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Studies of Antimicrobial Activity of 2′,4′,5′- and 2′,3′,4′-Trimethoxy Flavanones

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Abstract

2′,4′,5′- and 2′,3′,4′-trimethoxy flavanones have been synthesized starting with 2-hydroxyacetophone and substituted aldehyde. Antibacterial activities of the flavanones have been tested along with their corresponding chalcones against two human pathogenic bacteria (*Streptococcus-β-haemolyticus* and *Klebsiella sp. (G-)*). Antifungal activities of the flavanones have also been investigated against two plants pathogenic mold fungi (*Rhizactonia solani Sclerotium rolfsii*). The structures of the synthesized compounds have been characterized with the help of UV, IR and ^{1}H-NMR and ^{13}C-NMR spectra. The antibacterial and antifungal screening were performed *in vitro* by the filter paper disc diffusion method and poisoned food technique. The flavanones showed antibacterial activity while no activity was observed to their corresponding chalcones against the tested bacteria. On the other hand, chalcones and their corresponding flavanones both showed fungicidal activities.

Keywords: Aldol reaction; Chalcone; Flavanone; Antibacterial activity; Antifungal activity.

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1. Introduction

Flavonoids are widely occurring in natural plant pigments and medicinal plants [1-4]. The flavonoid compounds are a group of natural products found in fruits, vegetables, nuts, seeds and flowers as well as in teas and wines and are important constituent of human diet [5]. Synthesis of flavonoid compounds has attracted considerable attention because of their significant biocidal [6-8] and pharmaceutical [9-13] effects. Because of the exciting biological activities, many flavonoid compounds have been synthesized and studied their antibacterial and antifungal activities [14-19].

In this paper, we describe syntheses of 2′,4′,5′-trimethoxy flavanone **6** and 2′,3′,4′-trimethoxy flavanone **7** from their corresponding chalcones **4** and **5**, and antibacterial and antifungal activities of these flavanones along with their chalcone precursors.

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3. Experimental

3.1. *Materials and Instruments*

All melting points were recorded on Gallenkamp apparatus and were uncorrected. The IR spectra (KBr) were measured using a Shimadzu, DR-8001 spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra ($CDCl_3$) were recorded on a Brucker WH 400 MHZ instrument with TMS as an internal standard. The UV-Vis spectra were recorded on a LKB 4053 spectrophotometer using MeOH as a solvent. The purity of the compounds was checked by TLC.

3.2. *Synthesis of 2′-hydroxy-2,4,5-trimethoxychalcone (4, $C_{18}H_{18}O_5$)*

2-hydroxyacetophenone (**1**, 0.27 g) and 2,4,5-trimethoxybenzaldehyde (**2**, 0.43 g) in ethanolic solution of KOH (5%, 15 ml) were mixed together and 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 (420 mg), yield 60%, m.p. 124-125°C, R_f 0.77 (benzene:acetone = 4:1).

Anal Found: C, 69.17; H, 6.17%; Calc. for $C_{18}H_{18}O_5$; C, 69.47; H, 6.37%. UV λ_{max}^{MeOH} **:** 235, 260 and 365 nm. IR (KBr): 3400, 2930, 2840, 1680, 1600, 1501, 1460, 1375, 1345, 1304, 1275, 1225, 1145, 1095, 1020, 950, 903, 880, 810, 767, 608 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.93 (s, 3H, -OCH_3), 3.80 (s, 6H, –OCH_3 × 2), 5.90 (s, 1H, C_6-H), 6.93 (d, 2H, $C_{3'}$ and $C_{6'}$-H), 7.00 (s, 1H, C_α-H), 7.45 (s, 1H, C_β-H), 13.06 (s, 1H, -OH). ^{13}C ($CDCl_3$): δ 117 (C-1′), 155 (C-2′), 111 (C-3′), 120 (C-4′), 128 (C-5′), 135 (C-6′), 163 (>C=O), 140 (C-α), 145 (C-β), 152 (C-1), 155 (C-2), 115 (C-3), 117 (C-4), 123 (C-5), 119 (C-6), 56.55 ($C_{2'}$-OCH_3), 55.05 ($C_{4'}$-OCH_3), 56.28 ($C_{5'}$-OCH_3).

