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Flavonoids from mango leaves with antibacterial activity

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Abstract: Five flavonoids, *viz.* (–)-epicatechin-3-*O*-*β*-glucopyranoside (**1**), 5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-*g*]chromene-4(8*H*)-one (**2**), 6-(*p*-hydroxybenzyl)taxifolin-7-*O*-*β*-D-glucoside (tricuspid) (**3**), quercetin-3-*O*-*α*-glucopyranosyl-(1→2)-*β*-glucopyranoside (**4**) and (–)-epicatechin(2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol) (**5**), were isolated from the leaves of mango (*Mangifera indica* L.). The antibacterial activity of different concentrations of these flavonoids (100, 300, 500, 700, 900 and 1000 ppm) was evaluated against four bacterial species, namely *Lactobacillus* sp., *Escherichia coli*, *Azospirillium lipoferum* and *Bacillus* sp. All the tested concentrations of the five flavonoids significantly reduced the growth of all the five tested bacterial species. However, differences in the antibacterial activity of the flavonoids were evident. Compound **1** exhibited the lowest antibacterial activity, resulting in a 7–75 % reduction in the growth of the different bacterial species. Compound **5** showed the greatest antibacterial activity and the different concentrations reduced the bacterial growth by 45–99.9 %. *A. lipoferum* and *Bacillus* sp. showed the highest susceptibility to this compound. Compounds **2**–**4** also depicted pronounced antibacterial activity. Different concentrations of these compounds decreased bacterial growth by 52–96 %. From the present study, it can be concluded that compound **5** is the most effective of the tested flavonoids against *A. lipoferum* and *Bacillus* sp.

Keywords: antibacterial; *Mangifera indica*; mango; flavonoids; leaves.

INTRODUCTION

Flavonoids are a major class of oxygen-containing heterocyclic natural products that are widespread in green plants.[1] Generally, they are found as plant pigments in a broad range of fruits and vegetables.[2] These are C_{15} compounds composed of two aromatic rings linked through a three-carbon bridge with a carbonyl

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functional group located at one end of the bridge. Flavonoids have been recognized as having a protective effect in plants against microbial invasion by plant pathogens.[3,4] Flavonoid-rich plant extracts have been used for centuries to treat human disease.[5] Isolated flavonoids have been shown to possess a host of important biological activities, including antifungal and antibacterial activities.[6–8] The potential of naturally occurring flavonoids as anti-infective agents has been recognized.[9] However, reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variations in the susceptibility testing.[5]

Mango (*Mangifera indica* L.) is an economically important tropical fruit found throughout the world. It is very popular due to its excellent eating quality (bright colour, sweet taste and luscious flavour) and nutritional composition (vitamins, minerals, fibre and other phytochemical compounds).[10] Mango contains various classes of polyphenols, carotenoids, and ascorbic acid, which demonstrate different health-promoting properties, mainly from their antioxidant activities.[11] The present study was aimed at investigating the antibacterial activity of five flavonoids isolated from mango leaves, against four bacterial species.

EXPERIMENTAL

General procedure

All the reagents and the solvents used in the present study were procured from E. Merck Germany, Fluka Switzerland, BDH Chemicals England and Sigma-Aldrich Chemicals Co. USA. The solvents used were of analytical grade. For column chromatography, silica gel 60 (Merck 230–400 mesh) was used and TLC was performed on silica gel (Merck, Keiselgel 60F256). The melting points were determined by the sealed capillary method using a Gallenkamp melting point apparatus. However, the melting points were uncorrected. The optical rotation was measured by a polarimeter (modal wxg-4 Dise polarimeter).

The IR spectra of the compounds in KBr discs were recorded on a Fourier Transform Shimadzu 4200 instrument. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 14.1 TNMR spectrometer, operating at a frequency of 600 MHz. The DEPT experiments were performed using polarization transfer pulses of 90 and 135°. The EI–MS spectra were measured with a JEOL JMS-AX 505 HAD mass spectrometer at an ionization voltage of 70 eV.

