443 (Ar C=C str.), 1178 (C-O-C str.), 689 (C-Cl str.)	6.86-7.78 (m, 7H, Ar-H), 6.82 (s, 1H, *α*-H), 11.46 (s, 1H, OH)
9	F4	3200 (O-H str.), 3117 (Ar=C-H str.), 1670 (C=O str.), 1612 (C-C str.), 1576, 1456, 1426 (Ar C=C str.), 1488 (N-O asym. str.), 1369 (N-O sym. str.), 1167 (C-O-C str.)	7.66-7.31 (m, 7H, Ar-H), 6.73(s, 1H, *α*-H), 12.71(s, 1H, OH)
10	F5	3342 (O-H str.), 3038 (Ar=C-H str.), 2846 (C-H str.), 1669 (C=O str.), 1587 (C-C str.), 1553, 1460, 1432 (Ar C=C str.), 1214 (C-O str.), 1117 (C-O-C str.)	3.57 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.90-7.29 (m, 6H, Ar-H), 6.86 (s, 1H, *α*-H), 12.59 (s, 1H, OH)

***In-vitro* antioxidant activity of synthesized chalcones and flavanoids**

In the present study, *in-vitro* antioxidant activity of newly synthesized compounds was performed by DPPH model[25,26]. The method employed was by determining the free radical inhibitory ability of different antioxidant by using very stable free radical such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. Stock solution of DPPH (1.3 mg/mL) in methanol was prepared. Stock solution of DPPH 100 µL was added to 3.0 mL of methanol and absorbance was recorded at 516 nm. The various concentrations of compounds (25, 50 and 100 µg/mL) were prepared. All sample solutions 1.0 mL each is diluted with 3.0 mL with methanol and 100 µL of

stock solution of DPPH was added. Test tubes were kept for 30 min in light to complete the reaction. After 30 min, absorbance of each test tube was recorded at 516 nm on UV-VIS spectrophotometer against methanol as a blank. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations (figure: 1 & 2). Regression equations to derived IC_{50} values showed inverse relationship between IC_{50} values and percentage scavenging potential of compound.

The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Where;

Control is absorbance of a DPPH solution without compound;

Test is the absorbance of the test compound with DPPH.

The degree of discoloration indicates the free radical scavenging efficiency of the compound. Ascorbic acid was used as the free radical scavenger reference compound.

Table 3: Observation for *in-vitro* antioxidant activity of synthesized compounds

S. No.	Code No.	% Inhibition (DPPH-Scavenging)			IC_{50} (µg/mL)
		25 µg/mL	50 µg/mL	100 µg/mL	
1	C1	41.05±0.02	55.12±0.04	69.08±0.17	40.52
2	C2	38.04±0.01	51.05±0.23	65.05±0.11	47.45
3	C3	34.22±0.37	48.44±0.15	61.09±0.16	57.98
4	C4	19.01±0.12	26.00±0.2	32.50±0.09	>100
5	C5	36.80±0.14	50.60±0.03	63.54±0.12	49.12
6	F1	39.16±0.14	53.06±0.08	67.07±0.1	42.90
7	F2	35.01±0.06	48.30±0.01	61.13±0.02	58.64
8	F3	32.89±0.21	44.51±0.12	59.29±0.11	70.50
9	F4	17.11±0.16	23.31±0.01	30.00±0.08	>100
10	F5	34.12±0.08	48.60±0.26	60.48±0.25	61.46
Std. drug (Ascorbic acid)		46.12±0.16	60.14±0.1	78.30±0.01	31.48

Data represents mean ± S.D. of triplicate analysis

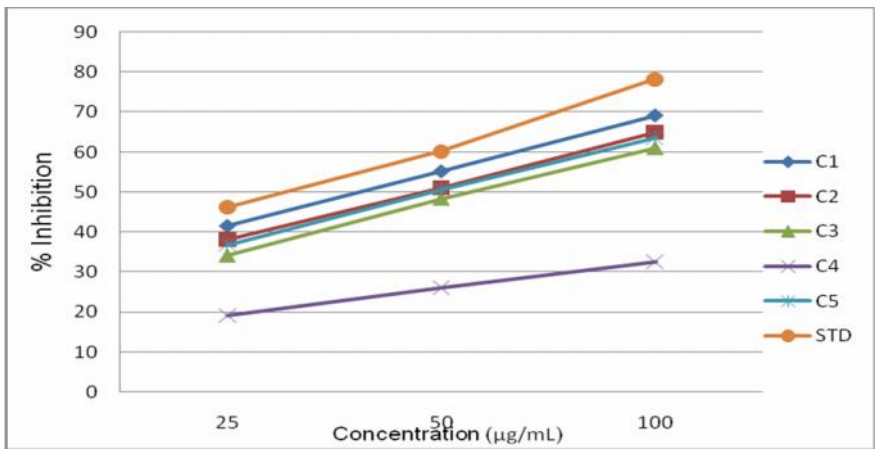


Figure:1 Graph showing DPPH scavenging activity of chalcones (C1-C5)