3.3. *Synthesis of 2′,4′,5′-trimethoxyflavanone (6, $C_{18}H_{18}O_5$)*

The chalcone (**4**, 1 g) was cyclized by refluxing with 5% ethanolic sulphuric acid (50 mL) for 72 h. The reaction mixture was kept at room temperature for 24 h. and worked-up as usual. TLC examination of the residue showed several spots and the major product was purified by preparative TLC using n-hexane-acetone (5:1) as developing solvent. The flavanone crystallized from benzene-petroleum spirit mixture as yellow flakes (560 mg), yield 56%, m.p. 138°C. It showed blue florescence spot in UV light, R_f 0.47 (n-hexane-acetone = 4:1). It gave greenish brown color with ferric chloride solution.

Anal Found: C, 68.56; H, 5.64%; Calc. for $C_{18}H_{18}O_5$; C, 68.79; H, 5.73%. UV λ_{max}^{MeOH} **:** 245, 270 and 380 nm. IR (KBr): 2980, 2940, 2850, 1645, 1590, 1562, 1492, 1466, 1416, 1369, 1292, 1261, 1107, 1072, 1025, 1006, 905, 870, 798, 770, 755, 620 cm^{-1}. ^{1}H-NMR

($CDCl_3$): δ 3.95 (s, 3H, -OCH_3), 3.85 (s, 6H, -OCH_3 × 2), 6.16 (s, 2H, $C_{3'}$-H and $C_{6'}$-H), 3.13 (dd, 2H, C_3-H), 5.44 (dd, 1H, C_2-H), 7.16 (m, 2H, C_6 and C_7-H), 7.89 (dd, 1H, C_5-H), 7.48 (d, 1H, C_8-H). ^{13}C-NMR ($CDCl_3$): δ 61.7 (C-2), 48.2 (C-3), 195.3 (C-4), 127.9 (C-5), 118.2 (C-6), 131.3 (C-7), 115.2 (C-8), 120.6 (C-1′), 153.3 (C-2′), 99.9 (C-3′), 148.1 (C-4′), 139.2 (C-5′), 113.8 (C-6′), 56.11 ($C_{2'}$-OCH_3), 56.32 ($C_{4'}$-OCH_3), 58.7 ($C_{5'}$-OCH_3).

3.4. *Synthesis of 2′-hydorxy-2,3,4-trimethoxychalcone (5, $C_{18}H_{18}O_5$)*

2′-hydorxy-2,3,4-trimethoxychalcone **5** was synthesized from 2-hydroxyacetophenone (**1**, 0.27 g) and 2,3,4-trimethoxybenzaldehyde (0.43 g) as mentioned above. The elution was done with ether-acetone (8:1) and crystallized from benzene-acetone mixture as yellow crystals (406 mg, yield 60%), m.p. 105-107°C, R_f 0.76 (benzene:acetone = 4:1).

Anal Found: C, 69.17; H, 6.17%; Calc. for $C_{18}H_{18}O_5$; C, 68.77; H, 5.77%. UV λ_{max}^{MeOH}: 230, 270 and 370 nm. IR (KBr): 3420, 2943, 2839, 1685, 1601, 1500, 1462, 1423, 1373, 1342, 1304, 1277, 1227, 1146, 1099, 1022, 946, 903, 879, 810, 767, 690 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.85 (s, 3H, -OCH_3), 3.78 (s, 6H, -OCH_3 × 2), 4.58 (s, 2H, C_4 and C_5-H), 5.69 (d, 1H, C_6-H), 5.73 (d, 1H, $C_{5'}$-H), 6.57 (d, 1H, C_α-H), 7.47 (d, 1H, C_β -H), 12.20 (s, 1H, -OH). ^{13}C-NMR ($CDCl_3$): δ 102.1 (C-1), 160.5 (C-2), 113.2 (C-3), 118.2 (C-4), 120.5 (C-5), 160.2 (C-6), 175.9 (>C=O), 125.2 (C-α), 140.5 (C-β), 99.4 (C-1′), 161.7 (C-2′), 152.4 (C-3′), 163.5 (C-4′), 133.2 (C-5′), 145.3 (C-6′), 56.1 ($C_{2'}$-OCH_3), 56.5 ($C_{3'}$-OCH_3), 56.9 ($C_{4'}$-OCH_3).