Isolation of bioactive compounds from mango leaves

Five hundred grams of fresh mango leaves (equivalent to 220 g dry weight) were collected from the University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan in May 2007. The leaves were washed with distilled water, dried in the shade and soaked in 1 L methanol for 15 min to remove chlorophyll. The leaves were then blended with 1.5 L methanol, left overnight, filtered with Whatman No. 1 filter paper under vacuum, centrifuged at 2000 rpm for 5 min and the supernatant was concentrated to 100 mL under vacuum at 50 °C. The concentrated solution was diluted with water (1:1), for precipitation to occur. These precipitates were filtered, washed with ether, dried in a vacuum desiccator to yield compound **1** (215 mg). The filtrate was then concentrated to reduce the volume to 100 mL, extracted with 100 mL of acetone, filtered and the residue was removed. The residue was purified by preparatory TLC (MeOH:$CHCl_3$, 1:99) and recrystallized in $CHCl_3$:MeOH (4:1) to yield com-

pound **2** (323 mg). The remaining filtrate was successively extracted with 150 mL $CHCl_3$ and *n*-butanol each. The $CHCl_3$ extract was subjected to silica gel column chromatography using a solvent system of ethyl acetate:MeOH:H_2O (4:1:1). From this column, compound **3** (1.75 g) was isolated and subsequently purified by preparative TLC using the solvent system EtOAc:MeOH (1:4). The butanolic extract was fractionated by silica gel column (90×4 cm) chromatography using an isocratic solvent system of MeOH:$CHCl_3$:H_2O (3:1:1) to yield compounds **4** (720 mg) and **5** (1.1 g).

Acid hydrolysis

Each flavonoid glycoside (3 mg) was refluxed with 2 M HCl (3 ml) for one hour. The aglycon part was extracted with EtOAc and identified with the help of IR, UV and NMR spectral analysis. The sugar part was isolated from the aqueous layer and identified by co-TLC and comparison with authentic samples.

Antibacterial activity

Four bacterial species, *viz*. *Lactobacillus* sp. 004, *Escherichia coli* 019, *Azospirillium lipoferum* 022 and *Bacillus* sp. 018, were procured from the Fungal Culture Bank, Institute of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan. After autoclaving at 121 °C, LBA broth medium was cooled to room temperature and 10 mL aliquots of the medium were added to 20 mL culture tubes. Appropriate quantities of the five flavonoids were added to the LBA broth medium in the culture tubes to achieve final concentrations of 100, 300, 500, 700, 900 and 1000 ppm. The test compounds were not added to the control tube. One drop of overnight broth culture of each bacterial species was added to the culture tubes prior to incubation at 37 °C for 24 h. Each treatment was performed in triplicate. Afterwards, the optical density of each suspension was recorded at 630 nm on a modal UT 2100UV spectrophotometer (Utechproducts Inc., USA). The effectiveness of the substances was inversely related to the optical density of the suspension.

Statistical analysis

All the data were subjected to analysis of variance followed by the Student–Newman––Keuls test ($p \leq 0.05$) to separate the treatment means using computer software COSTAT.

RESULTS AND DISCUSSION

Structures of the isolated compounds

*Compound **1***. Greenish brown powder; m.p. 202–205 °C. IR (KBr, cm^{-1}): 3431, 2923, 2922, 1650,1600. ^{1}H-NMR (600 MHz, MeOH-d_4, δ / ppm): 5.10 (1H, *d*, *J* = 2.2 Hz, H-2), 4.45 (1H, *dtd*, *J* = 2.2, 5.0, 3.4 Hz, H-3), 2.75 (2H, *d*, *J* = 3.4 Hz, H-4), 6.03 (1H, *d*, *J* = 2.2 Hz, H-6), 5.89 (1H, *d*, *J* = 2.2 Hz, H-8), 6.78 (1H, *br s*, H-2'), 6.97 (1H, *d*, *J* = 10.0 Hz, H-5'), 6.60 (1H, *dd*, *J* = 10.0, 1.8 Hz, H-6'), 6.54 (1H, *br s*, H-1"), 4.83 (1H, *br s*, H-2"), 4.65 (1H, *t*, *J* = 8.1 Hz, H-3"), 4.34 (1H, *t*, *J* = 8.2 Hz, H-4'), 4.77 (1H, *m*, H-5"), 4.20 (1H, *m*, H-6"α), 4.46 (1H, *m*, H-6"β). ^{13}C-NMR (MeOH-d_4, δ / ppm): 78.9 (C-2), 68.0 (C-3), 30.4 (C-4), 160.5 (C-5), 99.1 (C-6), 155.1 (C-7), 95.9 (C-8), 155.8 (C-9), 104.0 (C-10), 132.9 (C-1'), 115.1(C-2'), 146.3 (C-3'), 146.4 (C-4'),116.0 (C-5'), 115.5 (C-6'), 106.0 (C-1"), 73.0 (C-2"), 75.9 (C-3"), 71.8 (C-4"), 78.4 (C-5"), 62.9 (C-6").

