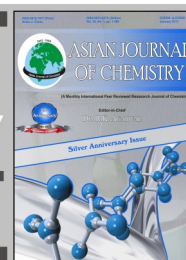


Asian Journal of Chemistry; Vol. 25, No. 4 (2013), 1927-1933

ASIAN JOURNAL OF CHEMISTRY

<http://dx.doi.org/10.14233/ajchem.2013.13242>



Synthesis of Some 4'-O Substituted Derivatives of Natural Naringin and Their Biological Screening

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(Received: 13 December 2011;

Accepted: 8 October 2012)

AJC-12252

This manuscript reports the synthesis of a series of new 4'-O-substituted derivatives of naringin (**1**) which is a naturally occurring flavanone glycoside isolated from the plant *Rhynchosia pseudo-cajan* Cambess. belonging to family of Papilionaceae. The structure of **1** was established through ¹D and ²D NMR analysis and by comparison with its published data while its synthetic derivatives **3a-l**, were characterized by ¹H NMR spectra and all these were screened against four enzymes namely, acetylcholinesterase, butyrylcholinesterase lipoxigenase and chymotrypsin. Their antioxidant potential was also evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and only 4'-O-ethyl naringin (**3a**) showed antioxidant potential (IC₅₀ 36.21 ± 0.32 μM). 4'-O-Ethyl naringin (**3a**), 4'-O-(2'''-phenylethyl) naringin (**3f**), 4'-O-(3'''-phenylpropyl) naringin (**3g**) and 4'-O-(*p*-fluorobenzyl) naringin (**3i**) were found to be moderate inhibitors of butyrylcholinesterase while naringin (**1**) itself, 4'-O-*n*-butyl naringin (**3b**), 4'-O-iso-propyl naringin (**3d**) and 4'-O-(*N*-3''', 5'''-dimethylphenyl-C-acetamido) naringin (**3l**) were identified as moderate inhibitors of lipoxigenase. Against chymotrypsin only 4'-O-(*p*-bromobenzyl) naringin (**3h**) was found to be a moderate inhibitor.

Key Words: Naringin, Flavanone glycoside, *Rhynchosia pseudo-cajan* Cambess., Acetylcholinesterase, Butyrylcholinesterase, Lipoxigenase.

INTRODUCTION

Rhynchosia pseudo-cajan Cambess. (papilionaceae) is a common weed which is medicinally important. It is an erect shrub having whitish leaflets on lower side. Its common name is Lahrr. It is distributed in Jammu & Kashmir and Pakistan. Its leaves are used as tonic and stomach disorders, leaves and bark powder is useful for peptic ulcer and other digestive problems¹. The crude drugs of *Rhynchosia* are used in the formation of fermented liquor having antioxidative, antitumor, skin whitening and arthritis preventing and treating effects². Since now no phytochemical studies have been carried out on this species but many important compounds have been discovered from other species of genus *Rhynchosia*. Tirumalin, a prenylated dihydroflavanol was isolated from *Rhynchosia cyanosperma*³. Rhynchosin, a new 5-deoxyflavanol was isolated from leaves of *Rhynchosia beddomei*⁴. The leaves of *Rhynchosia rufescens* contain methylated flavonols such as kaempferol, kaempferol 3-methyl ether and quercetin 3-methyl ether, which are of chemotaxonomic importance⁵. Many flavonoides have been

reported from various *Rhynchosia species*⁶. Similarly many glycosides have also been isolated from *Rhynchosia rothii*⁷.

Plants have been a source of medicine for thousands of years and phytochemicals continue to play an essential role in medicine⁸. The therapeutic use of development of human knowledge, scientists endeavored to isolate different chemical constituents from plant, put them to biological and pharmacological tests and thus have been used to prepare modern medicines⁹. As described above, the plant *Rhynchosia pseudo-cajan* is an important medicinal plant, so in this work, we report isolation of naringin (**1**) from this plant and subsequent synthesis of its various 4'-O-substituted derivatives and then their screening against four enzymes namely, acetylcholinesterase, butyrylcholinesterase, lipoxigenase and chymotrypsin. Their antioxidant potential was also assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

EXPERIMENTAL

All chemicals used in the experiments were of analytical grade and were used without further purification otherwise

specified. The solvents used were purified with distillation before use. For column chromatography (CC), silica gel (70-230 mesh) was used. TLC was performed on pre-coated silica gel G-25-UV254 plates. Detection was carried out at 254 nm and by ceric sulphate reagent. Purity was checked on TLC with different solvent systems using methanol, acetone and CHCl_3 giving single spot. ^1H NMR and ^{13}C NMR Spectra were recorded in CD_3OD run on Bruker spectrometers operating at 400 MHz. The chemical shifts are given in δ in ppm and coupling constants in Hz.

The plant *Rhynchosia pseudo-cajan* Cambess. was collected from Kotli, Kashmir (P.O.K.) in June 2009 and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore. A voucher specimen (GC.Herb.Bot. 623) has been deposited in the herbarium of the same university.

