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Cytotoxic and antimicrobial activities of two new synthetic 2'-oxygenated flavones reported from *Andrographis viscosula*

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Abstract: Two new 2'-oxygenated flavones have been synthesized *via* chalcone precursors and the biocidal activity of these two flavones, along with the corresponding chalcones against microbes (bacteria and fungi) and brine shrimp nauplii were investigated. Both the flavones (compounds **6** and **7**) and their corresponding chalcones (compounds **4** and **5**) showed good activity against all the tested bacterial and fungal strains. The LC_{50} values of compounds **4– 7** were found to be 2.31, 0.94, 1.39 and 0.58 μg mL^{-1}, respectively. The synthesized compounds were characterized using UV–Vis, IR, ^{1}H-NMR and ^{13}C-NMR spectral data, together with elemental analysis.

Keywords: 2'-oxygenated flavone, *Andrographis viscosula*, cytotoxicity, antibacterial and antifungal activity, inhibition zone.

INTRODUCTION

Rao *et al.* reported the isolation and characterization of two new 2'-oxygenated flavones, *viz*., 2',5,7-trimethoxyflavone (**6**) and 2',4',5,6',7-pentamethoxyflavone (**7**), from the whole plant of *Andrographis viscosula*,[1] but their synthesis has not yet been reported. *Andrographis viscosula* Nees (Acanthaceae) is an erect herb found in the Hills of Tinnevellt, South India[2] as well as in Bangladesh.[3] In traditional Indian medicine some of the *Andrographis* species are used in the treatment of dyspepsia, influenza, dysentery, malaria and respiratory infections.[3,4] The flavonoid compounds have been demonstrated to possess many biological and pharmacological activities, such as antibacterial, antifungal, antiviral, antioxidant, antiinflammatory, antimutagenic and antiallergic activities and inhibitory activities on several enzymes.[5,6] In view of the interesting biological activities observed in other flavonoid compounds, some flavonoid compounds have already been synthesized and their antimicrobial activity studied.[7–12] This paper reports the new synthesis of 2',5,7-trimethoxyflavone (**6**) and 2',4',5,6',7-pentamethoxyflavone

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(**7**). Aldol condensation of 2-hydroxy-4,6-dimethoxyacetophenone[13] (**1**) with 2-methoxybenzaldehyde (**2**) and 2,4,6-trimethoxybenzaldehyde (**3**) would produce 2'-hydroxy-2,4',6'-trimethoxychalcone (**4**) and 2'-hydroxy-2,4,4',6,6'-pentamethoxychalcone (**5**), respectively. Cyclization of the chalcones **4** and **5** using DMSO/I_2 as an oxidizing agent[14] furnished the above two flavones (compounds **6** and **7**). Both the flavones and their corresponding chalcones were screened *in vitro* for their antibacterial activity against four human pathogenic bacteria, *viz., Bacillus megaterium* (G^+), *Streptococcus β-haemolyticus* (G^+), *Escherichia coli* (G^-), *Klebsiella* sp. (G^-) and antifungal activity against two plant as well as mold fungi, *viz., Aspergillus niger* and *Aspergillus fumigatus*. 2',5,7-Trimethoxyflavone (**6**) and 2',4',5,6',7-pentamethoxyflavone (**7**) have also been reported for their potent cytotoxic activity. Hence the aim of the present studies was to investigate the cytotoxicity as well as the antimicrobial properties of two 2'-oxygenated flavones with the hope of adding new and potent chemotherapeutic agents to the arsenal of weapons used against resistant organisms, as well as other most lethal infectious diseases.

EXPERIMENTAL

Instrumental

All melting points were recorded on a 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 were recorded on a Bruker WH (^{1}H-NMR at 250 MHz, ^{13}C-NMR at 75 MHz) instrument with TMS as the internal standard. The UV–Vis spectra were recorded on a LKB 4053 spectrophotometer using MeOH as the solvent. The purity of the compounds was checked by TLC.