EI–MS (*m*/*z*): 452 (M^+), 256, 213, 170, 153, 125, 97. UV (MeOH) (λ_{max} / nm): 212, 280. $[\alpha]^{20}$ (589 nm) = –30.4° (*c* = 0.1 g/100 ml, MeOH).

Compound ***2***. Brown solid; m.p. 220–221 °C. IR (KBr, cm^{-1}): 3410 (*br*), 2923, 2916, 1670, 1650, 1600. ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.83 (1H, *s*, H-2), 6.34 (1H, *s*, H-8), 7.35 (1H, *dd*, *J* = 8.6, 2.6 Hz, H-2'), 6.83 (1H, *dd*, *J* = 8.3 Hz, 2.6 Hz, H-3', H-5'), 7.37 (1H, *dd*, *J* = 8.3, 2.6 Hz, H-6'), 4.72, 4.89 (2H, *dd*, *J* = = 3.5 Hz, 16.4 Hz, H-2"), 6.71 (1H, *m*, H-3"), 5.81 (1H, *d*, *J* = 3.0 Hz, H-4"). ^{13}C--NMR ($CDCl_3$, δ / ppm): 148.8 (C-2), 125.5 (C-3), 197.7 (C-4), 161.9 (C-5), 105.4 (C-6), 160.9 (C-7), 96.9 (C-8), 158.0 (C-9), 104.9 (C-10),126.2 (C-1'), 132.3 (C-2'), 115.3 (C-3'), 146.2 (C-4'), 116.2 (C-5'), 132.2 (C-6'), 77.0.(C-2"), 132.6 (C-3"), 115.2 (C-4"). UV (MeOH) (λ_{max} / nm): 270, 256; (NaOAc) (λ_{max} / nm): 276. EI–MS (*m*/*z*): 308 (M^+), 245, 184, 170, 153, 129, 109, 108, 107, 79, 55.

Compound ***3***. Yellow brown crystalline, m.p. 145 °C. IR (KBr, cm^{-1}): 2914, 2724, 2357, 1697, 1610, 1454, 1376, 1202, 1030. ^{1}H-NMR (600 MHz, MeOH-d_4, δ / ppm): 4.97 (1H, *d*, *J* =11.4 Hz, H-2), 4.45 (1H, *d*, *J* =11.4 Hz, H-3), 6.25 (1H, *s*, H-8), 6.90 (1H, *d*, *J* = 1.82, H-2'), 6.77 (1H, *d*, *J* = = 7.4 Hz, H-5'), 6.76 (1H, *dd*, *J* = 7.4, 1.82 Hz, H-6'), 3.55 (2H, *s*, H-1"), 6.62 (2H, *dd*, *J* = 8.4, 2.1 Hz, H-3", H-7"), 6.61 (2H, *dd*, *J* = 8.4, 2.1 Hz, H-4", H-6"), 6.52 (1H, *br s*, H-1'"), 4.80 (1H, *br s*, H-2'"), 4.62 (1H, *t*, *J* = 8.1 Hz, H-3'"), 4.31 (1H, *t*, *J* = 8.2 Hz, H-4'"), 4.73 (1H, *m*, H-5'"), 3.55 (1H, *m*, H-6'"α), 4.52 (1H, *m*, H-6'"β). ^{13}C-NMR (MeOH-d_4, δ / ppm): 82.8 (C-2), 71.5 (C-3), 198.8 (C-4), 160.7 (C-5), 110.9 (C-6), 96.4 (C-8), 162.5 (C-7), 160.7 (C-9), 104.5 (C-10), 130.7 (C-1'), 115.4 (C-2'), 146.0 (C-3'), 146.4 (C-4'), 114.9 (C-5'), 114.7 (C-6'), 31.6 (C-1"), 136.1 (C-2"), 132.8 (C-3") 115.8 (C-6", C-4"), 160.7 (C-5"), 133.1 (C-7"), 102.8 (C-1'"), 73.6 (C-2'"), 76.4 (C-3'"), 69.7 (C-4'"), 77.6 (C-5'"), 62.6 (C-6'"). EI–MS (*m*/*z*): 595 (M^+), 184, 170, 153, 134.9, 125, 109, 108, 107, 97, 79. UV (MeOH) (λ_{max} / nm): 228, 287. $[\alpha]^{25}$ (589 nm) = –7.62° (*c* 0.5 g/100 ml, MeOH)