3.5. *Synthesis of 2′,3′,4′-trimethoxyflavanone (7, $C_{18}H_{18}O_5$)*

The chalcone (**5**, 1g) was cyclized by refluxing with 5% H_2SO_4 for 72 h and product obtained from preparative TLC (310 mg), yield 31%, as pale yellow flakes, m.p. 118°C. It showed blue florescence spot in UV light, R_f 0.41 (n-hexane:acetone = 4:1).

Anal Found: C, 68.53, H, 5.61%; Calc. for $C_{18}H_{18}O_5$; C, 68.79; H, 5.73%. UV λ_{max}^{MeOH}: 235, 280 and 380 nm. IR (KBr): 2970, 2939, 2850, 1647, 1593, 1562, 1492, 1466, 1416, 1369, 1292, 1261, 1134, 1107, 1072, 1026, 1006, 906, 872, 798, 771, 756, 621 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.88 (s, 3H, -OCH_3), 3.81 (s, 6H, -OCH_3 × 2), 2.81-3.13 (2d, 1H, C_3-H), 5.44 (dd, 1H, C_3-H), 6.89 (d, 1H, $C_{5'}$-H), 7.11 (d, 1H, $C_{6'}$-H), 7.29 (d, 1H, C_5-H), 6.61 (d, 1H, C_8-H), 6.89 (m, 1H, C_6-H), 7.22 (m, 1H, C_7-H). ^{13}C-NMR ($CDCl_3$): 65.5 (C-2), 48.9 (C-3), 198.8 (C-4), 122.2 (C-4a), 128.3 (C-5), 119.2 (C-6), 135.5 (C-7), 115.3 (C-8), 160.5 (C-8a), 122.8 (C-1′), 149.6 (C-2′), 135.3 (C-3′), 149.4 (C-4′), 108.2 (C-5′), 121.3 (C-6′), 55.2 ($C_{2'}$-O<u>C</u>H_3), 56.5 ($C_{3'}$-O<u>C</u>H_3), 56.9 ($C_{4'}$-O<u>C</u>H_3).

3.6. *Antibacterial screening tests*

The antibacterial activity of synthesized compounds **4-7** were studied against two human pathogenic bacteria, viz., *Streptococcus-β-haemolyticus* (G^+) and *Klebsiella* sp. (G^-). For detection of antibacterial activities, the filter paper disc diffusion method [20,21] was

performed. Kanamycin was used as standard antibiotics for the antibacterial activities. Nutrient Agar (NA) was used as basal medium for antibacterial test. These agar media were inoculated with 0.5 mL of the 24 h liquid cultures containing 10^7 microorganism h mL^{-1}. The diffusion time was 24 h at 5°C for bacteria. The incubation time was assigned to be 12 h. at 37°C for bacteria. Discs with only DMSO were used as control. The diameter (in mm) of the observed inhibition zones were taken as a measure of inhibitor activity.