Synthesis of 2'-hydroxy-2,4',6'-trimethoxychalcone (***4***)

A mixture of 2-hydroxy-4,6-dimethoxyacetophenone[13] (**1**, 1.96 g, 10 mmole) and 2-methoxybenzaldehyde (**2**, 1.1. eq., 1.50 g, 11 mmol) 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 the solvent evaporated. The reaction mixture was purified by preparative TLC over silica gel GF_{254} using ether–acetone (8:1) as the developing solvent and the compound was recrystallized from ether as yellow crystals (2.11 g, 61%), with m. p. 56–7 °C, R_f 0.69 (ether–acetone; 9:1). Anal. Found: C, 68.45; H, 5.96; $C_{18}H_{18}O_5$ requires C, 68.78; H, 5.77 %; UV–Vis/nm: 248 and 322; IR (KBr): 3395, 2938, 2819, 1658, 1608, 1442, 1358, 1340, 1268, 1208, 1165, 1120, 1078, 985, 959, 939, 887, 805, 740, 716 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.79 (3H, *s*, $C_{6'}$-OCH_3), 3.84 (3H, *s*, $C_{4'}$-OCH_3), 3.91 (3H, *s*, C_2-OCH_3), 6.46 (1H, *d*, J = 2.6 Hz, $C_{3'}$-H), 6.73 (1H, *d*, J = 2.6 Hz, $C_{5'}$-H), 7.09 (1H, *dt*, J = 7.6 and 0.9 Hz, C_5-H), 7.19 (1H, *dd*, J = 8.2 and 0.9 Hz, C_3-H), 7.48 (1H, *dt*, J = 8.2 and 1.7 Hz, C_4-H), 7.82 (1H, *dd*, J = 8.2 and 1.7 Hz, C_6-H), 7.39 (1H, *d*, J = 16 Hz, C_α-H), 8.01 (1H, *d*, J = 16 Hz, C_β-H), 12.36 (1H, *s*, $C_{2'}$-OH); ^{13}C-NMR (DMSO-d_6): δ 102.8 (C-1'), 158.3 (C-2'), 95.6 (C-3'), 170.2 (C-4'), 92.6 (C-5'), 164.4 (C-6'), 178.4 (>C=O), 123.9 (C-α), 143.5 (C-β), 118.5 (C-1), 158.7 (C-2), 114 (C-3), 128.2 (C-4), 120.7 (C-5), 126.1 (C-6), 59.7 ($C_{6'}$-O$\underline{C}H_3$), 56.0 ($C_{4'}$-O$\underline{C}H_3$), 56.4 (C_2-O$\underline{C}H_3$).

Synthesis of 2'-hydroxy-2,4,4',6,6'-pentamethoxychalcone (***5***)

Chalcone **5** was synthesized by the method employed for chalcone **4**. It was purified by recrystallization from petroleum spirit as yellow crystals (2.38 g, 51%), with m. p. 70–71 °C, R_f 0.67

(*n*-hexane–acetone; 5:1). Anal Found: C, 64.67; H 5.43; $C_{20}H_{22}O_7$ requires C, 64.16; H, 5.92 %; UV–Vis/nm: 243 and 315; IR (KBr): 3420, 2943, 2824, 1667, 1612, 1438, 1365, 1330, 1272, 1218, 1157, 1118, 1080, 983, 960, 941, 894, 810, 744, 713 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.75 (6H, *s*, C_2-OCH_3 and C_6-OCH_3), 3.79 (3H, *s*, $C_{6'}$-OCH_3), 3.84 (3H, *s*, C_4-OCH_3), 3.93 (3H, *s*, $C_{4'}$-OCH_3), 6.18 (2H, *s*, C_3-H and C_5-H), 6.38 (1H, *d*, J = 2.4 Hz, $C_{3'}$-H), 6.44 (1H, *d*, J = 2.4 Hz, $C_{5'}$-H), 7.45 (1H, *d*, J = 16 Hz, C_α-H), 8.06 (1H, *d*, J = 16 Hz, C_β-H), 12.28 (1H, *s*, $C_{2'}$-OH); ^{13}C NMR ($CDCl_3$): δ 101.8 (C-1), 160.5 (C-2), 94.1 (C-3), 170.2 (C-4), 92.8 (C-5), 165.6 (C-6), 176.8 (>C=O), 123.3 (C-α), 142.8 (C-β), 99.4 (C-1'), 161.7 (C-2'), 92.9 (C-3'), 163.2 (C-4'), 91.8 (C-5'), 161.7 (C-6'), 56.1 ($C_{6'}$-O$\underline{C}H_3$), 56.4 ($C_{4'}$-O$\underline{C}H_3$), 55.8 (C_2-O$\underline{C}H_3$), 55.6 (C_4-O$\underline{C}H_3$), 55.8 (C_6-O$\underline{C}H_3$).