Compound ***4***. Reddish pink powder; m.p. 210–214 °C. IR (KBr, cm^{-1}): 3332, 2950, 2922, 1652, 1600, 1300, 1210, 1147, 1050, 878. ^{1}H-NMR (600 MHz, MeOH-d_4, δ / ppm): 6.32 (1H, *d*, *J* = 2.1Hz, H-6), 6.51 (1H, *d*, *J* = 2.10 Hz, H-8), 6.78 (1H, *d*, *J* = 1.8 Hz, H-2'), 7.63 (1H, *d*, *J* = 10.0 Hz, H-5'), 7.62 (1H, *dd*, *J* = 10.0, 1.8 Hz, H-6'), 5.71 (1H, *d*, *J* = 7.6 Hz, H- 1"), 4.83 (1H, *br s*, H-2"), 4.65 (1H, *t*, *J* = 8.1 Hz, H-3"), 4.34 (1H, *t*, *J* = 8.2 Hz, H-4"), 4.77 (1H, *m*, H-5"), 3.50 (1H, *m*, H-6"α), 4.46 (1 H, *m*, H-6"β), 5.10 (1H, *d*, *J* = 7.8 Hz, H-1'"), 4.28 (1H, *d*, *J* = 8.2 Hz, H-2'"), 4.63 (1H, *br s*, H-3'"), 4.13 (1H, *t*, 8.2 Hz, H-4'"), 4.52 (1H, *m*, H-5'"), 4.36, 4.50 (2H, *m*, H-6'"α, H-6'"β). ^{13}C-NMR (MeOH-d_4, δ / ppm): 160.0 (C-2), 133.3 (C-3), 198.9 (C-4), 162.1 (C-5), 100.4 (C-6), 162.8 (C-7), 96.4 (C-8), 161.1 (C-9), 104.6 (C-10), 132.9 (C-1'), 115.4 (C-2'),146.4 (C-3'), 146.2 (C-4'), 115.8 (C-5'), 123.9 (C-6'), 105.6 (C-1"), 101.5 (C-1'"), 72.6 (C-4", C-4'"), 62.5 (2C, C-6", C-6'") 79.9 (C-5"), 78.1 (C-5'"), 77.7 (C-2"), 72.3 (C-2'"), 77.7 (C-3"), 72.8 (C-3'"). EI–MS (*m*/*z*): 626 (M^+), 390, 354, 327, 302, 299,

192, 153, 125,121, 93. UV (λ_{max} / nm) (MeOH): 357, 307, 256, (λ_{max} / nm) (NaOAc): 372, 260.

Compound ***5***. Off-white powder, m.p. 241–245 °C. IR (KBr, cm^{-1}): 3331, 2923, 1650, 1240, 1070, 880. ^{1}H-NMR (600 MHz, MeOH-d_4, δ / ppm): 4.87 (1H, *d*, *J* = 2.4 Hz, H-2), 3.98 (1H, *m*, H-3), 2.85 (1H, *dd*, *J* = 5.4, 16.2 Hz, H-4α), 2.51 (1H, *dd*, *J* = 4.2, 16.2 Hz, H-4β), 5.88 (1H, *d*, *J* = 2.1 Hz, H-8), 6.03 (1H, *d*, *J* = 2.1 Hz, H-6), 6.89 (1H, *d*, *J* = 1.7 Hz, H-2'), 6.77 (1H, *d*, *J* = 7.4 Hz, H-5'), 6.73 (1H, *dd*, *J* = 7.4, 1.7 Hz, H-6'). ^{13}C-NMR (MeOH-d_4, δ / ppm): 74.8 (C-2), 71.8 (C-3), 31.4 (C-4), 160.1 (C-5), 96.8 (C-6), 156.1 (C-7), 96.5 (C-8), 155.3 (C-9), 104.0 (C-10), 132.1 (C-1'), 110.1 (C-2'), 145.5 (C-3') 146.4 (C-4'), 116.0 (C-5'), 115.2 (C-6'). EI–MS (*m*/*z*): 290 (M^+), 245, 227, 170, 153, 126. UV (MeOH) (λ_{max} / nm): 280, 212. $[\alpha]^{25}$ (589 nm) = –14.90°.