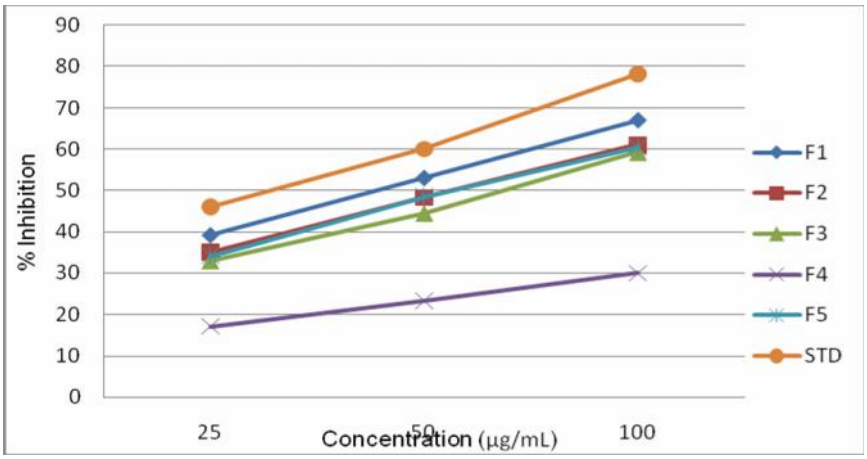


Figure:2 Graph showing DPPH scavenging activity of flavonoids (F1-F5)

Results And Discussion

In the present study, the synthesis of title compounds has been carried out successfully from the starting material 2,4-dihydroxy acetophenone. The purity of the newly synthesized compounds was established by TLC and melting point. The structures of chalcones and flavanoids were confirmed on the basis of spectral data (IR, ^{1}H NMR). Chalcones showed the IR absorptions characteristics of phenolic -OH (3200-3350 cm^{-1}), carbonyl >C=O (1685-1600 cm^{-1}) and aromatic C=C (1580-1400 cm^{-1}) functionalities. The ^{1}H NMR spectra of chalcones displayed multiplet due to aromatic protons at 6.92-8.00 δ (m, Ar-H) and singlet due to phenolic –OH at 11.94-12.75 δ (s, 2H, 2 x OH). The characteristics signals for a chalcone moiety appeared as two doublets at 7.55-7.79 δ (d, 1H, *α*-H) and 7.82-7.95 δ (d, 1H, *β*-H). Similarly IR spectra of flavanoids shows a broad peak of at 3342-3200 cm^{-1} (Ar-OH) due to presence of phenolic –OH group, 3117-3010 cm^{-1} (aromatic stretching), 1686-1610 cm^{-1} (C=O pyrone ring). The presence of >C=O stretching for pyrone ring confirmed the formation of benzopyrone ring present in flavanoids. The ^{1}H NMR spectra of flavanoids displayed multiplet due to aromatic protons at 6.89-8.53 δ (m, H, Ar-H) and singlet for phenolic –OH at13.1-11.46 δ (s, H, OH) instead of doublet as showed in respective chalcone also confirmed the cyclization of chalcones into flavanoids. The characteristics signals for -CO-C*H*=C< appeared as singlets at 6.73-6.86 δ (s, 1H, *α*-H) and also absence of peak for -CO-CH=C*H*- proton gave evidence for the use of *β*-H in the cyclization process of chalcone. All the compounds (C1-C5 & F1-F5) gave satisfactory IR, ^{1}H NMR and elemental analysis data correlation with the assign structure.

The antioxidant activity results in table3 show that most of the compounds exhibited moderate to good antioxidant activity compared to that of standard (ascorbic acid) (IC_{50}=31.48 µg/mL). The data indicate that the compounds with good activity have a para electron-withdrawing substituent on B ring. As a typical compound, C1 and F1 with para hydroxy group on the ring B had the strongest activity (IC_{50}= 40.52 µg/mL and 42.90 µg/mL respectively). By contrast, compounds C4 and F4, with a meta electron-withdrawing nitro group, displayed weak activity (IC_{50}>100 µg/mL). These results suggested that the substitution pattern of hydroxyl group on ring A and B may be crucial for their antioxidant activity enhancement. The para substituted group exhibited better free radical scavenging activity than ortho and meta substituted systems as C5 and F5 compounds (IC_{50}= 49.12 µg/mL and 61.46 µg/mL respectively) showed less activity having ortho, meta-disubstituted methoxy derivative compared toC2 and F2 having para-substituted methoxy derivative (IC_{50}= 47.45 µM and 58.64 µM respectively). Standard drug ascorbic acid showed IC_{50}=31.48 µM

In conclusion, on the basis of the above findings, the substituted hydroxy chalcones and substituted hydroxyl flavanoids scaffolds were selected as skeleton for the development of flavanoid structurally-related compounds having antioxidant activity. In spite of this, chalcones could also be selected in our synthetic lead-optimization study because they were also shown good antioxidant activity. However, a further evaluation of chalcones and flavanoids will be undertaken, concerning the structural requirements on B ring for antioxidant activity.

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