Synthesis of 2',5,7-trimethoxyflavone (***6***)

Treatment of chalcone **4** (628 mg, 2 mmol) with a catalytic amount of iodine in dimethyl sulphoxide[14] (DMSO, 25 mL) gave the corresponding flavone **6**. The mixture was refluxed for 20 min in a silicon oil bath and diluted with water. The solid obtained was filtered off and washed with 20 % aq. sodium thiosulphate. The flavone was purified by preparative TLC over silica gel GF_{254} using petroleum spirit–acetone (7 : 1) as the developing solvent and the compound was purified by recrystallization from chloroform as light yellow needles (420 mg, 67%), with m. p. 177–78 °C (Lit.[1] m. p. 177–78 °C), R_f 0.59 (petroleum spirit–acetone; 4 : 1). It gave blue fluorescence in UV light and positive Mg/HCl test. Anal. Found: C, 69.48; H, 4.90; $C_{18}H_{16}O_5$ requires C, 69.22; H, 5.16 %, UV–Vis/nm: 258 and 328; IR (KBr): 1699, 1602, 1491, 1458, 1427, 1341 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 (3H, *s*, C_5-OCH_3), 3.87 (3H, *s*, C_7-OCH_3), 3.90 (3H, *s*, $C_{2'}$-OCH_3), 6.48 (1H, *d*, J = 2.3 Hz, C_6-H), 6.78 (1H, *d*, J = 2.3 Hz, C_8-H), 6.69 (1H, *s*, C_3 -H), 7.13 (1H, *dt*, J = 7.8 and 0.9 Hz, $C_{5'}$-H), 7.22 (1H, *dd*, J = 8.1 and 0.9 Hz, $C_{3'}$-H), 7.53 (1H, *dt*, J = 8.1 and 1.7 Hz, $C_{4'}$-H), 7.89 (1H, *dd*, J = 8.1 and 1.7 Hz, $C_{6'}$-H); ^{13}C NMR (DMSO-d_6): δ 157.4 (C-2), 112.4 (C-3), 175.8 (C-4), 108.1 (C-4a), 160.2 (C-5), 96.2 (C-6), 163.7 (C-7), 93.2 (C-8), 159.3 (C-8a), 119.4 (C-1'), 157.5 (C-2'), 113.1 (C-3'), 132.6 (C-4'), 120.7 (C-5'), 128.6 (C-6'), 56.1 (C_5-O$\underline{C}H_3$), 55.9 (C_7-O$\underline{C}H_3$), 56.0 ($C_{2'}$-O$\underline{C}H_3$).