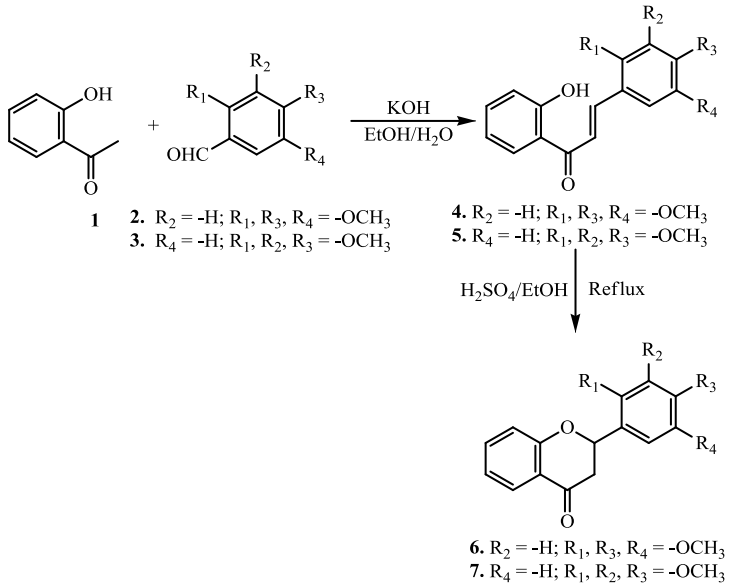
3.7. *Antifungal screening tests*

The antifungal activity of the synthesized compounds **4-7** were studied against two plants pathogenic and molds fungi, viz. *Rhizactonia solani* and *Sclerotium rolfsii*. The antifungal activity was assessed by poisoned food technique [22] with modified condition [23]. Fluconazole (200 μg/disc) and Potato Dextrose Agar (PDA) were used as standard fungicide for the antifungal activity and basal medium for test fungi, respectively. Glass petri dishes were sterilized and 15 mL of sterilized melted PDA medium (~45°C) was poured into each petridish (90 mm). After solidification of the medium, a small portion of mycelium of each fungus was spread carefully over the centre 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 disc of samples was 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 to get sufficient time to diffuse 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.

4. Results and Discussions

The synthesis of 2′,4′,5′-trimethoxy flavanone **6** and 2′,3′,4′-trimethoxy flavanone 7 were accomplished starting from 2-hydroxyacetophenone **1** as shown in Scheme 1. Alkaline condensation of o-hydroxyacetophenone **1** and 2,4,5-trimethoxy benzaldehyde **2** produced 2′-hydroxy-2,4,5-trimethxychalcone **4**. It was obtained as yellow crystals, m.p. 124-125°C. The structure of this chalcone **4** has been confirmed by spectral data and elemental analysis. The UV-Vis spectrum of the chalcone **4** gave absorption bands at λ_{max} 235, 260 and 365 nm in methanol, which suggested the presence of a chalcone skeleton [24]. The IR absorption frequency at ν 3400 cm^{-1} indicated the presence of hydroxyl group and it gave light brown color with alcoholic ferric chloride solution which confirmed that compound **4** has free hydroxyl group. The absorption frequency at 1680 cm^{-1} showed the presence of conjugated carbonyl (> C=O) group. The ^{1}H-NMR spectrum of the compound **4** indicated the presence of three methoxy groups at δ 3.93 (C_2-OCH_3), 3.80 (C_3-OCH_3

and C_4-OCH_3) as two singlets integrating for three and six protons, respectively. The two aromatic protons of B-ring appeared as two doublets at δ 6.93 assigned to $C_{3'}$-H and $C_{6'}$-H integrating for two protons. The C_α-H and C_β-H protons appeared as two doublets at δ 7.00 and 7.45 integrating for one proton each. A singlet at δ 13.06 indicated the presence of a chelated phenolic proton at C_2 integrating for one proton.



Scheme 1. Synthesis of chalcone and flavanone derivatives.

Cyclization of chalcone **4** to the corresponding triomethoxy flavanone **6** was carried out by using H_2SO_4/EtOH as an oxidizing agent. The yield of this flavanone **6** was 56% and crystallized from benzene-petroleum spirit as pale yellow flakes, m.p. 138°C. The structure of this flavanone **6** has been confirmed by spectral data. The UV-Vis absorption bands of compound **6** in methanol at λ_{max} 245, 270 and 380 nm suggested the flavanone skeleton. IR absorption band appeared at δ 1645 cm^{-1} indicated the presence of a chelated carbonyl group (>C=O). Similar IR band (in the range of 1640-1652) for chelated carbonyl group was observed for other flavanone compounds [25,26]. The absence of IR absorption band of hydroxyl group confirmed the oxidation of chalcone **4** into flavanone **6**. The ^{1}H-NMR spectrum of flavanone **6** indicated the presence of three methoxy groups at δ 3.95 ($C_{2'}$-OCH_3) and 3.85 ($C_{4'}$-OCH_3 and $C_{5'}$-OCH_3) at two singlets integrating for three and six protons, respectively. The two aromatic protons of B-ring appeared as singlet at 6.16 assigned to $C_{3'}$-H and $C_{6'}$-H integrating for two protons. The flavanone **6** also gave a characteristics double doublet at 5.44 for C_2-H proton and two doublets at 3.13 for C_3-H protons integrating for one and two protons, respectively. The C_5-H and C_8-H protons on A-ring appeared at doublet at δ 7.89 and 7.48 integrating for one proton of each, respectively.