Compound **1** was obtained as a greenish brown amorphous powder having m.p. 202–205 °C, positive to the butanol/HCl and vanillin/HCl tests. It gave a dark greenish black colour with $FeCl_3$. A positive molecular ion peaks (M^+) appeared at *m*/*z* 452. The IR spectrum showed bonded OH at (3431 cm^{-1}) and an aromatic group at 1600 and 1650^{-1}. The ^{1}H-NMR spectrum showed a pair of doublets at δ 2.7 and 2.8 ppm, assigned to the H-4 protons (coupled to each other with *J* = 16.7 Hz and to H-3 with *J* = 4.5 and 2.5 Hz), a doublet at 5.10 ppm (*J* = = 2.2 Hz, H-2), a *dtd* signal at 4.45 ppm (*J* = 2.2, 3.4 Hz, H-3) and a pair of meta coupled doublets (*J* = 2.2 Hz) at 6.0 ppm (H-6) and 5.89 ppm (H-8). The ^{1}H-NMR spectrum showed a resonance due to an anomeric proton at 6.54 ppm (*br*, *s*, H-1"), a broad signal at 4.80 ppm (H-2") and four other peaks, indicating that the glucose moiety is a β-D-glucopyranosyl group. The glucosidation at position 3 was also concluded from a ^{1}H-heteronuclear multiple band correlation (MBC) correlation between the anomeric proton of glucose at 6.54 ppm and the C-3 at 68.0 ppm. Also, the ^{13}C-NMR signals at C-2 and C-3 confirm that the compound suggested is (–)-epicatechin with a glucose moiety at C-3.[12]

Compound **2** was isolated as a brown solid having a m.p. 220–221 °C. The molecular formula $C_{18}H_{12}O_5$ was deduced from elemental analysis and the EI––MS mass spectrum, which exhibited a (M^+) at *m*/*z* 308. The compound gave a bluish black colour with $FeCl_3$. Bands at 3410 (OH), 1670 (C=O) and 1650 and 1600 cm^{-1} (phenyl group) were observed in the IR spectrum. The ^{1}H-NMR spectrum exhibited a singlet at 7.83 ppm (H-2) and the ^{13}C-NMR spectrum, a signal at 148.8 ppm (C-2), which are characteristic for the isoflavone skeleton.[13] This was further supported by the UV spectrum with λ_{max} at 270 nm. The ^{1}H-NMR data indicated a doublet of doublets (*J* = 8.6, 2.6 Hz) at 7.35 (H-2'), 6.83 (H-3',H-5') and 7.37 ppm (H-6'), showing the presence of a 4'-OH on ring B of isoflavonoid. The OH at C-7 was not free as NaOAc failed to produce any bathochromic shift.[14] The ^{1}H-NMR and ^{13}C-NMR spectra showed the presence of a pyran ring at 4.72 and 4.89 (2H, H-2"), 6.71 (H-3") and 5.81 ppm (H-4") and at 76.9 (H-2"),

132.6 (H-3") and 115 ppm (H-4"), respectively. These data suggested that compound **2** was 5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-*g*]chromene-4(8*H*)-one. This compound was previously reported form *Erythrina lysistemon.*[15]

Compound **3** was isolated as a yellow powder, m.p. 145 °C. EI-MS gave the (M^+) peak at *m*/*z* 595. Its UV and IR data were similar to that of the reported data.[16] The ^{1}H-NMR signals at 4.97 (1H, *d*, *J* = 11.4 Hz , H-2) and 4.45 ppm (1H, *d*, *J* = 11.4 Hz, H-3) are specific for trans stereochemistry of the dihydroflavonol skeleton. Two sets of doublets *(J* = 8.4, 2.1 Hz) at 6.62 (H-3", H-7") and 6.61 ppm (H-4", H-6") indicate the presence of a *p*-substituted phenyl group. The proton ^{1}H-NMR resonance of the anomeric carbon at 6.52 ppm (1H, *br s*, H-1'") suggest the glucose moiety has the *β*-configuration. The ^{13}C-NMR upfield signals of C-8 and C-6 and the downfield resonance of C-7 indicate that the glucose moiety was attached with that of (C-7). Regarding the data described, compound 3 is suggested to be 6-(*p*-hydroxybenzyl)taxifolin-7-*β*-D-glucoside. This compound was previously identified from *Cudrania tricuspidata*.[17]

Compound **4** was isolated as a reddish pink powder, m.p. 210–214 °C. The UV, IR and NMR data resembled those of a reported flavonol.[18] The IR spectrum showed bands at 3332 (OH) and 1652 cm^{-1} (C=O). The UV spectrum showed a maximum absorbance with NaOAc at 260 and 372 nm, indicating the presence of free OH groups at position 5 and 7 of ring A.[19] In the ^{1}H-NMR spectrum, two anomeric protons appeared at 5.71 ppm (1H, *d*, *J* = 7.7, H-1") and 5.10 (1H, *d*, *J* = 7.8, Hz H-1'"), indicating the presence of glucose moieties having a *β*-configuration. The ^{1}H-NMR and ^{13}C-NMR data of model sugars identified that the sugar moiety may be D-glucopyranoside. The ^{13}C-NMR upfield signal at 77.7 (C-2") and downfield signal at 105.6 ppm (C-1") confirmed the presence of a 1→2 interglucoside linkage.[20] This compound was previously isolated from *Cadaba glandulosa*.[21]