Synthesis of 2',4',5,6',7-pentamethoxyflavone (***7***)

The method used for the synthesis of **7** was similar to that described for compound **6**. It was purified by preparative TLC over silica gel GF_{254} using *n*-hexane–acetone (4:1) as the developing solvent and the compound was purified by recrystallization from petroleum spirit as pale yellow needles (499 mg, 79 %), m. p. 193–94 °C (Lit.[1] m. p. 192–94 °C), R_f 0.49 (*n*-hexane–acetone; 5 : 1). It gave blue fluorescence in UV light and a positive Mg/HCl test. Anal. Found: C, 64.25; H, 5.62; $C_{20}H_{20}O_7$ requires C, 64.51; H, 5.41 %; UV–Vis/nm: 254 and 306; IR (KBr): 2924, 2850, 1639, 1608, 1590, 1461, 1428, 1384, 1340, 1261, 1240, 1157, 1107, 1026, 984, 806 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.74 (6H, *s*, $C_{2'}$-OCH_3 and $C_{6'}$-OCH_3), 3.82 (3H, *s*, C_7-OCH_3), 3.83 (3H, *s*, $C_{4'}$-OCH_3), 3.91 (3H, *s*, C_5-OCH_3), 6.13 (2H, *s*, $C_{3'}$-H and $C_{5'}$-H); ^{13}C NMR ($CDCl_3$): δ 157.7 (C-2), 116.4 (C-3), 177.8 (C-4), 109.2 (C-4a), 160.8 (C-5), 95.9 (C-6), 163.6 (C-7), 92.9 (C-8), 160.5 (C-8a), 104.4 (C-1'), 159.5 (C-2'), 90.6 (C-3'), 163.2 (C-4'), 90.5 (C-5'), 159.6 (C-6'), 56.3 (C_5-O$\underline{C}H_3$), 55.6 (C_7-O$\underline{C}H_3$), 55.8 ($C_{2'}$-O$\underline{C}H_3$), 55.4 ($C_{4'}$-O$\underline{C}H_3$), 55.9 ($C_{6'}$-O$\underline{C}H_3$).

Antibacterial screening

The antibacterial activities of the synthesized compounds **4–7** were studied against four human pathogenic bacteria, *viz., B. megaterium* (G^+), *S. β-haemolyticus* (G^+), *E. coli* (G^-), *Klebsiella* sp. (G^-). For the detection of the antibacterial activity, the filter paper disc diffusion method[15,16] was employed. Ciprofloxacin was used as standard antibiotic for the antibacterial test. Nutrient agar (NA) was used as the basal medium for the test bacteria. These agar media were inoculated with 0.5 mL of 24 h liquid cultures containing 10^7 microorganisms/mL. The diffusion time was 24 h at 5 °C and the incubation time was 12 h at 37 °C for the bacteria. Discs with only DMSO were used as control. The diameter (in mm) of the observed inhibition zones were taken as a measure of the inhibitory activity.

Determination of the minimum inhibiory concentration (MIC)

A current definition of the minimum inhibitory concentration, MIC, is "the lowest concentration which resulted in maintenance or reduction of inoculum viability".[17] 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.[18,19] The serial dilution technique[20] was applied for the determination of minimum inhibitory concentration of the compounds against the four tested bacteria, *viz., B. megaterium, S. β-haemolyticus, E. coli, Klebsiella* sp. The media used in this respect were nutrient broth (Difco). Dilution series were set up 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 test bacteria (10^7 cell/mL) were added and incubated at 30 °C for 24 h.

Antifungal screening

The antifungal activities of compounds **4–7** were evaluated towards two plants pathogenic and mold fungi, *viz., A. niger* and *A. fumigatus*. The antifungal activity was assessed by the poisoned food technique[21] with some modification.[22] Nystatin (50 μg $disc^{-1}$) was used as the standard fungicide for the antifungal test. Potato dextrose agar (PDA) was used as the basal medium for the 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) °C and after five days of incubation they were ready for use. Prepared discs of the 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 refigerator 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 the solvent to prepare the desired solution (10 mg mL^{-1}) of the compounds initially.

Cytotoxicity bioassay

Brine shrimp lethality bioassay[23–25] 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 in one side of a small tank divided by a net containing 3.8 %. NaCl solution for hatching. In 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 give a concentration of 5 mg/mL. From the stock solutions 0.5, 1, 2, 5, 10, 20, 40 and 80 μL were placed in 8 different vials, with the volume made up to 5 mL with NaCl solution. The final concentrations of the samples in the vials were 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 h 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 probit analysis[26] for the determination of the LC_{50} values for the extracts.