Alkaline condensation of o-hydroxyacetophenone **1** and 2,3,4-trimethoxybenzaldehyde **3** yielded 2′-hydroxy-2,3,4-trimethoxychalcone **5**. It was obtained as yellow crystals. The structure of this chalcone **5** has been confirmed by spectral data and elemental analysis. The interpretations of the spectral data were similar to those as stated for chalcones **4**.

Cyclization of chalcone **5** to the corresponding trimethoxy flavanone **7** was carried out using H_2SO_4/EtOH as an oxidizing agent. The yield of this flavanone **7** was 31% and it crystallized as pale yellow flakes, the structure of the flavanone **7** has been confirmed by the spectral and elemental analysis as described for the previous compound **6**.

4.1. *Antibacterial activities*

The antibacterial activities of the compounds **4-7** have been assayed at the concentration of 100, 200 and 300 μg/disc against two human pathogenic bacteria. Among them, one was gram-positive and other was gram-negative. The inhibitory effects of the compounds **4-7** against these organisms are given in Table 1.

The screening results indicate that compound **4** and **5** did not show any antibacterial activity to the tested bacteria. Compounds **6** and **7** showed moderate activity. From the above results it can be concluded that flavanone ring system is responsible for the antibacterial activity of the compounds **6** and **7**.

4.2. Antifungal activities

The antifungal activities of the compounds **4-7** have been assayed at the concentration of 100 and 200 μg/disc against two plants pathogenic and molds fungi. The inhibitory effects of the compounds **4-7** against these organisms are given in Table 2.

The screening results indicate that all the compounds **4-7** showed moderate antifungal activity against *Rhizactonia solani* and *Sclerotium rolfsii* in comparison with standard fungicides. The above results reveal that the flavanone ring system and presence of methoxy group (-OCH_3) are responsible for the antifungal effects.

Table 1. Results of the antibacterial activity of the compounds against *Streptococcus-β-haemolyticus(G^+)* and *Klebsiella sp.* (G^-).

Bacteria	Molecular formula and compound No.	Diameter of the zone of inhibition (mm)			
		100 μg $disc^{-1}$	200 μg $disc^{-1}$	300 μg $disc^{-1}$	*K-30 30 μg $disc^{-1}$
Streptococcus-β-haemolyticus(G^+)	$C_{18}H_{18}O_5$ (4)	-	-	-	31
	$C_{18}H_{18}O_5$ (6)	11	14	19	
	$C_{18}H_{18}O_5$ (5)	-	-	5	
	$C_{18}H_{18}O_5$ (7)	-	8	11	
Klebsiella sp. (G^-)	$C_{18}H_{18}O_5$ (4)	-	-	7	30
	$C_{18}H_{18}O_5$ (6)	7	13	16	
	$C_{18}H_{18}O_5$ (5)	-	-	-	
	$C_{18}H_{18}O_5$ (7)	8	14	19	

*K-30, Kanamycine standard disc 30μg $disc^{-1}$

Table 2. Results of the antifungal activity of the compounds against *Rhizactonia solani Sclerotium rolfsii.*

Fungi	Compound No.	Concentration (μg disc^{-1})	Diameter of the zone of inhibition (mm)	N*-50 50 μg disc^{-1}
Rhizactonia solani	4	100	10	19
		200	14	
	6	100	11	
		200	14	
	5	100	10	
		200	13	
	7	100	14	
		200	18	
Sclerotium rolfsii	4	100	13	21
		200	18	
	6	100	15	
		200	20	
	5	100	10	
		200	13	
	7	100	17	
		200	20	

*Nystatin standard disc (50 μg disc^{-1})

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