Compound **5** was isolated as an off-white amorphous powder, m.p. 241–245 °C. The compound was positive to butanol/HCl and vanillin/HCl reagents. The UV spectrum (MeOH) showed maximum absorbance at 280 and 212 nm. Its EI–MS *m*/*z* 313 $(Na+M)^+$ indicates a monomeric unit of *m*/*z* 290. Bands at 3331, 2923 and 1650 cm^{-1} were observed in the IR spectrum. The ^{1}H-NMR spectrum showed a pair of doublets at 2.51 and 2.85 ppm, assigned to H-4 proton (coupled to each other with *J* = 16.2 Hz and to H-3 with 5.4 and 4.2 Hz). A doublet at 4.87 ppm (*J* = 2.4 Hz, H-2), a signal at 3.98 ppm (1H, *m*, H-3) and a pair of meta coupled doublets (*J* = 2.1 Hz) at 6.03 (H-6) and 5.88 ppm (H-8) were observed. The ^{1}H-NMR data suggest that the compound was epicatechin, which was further supported by ^{13}C-NMR signals, especially at 74.8 (C-2) and 71.8 ppm (C-3).[12] This compound was previously isolated from *Adansonia digitata*.[22]

The structures of the five isolated compounds are given in Fig. 1.

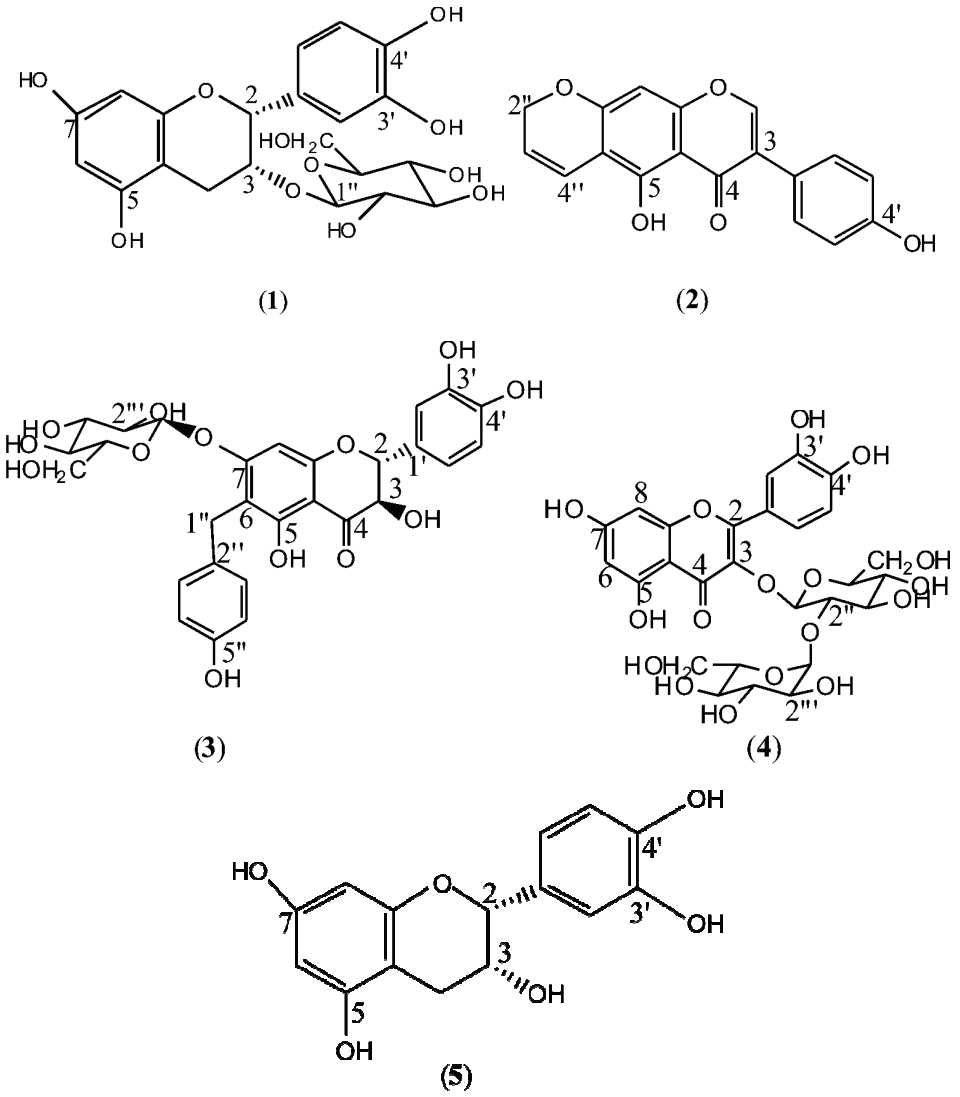

Fig. 1. Structures of flavonoids isolated from mango leaves.