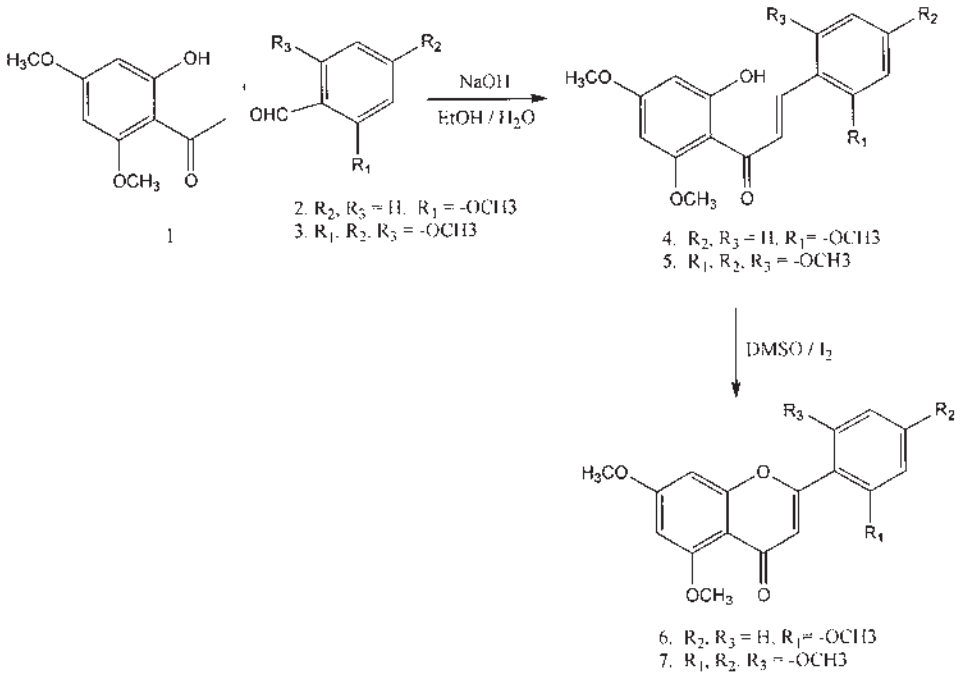
RESULTS AND DISCUSSION

This paper is concerned with the synthesis and antimicrobial activity of two new 2'-oxygenated flavones, *viz.*, 2',5,7-trimethoxyflavone (**6**) and 2',4',5,6',7-pentamethoxyflavone (**7**). The synthesis of the above two new flavones were accomplished starting from 2-hydroxy-4,6-dimethoxyacetophenone (**1**) as shown in Scheme 1.

Alkaline condensation of 2-hydroxy-4,6-dimethoxyacetophenone (**1**) with 2-methoxybenzaldehyde (**2**) gave the corresponding chalcone **4** in moderate yield. The UV–Vis spectrum of **4** in MeOH (248 and 322 nm) suggested a chalcone structure and the IR absorption band at 3395 cm^{-1} indicated the presence of hydroxyl groups. A positive ferric chloride test also indicated that compound **4** has a free hydroxyl group and the band at 1658 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1} H-NMR spectrum of **4** indicated the presence of three aromatic methoxyl groups as singlets at δ 3.79, 3.84 and 3.91. Two doublets at δ 7.39 (C-α) and 8.01 (C-β), are characteristic of the chalcone skeleton. Two *meta*-coupled doublets at δ 6.46 and 6.73, each integrating to one proton, were assigned to H-3' and H-5', respectively. The spectrum also showed a typical ABCD spectrum for the four aromatic protons of ring B at δ 7.09, 7.19, 7.48 and 7.82 for H-5, H-3, H-4 and H-6, respectively. One hydroxyl proton appeared as a singlet at δ 12.36.