Extraction, fractionation and isolation: The shade-dried ground whole plant (15 kg) was exhaustively extracted with methanol (20 L $\times$ 4) at room temperature. The extract was evaporated to yield the residue (950 g), which was dissolved in distilled water (2 L) and partitioned with *n*-hexane (1.5 L $\times$ 4), chloroform (1.5 L $\times$ 4), ethyl acetate (1.5 L $\times$ 4) and *n*-butanol (1.5 L $\times$ 4) respectively. These organic fractions and remaining water fraction was concentrated separately on rotary evaporator. The yields of *n*-hexane soluble fraction, chloroform soluble fraction, ethyl acetate soluble fraction, *n*-butanol soluble fraction and remaining aqueous fraction were 203, 226, 198, 185 and 138 g respectively. The ethylacetate soluble fraction was subjected to column chromatography over silica gel (70-230 mesh) using *n*-hexane with gradient of CHCl_3 (from 50:50, 30: 70, 20:80, 10:90, 0:100) (5000 mL for each gradient) and followed by methanol up to 100 %. Twelve fractions (Fr. 1-12) were collected, having elution volumes as 3100, 4650, 3350, 3200, 3650, 4400, 2500, 2600, 3200, 4300, 3500 and 4200 mL respectively. The Fr. 11 (3500 mL) from the first column was loaded on silica gel (70-230 mesh) (64 g) and eluted with methanol:acetone: CHCl_3 (10:10:80) (2900 mL) to isolate **1** (1.6 g), which was characterized and then subjected to derivatization.

Synthesis of naringin derivatives in DMF: The calculated amount of naringin (50 mg; **1**) was taken in a round bottomed flask (50 mL) and DMF (10 mL) was added to dissolve it, followed by the addition of lithium hydride (50 mg) to the mixture therein. The blend was stirred for 0.5 h at ambient temperature and then slowly added the second reactant, **2a-l**, to the mixture and it was further stirred for 4 h. The progress of reaction was monitored *via* TLC. The product was dissolved in water and separated by solvent extraction with ethyl acetate. TLC tests of the various products were performed using solvents of methanol and chloroform in various ratios. The appearance of single spot in each case on the TLC plate with different R_f value relative to **1**, corroborated the purity and the formation of desired product. The purity of the desired products was also evident from their clear NMR spectra.

Structural identification

Naringin (1): Amorphous white solid (1.6g) ^1H NMR (400 MHz, CD_3OD): δ 7.28 (d, J = 8.4 Hz, 2H, H-2' and H-6'), 6.79 (d, J = 8.7 Hz, 2H, H-3' & H-5'), 6.16 (d, J = 2.1 Hz,

1H, H-8), 6.14 (d, J = 2.1 Hz, 1H, H-6), 5.33 (dd, J = 2.7, 12.9 Hz, 1H, H-2), 5.24 (d, J = 1.2 Hz, 1H, H-1'''), 5.06 (d, J = 7.2 Hz, 1H, H-1''), 4.29 (m, 1H, H_a-6''), 4.18 (m, 1H, H_b-6''), 3.92 (m, 1H, H-5'''), 3.80 (m, 1H, H-5''), 3.43 (m, 1H, H-3''), 3.34 (m, 1H, H-3'''), 3.32 (m, 1H, H-2'''), 3.30 (m, 1H, H-2''), 3.27 (m, 1H, H-4'''), 3.16 (dd, J = 17.1, 12.9 Hz, 1H, H_a-3), 3.13 (m, 1H, H-4''), 2.72 (dd, J = 17.1, 2.7 Hz, 1H, H_b-3), 1.27 (d, J = 6.3, 3H, CH₃-6'''). ^{13}C NMR (100 MHz, CD_3OD): δ 198.51 (C-4), 166.58 (C-7), 164.98 (C-5), 164.61 (C-9), 159.07 (C-4'), 130.84 (C-1'), 129.10 (C-2' and C-6'), 116.35 (C-3' and C-5'), 104.90 (C-10), 102.56 (C-1''), 99.41 (C-1'''), 97.85 (C-6), 96.77 (C-8), 80.70 (C-2), 79.04 (C-2''), 78.95 (C-5''), 78.11 (C-3''), 73.92 (C-4'''), 72.17 (C-2''' and C-3'''), 71.24 (C-4''), 69.98 (C-5'''), 62.27 (C-6''), 44.14 (C-3), 18.21 (C-6''').