Antibacterial activity

Analysis of variance showed that the effect of flavonoids, bacterial species, concentration and their interaction was highly significant ($p \leq 0.001$) for bacterial growth (Table I). The data presented in Fig. 2 indicates that all the concentrations of the five isolated flavonoids significantly suppressed the growth of all the four tested bacterial species, however, variation in antibacterial activity of the isolated compounds was evident. Compound **1** exhibited the least antibacterial activity. Various concentrations of compound **1** reduced the bacterial growth by 7–75 %

(Fig. 2A). In contrast, compound **5** was found to be the most effective in controlling bacterial growth. This compound was highly toxic to *A. lipoferum* and *Bacillus* sp. growth, resulting in 94–99.9 % and 73–99 % decreases in bacterial growth over corresponding control treatments, respectively. *Lactobacillus* sp. and *E. coli* were comparatively less susceptible to compound **5** where 59–96 % and 45–83 % suppression in bacterial growth, respectively, was recorded over the corresponding control treatments (Fig. 2E). Compounds **2**–**4** exhibited intermediate antibacterial activity between compound **1** and **5**. Different concentrations of compound **2**–**4** reduced the bacterial growth by 65–96 %, 52–80 % and 68–92 %, respectively (Figs. 2B–2D). Recently, similar antibacterial activities were also reported for other flavonoids isolated from different plant species.[8,23,24] Various antibacterial mechanisms of action of different flavonoids have been proposed, including inhibition of nucleic acid synthesis,[25] inhibition of cytoplasmic membrane function[26] and inhibition of energy metabolism.[27] Earlier compound **2** was known for its antimicrobial and radical scavenging activities.[15] In conclusion, the results of the present study revealed that the flavonoids isolated from mango leaves possess antibacterial activity. Compound **5** is the most effective flavonoid against *A. lipoferum* and *Bacillus* sp.

TABLE I. Analysis of variance for the effect of different concentrations of the five flavonoids isolated from mango leaves against four bacterial species

Sources of variation	df	SS	MS	F values[a]
Treatment	139	121	0.87	2285
Flavonoids (F)	4	26	6.54	17147
Bacterial species (B)	3	4.1	1.35	3554
Concentration (C)	6	77	12.91	33882
F×B	12	4.2	0.35	922
F×C	24	6.2	0.26	678
B×C	18	1.0	0.06	147
F×B×C	72	1.9	0.03	70
Error	280	0.1	0.0004	–
Total	420	335	–	–