Cyclization of the chalcone **4** into the corresponding 2'-oxygenated flavone **6** was carried out using DMSO/I_2 as the oxidizing agent.[14] The spectral data of compound **6** (UV–Vis, IR, ^{1}H-NMR and ^{13}C-NMR) were similar to that obtained for the natural sample.

Again, cross-aldol condensation of 2-hydroxy-4,6-dimethoxyacetophenone (**1**) with 2,4,6-trimethoxybenzaldehyde (**3**) yielded the corresponding chalcone **5** in moderate yield. UV–Vis spectrum of **5** in MeOH (243 and 315 nm) suggested



Scheme 1.

the chalcone skeleton and the IR absorption bands at 3420 cm^{-1} indicated the presence of a hydroxyl group. The positive ferric chloride test also indicated that compound **4** has a free hydroxyl group and the band at 1667 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H-NMR spectrum of **5** indicated the presence of five aromatic methoxyl groups as singlets at δ 3.75 (6H), 3.79 (3H), 3.84 (3H) and 3.93 (3H). Compound **5** also showed two characteristic doublets at δ 7.45 and 8.06, assigned to α-H and β-H protons, respectively. A two-proton singlet at δ 6.18 was assigned to H-3 and H-5 and two *meta*-coupled doublets at δ 6.38 and 6.44 were assigned to H-3' and H-5', respectively. One hydroxyl proton appeared as singlet at δ 12.28.

Oxidation of the chalcone **5** to the corresponding 2'-oxygenated flavone **7** was carried out using DMSO/I_2 as the oxidizing agent.[14] The yield of this flavone was very good (79 %). The spectral data of compound **7** (UV–Vis, IR, ^{1}H-NMR and ^{13}C-NMR) were similar to those obtained for the natural sample.

Antibacterial activities

The antibacterial activities of compounds **4–7** were assayed at the concentration of 100 and 200 μg $disc^{-1}$ against strains of both gram-positive and gram-negative pathogenic bacteria. Initially, the susceptibility testing was carried out by the conventional paper disc method on nutrient agar (NA). The inhibition zone diameters were read and rounded up to the nearest whole number (mm) for analysis. The inhibitory effects of compounds **4–7** against these organisms are given in Table I.

TABLE I. Antibacterial screening for the compounds **4–7**[*]

Compound	Concentration	*B. megaterium*	*S. β-haemolyticus*	*E. coli*	*Klebsiella* sp.
4	100 μg $disc^{-1}$	10	9	–	11
	200 μg $disc^{-1}$	14	15	17	17
5	100 μg $disc^{-1}$	19	15	–	12
	200 μg $disc^{-1}$	28	23	15	19
6	100 μg $disc^{-1}$	22	27	20	26
	200 μg $disc^{-1}$	29	31	29	33
7	100 μg $disc^{-1}$	24	27	26	29
	200 μg $disc^{-1}$	33	32	31	34
C-30[**]	30 μg $disc^{-1}$	29	31	27	28

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

The screening results indicate that all compounds showed good antibacterial activity to the tested bacteria, except compounds **4** and **5** showed no antibacterial activity at the concentration of 100 μg $disc^{-1}$ against *E. coli.* From the inhibition zone diameter data analysis, the flavones **5** and **7** were identified as being more active than their cor-

responding chalcones. Of the flavones, 2',4',5,6',7-pentamethoxyflavone (**7**) showed higher antibacterial activity than 2',5,7-trimethoxyflavone (**6**), which might be due to the presence of more methoxyl groups in the B-ring of flavone **7**.

Minimum inhibitory activity

The minimum inhibitory concentration (MICs, μg mL^{-1}) of compounds **4–7** in comparison to ciprofloxacin against antibiotic susceptible strains of both gram-positive and gram-negative bacteria, *i.e. B. megaterium, S. β-haemolyticus, E. coli, Klebsiella* sp. were determined. Of the compounds tested, compound **7** demonstrated moderate antibacterial activity against both the gram-positive and gram-negative bacteria strains. The MIC level of compounds **4–7** against these organisms are given in Table II.

TABLE II. MIC level of compounds **4–7**

Test organism	Minimum inhibitory concentration/μg ml^{-1}				
	4	**5**	**6**	**7**	Ciprofloxacin
B. megaterium	32	32	64	32	4
S. β-haemolyticus	64	32	32	32	4
E. coli	64	128	64	32	8
Klebsiella sp.	32	32	32	32	4

Antifungal activities

The antifungal activities of compounds **4–7** were assayed *in vitro* at the concentration of 100 μg $disc^{-1}$ against *A. niger* and *A. fumigatus.* The inhibitory effects of compounds **4–7** against these organisms are given in Table III.

TABLE III. Antifungal screening for the compounds **4–7**[*]

Compound	Concentration	*A. niger*	*A. fumigatus*
4	100 μg $disc^{-1}$	11	10
5	100 μg $disc^{-1}$	15	14
6	100 μg $disc^{-1}$	13	15
7	100 μg $disc^{-1}$	18	16
N-50[**]	50 μg $disc^{-1}$	21	19

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

The screening results indicate that all compounds exhibited antifungal activities to the tested fungi. It can be noted that flavones **(6** and **7**) showed a greater inhibitory effect against both the fungi compared to the corresponding chalcones (**4 a**nd **5**). It can also be seen that the flavone **7** showed higher fungicidal effects than the flavone **6** and that chalcone **5** showed higher fungicidal effects than the

chalcone **4**. From the above results, in can be concluded that the flavone ring system and the presence of methoxyl group (-OCH_3) are responsible for the greater antifungal effects.

TABLE IV. The results of cytotoxic effect of the compounds **4–7** and standards bleomycin and gallic acid

Test samples	LC_{50} (ppm)	95 % confidence limit ppm		Regression Equation	χ^2 (df)
		lower	upper		
4	2.31	1.30	4.10	$y = 4.36 + 1.78\,x$	0.32 (2)
5	0.94	0.42	1.80	$y = 3.60 + 1.49\,x$	0.17 (2)
6	1.39	0.69	2.82	$y = 3.54 + 1.29\,x$	0.41 (2)
7	0.58	0.19	1.77	$y = 4.08 + 1.22\,x$	0.20 (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)

Cytotoxic activity

The LC_{50} values of compounds **4–7** were found to be 2.31, 0.94, 1.39 and 0.58 μg mL^{-1}, respectively (Table IV). The standard anticancer drug bleomycin has an LC_{50} value of 0.41 μg mL^{-1}. The lowest LC_{50} value was found in the case of the flavone **7**, indicating its higher cytotoxicity than the other compounds **4, 5** and **6**. The flavonoid compounds **6** and **7** showed potent biocidal activity against brine shrimp nauplii when compared with the control DMSO and gallic acid used as a standard agent.[27]

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ИЗВОД

ЦИТОТОКСИЧНА И АНТИМИКРОБНА АКТИВНОСТ ДВА НОВА СИНТЕТИЧКА 2´-ОКСИГЕНОВАНА ФЛАВОНА ИЗ *Andrographis viscosula*

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Два нова 2´-оксигенована флавона синтетисана су преко халконских прекурсора, и испитана је биоцидна активност та два флавона и одговарајућих халкона на микробе (бактерије и гљивице) и науплије рачића *Artemia salina*. И флавони (једињења **6** и **7**) и одговарајући халкони (једињења **4** и **5**) показали су добру активност на све испитане бактеријске и гљивичне сојеве. Нађено је да су LC_{50} вредности за једињења **4, 5, 6** и **7** 2,31, 0,94, 1,39 и 0,58 μg mL^{-1}, редом. Синтетисана једињења окарактерисана су преко UV–Vis, IR, ^{1}H-NMR и ^{13}C-NMR спектралних података, као и елементалном анализом.

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