[a]Significant at $p \leq 0.001$

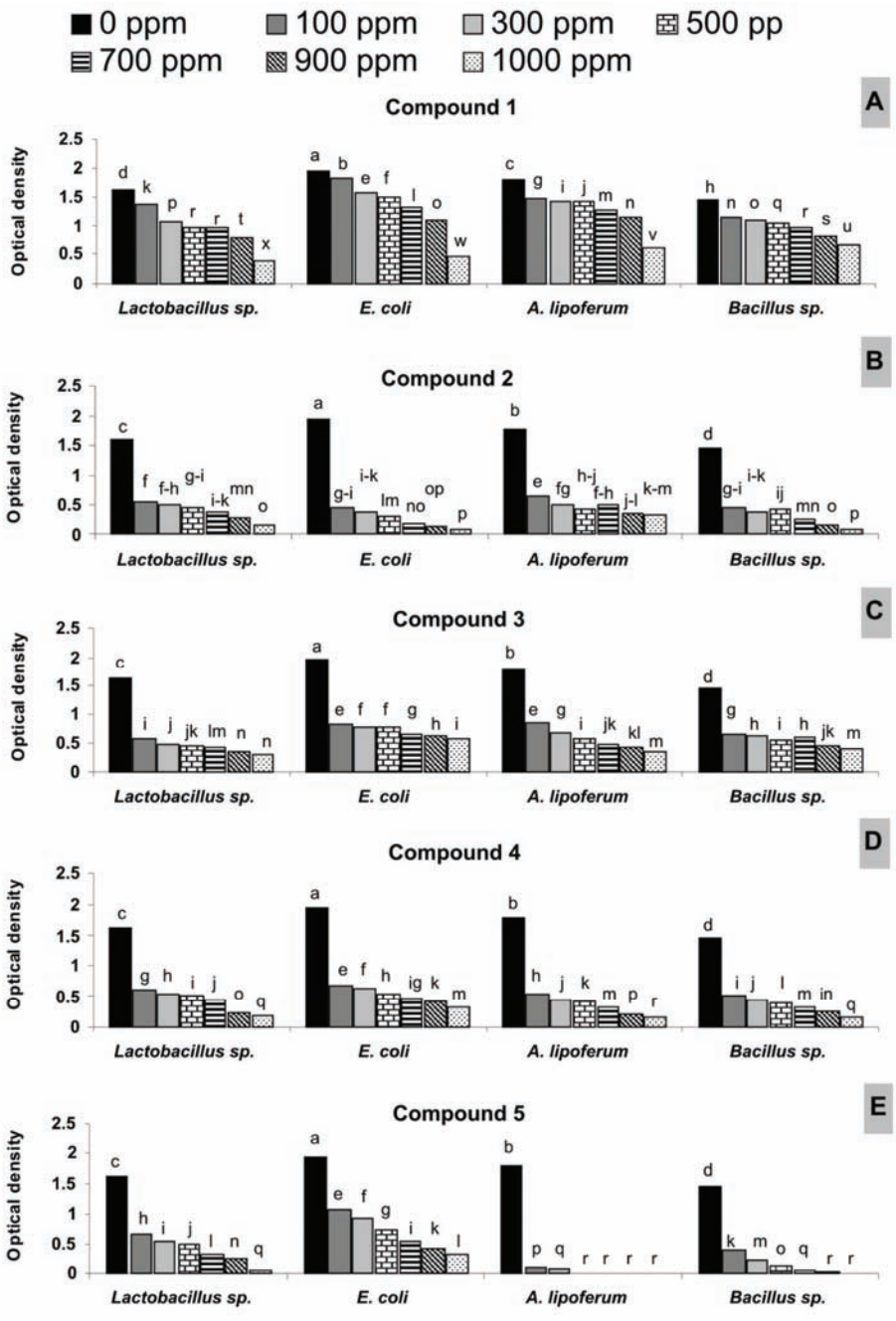

Fig. 2. Effect of different concentrations of five flavonoids on the growth of bacteria. In each graph, bars with different letters show significant difference ($p \leq 0.05$) as determined by the Student–Newman–Keuls test.

ИЗВОД

АНТИБАКТЕРИЈСКА АКТИВНОСТ ФЛАВОНОИДА ЛИСТА МАНГА

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Из листа манга (*Mangifera indica* L.) изоловано је пет флавоноида: (–)-епикатехин-3-*O*-*β*-глукопиранозид (**1**), 5-хидрокси-3-(4-хидроксифенил)пирано[3,2-*g*]хромен-4(8*H*)-он (**2**), 6-(*p*-хидроксибензил)таксифолин-7-*O*-*β*-D-глукозид (**3**), кверцетин-3-*O*-*α*-глукопиранозил-(1→2)-*β*-глукопиранозид (**4**) и (–)-епикатехин (2-(3,4-дихидроксифенил)-3,4-дихидро-2*H*-хромен-3,5,7-триол) (**5**). Антибактеријска активност различитих концентрација флавоноида (100, 300, 500, 700, 900 и 1000 ppm) је одређивана спрам четири бактеријске врсте: *Lactobacillus* sp., *Escherichia coli*, *Azospirillium lipoferum* и *Bacillus* sp. Сви флавоноиди су значајно смањивали раст тестираних бактерија, мада је постојала разлика у њиховој ефикасности. Једињење **1** је имало најмању антибактеријску активност (смањење раста различитих врста бактерија 7–75 %). Једињење **5** је имало највећу антибактеријску активност (редукција раста бактерија 45–99,9 %). Бактерије *A. lipoferum* и *Bacillus* sp. су биле најосетљивије на ово једињење. Једињења **2**–**4** су, такође, испољила изражену антибактеријску активност (редукција раста 52–96 %). На основу резултата ове студије може се закључити да је једињење **5** најефикасније од свих тестираних флавоноида и да је ефекат најизраженији спрам *A. lipoferum* и *Bacillus* sp.

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