4'-O-Ethyl naringin (3a): Gummy solid, yield 82 %. ^1H NMR (400 MHz, CD_3OD): δ 7.33 (d, J = 8.8 Hz, 2H, H-2' & H-6'), 6.87 (d, J = 8.5 Hz, 2H, H-3' & H-5'), 6.17 (d, J = 2.3 Hz, 1H, H-8), 6.15 (d, J = 2.3 Hz, 1H, H-6), 5.41 (dd, J = 2.5, 12.8 Hz, 1H, H-2), 5.32 (d, J = 1.3 Hz, 1H, H-1'''), 5.11 (d, J = 7.4 Hz, 1H, H-1''), 4.21 (m, 1H, H_a-6'a), 4.15 (m, 1H, H_b-6'b), 3.95 (m, 1H, H-5'''), 3.85 (q, J = 7.1 Hz, 2H, CH₂-1'''), 3.81 (m, 1H, H-5''), 3.46 (m, 1H, H-3''), 3.36 (m, 1H, H-3'''), 3.33 (m, 1H, H-2'''), 3.31 (m, 1H, H-2''), 3.28 (m, 1H, H-4'''), 3.15 (dd, J = 17.2, 12.8 Hz, 1H, H-3a), 3.14 (m, 1H, H-4''), 2.73 (dd, J = 17.2, 2.5 Hz, 1H, H-3b), 1.38 (t, J = 7.1 Hz, 2H, CH₃-2'''), 1.28 (d, J = 6.3, 3H, CH₃-6''').

4'-O-*n*-Butyl naringin (3b): Gummy solid, yield 81 %. ^1H NMR (400 MHz, CD_3OD): δ 7.65 (d, J = 8.4 Hz, 2H, H-2' & H-6'), 7.06 (d, J = 8.4 Hz, 2H, H-3' & H-5'), 6.60 (d, J = 2.0 Hz, 1H, H-8), 6.58 (d, J = 2.0 Hz, 1H, H-6), 5.39 (dd, J = 2.4, 12.7 Hz, 1H, H-2), 5.42 (d, J = 1.2 Hz, 1H, H-1'''), 5.25 (d, J = 7.5 Hz, 1H, H-1''), 4.20 (m, 1H, H_a-6''), 4.14 (m, 1H, H_b-6''), 3.90 (m, 1H, H-5'''), 3.71 (m, 1H, H-5''), 3.54 (m, 1H, H-3''), 3.45 (t, J = 8.4 Hz, 2H, CH₂-1'''), 3.43 (m, 1H, H-3'''), 3.41 (m, 1H, H-2'''), 3.29 (m, 1H, H-2''), 3.09 (m, 1H, H-4'''), 3.07 (dd, J = 17.3, 12.7 Hz, 1H, H_a-3), 3.05 (m, 1H, H-4''), 2.67 (dd, J = 17.3, 2.4 Hz, 1H, H_b-3), 1.75 (m, 2H, CH₂-2'''), 1.47 (m, 2H, CH₂-3'''), 1.32 (d, J = 6.8, 3H, CH₃-6'''), 0.98 (t, J = 7.2 Hz, 3H, CH₃-4''').

4'-O-*n*-Pentyl naringin (3c): Gummy solid, yield 83 %. ^1H NMR (400 MHz, CD_3OD): δ 7.24 (d, J = 8.0 Hz, 2H, H-2' & H-6'), 6.81 (d, J = 8.0 Hz, 2H, H-3' & H-5'), 6.22 (d, J = 2.3 Hz, 1H, H-8), 6.19 (d, J = 2.3 Hz, 1H, H-6), 5.36 (dd, J = 2.6, 12.5 Hz, 1H, H-2), 5.28 (d, J = 1.2 Hz, 1H, H-1'''), 5.11 (d, J = 7.2 Hz, 1H, H-1''), 4.21 (m, 1H, H_a-6''), 4.12 (m, 1H, H_b-6''), 3.84 (m, 1H, H-5'''), 3.76 (m, 1H, H-5''), 3.45 (m, 1H, H-3''), 3.38 (t, J = 8.0 Hz, 2H, CH₂-1'''), 3.31 (m, 1H, H-3'''), 3.29 (m, 1H, H-2'''), 3.27 (m, 1H, H-2''), 3.21 (m, 1H, H-4'''), 3.12 (dd, J = 17.4, 12.5 Hz, 1H, H_a-3), 3.06 (m, 1H, H-4''), 2.67 (dd, J = 17.4, 2.6 Hz, 1H, H_b-3), 1.73 (m 2H, CH₂-2'''), 1.52 (m 2H, CH₂-3'''), 1.35 (d, J = 6.3, 3H, CH₃-6'''), 1.52 (m 2H, CH₂-4'''), 0.65 (t, J = 6.2 Hz, 3H, CH₃-5''').

4'-O-*iso*-Propyl naringin (3d): Gummy solid, yield 80 %. ^1H NMR (400 MHz, CD_3OD): δ 7.32 (d, J = 8.4 Hz, 2H, H-2' & H-6'), 6.92 (d, J = 8.8 Hz, 2H, H-3' & H-5'), 6.01 (d, J = 2.0 Hz, 1H, H-8), 5.98 (d, J = 2.0 Hz, 1H, H-6), 5.34 (dd, J = 2.8, 12.7 Hz, 1H, H-2), 5.28 (d, J = 2.8 Hz, 1H, H-1'''), 5.11 (d, J = 7.2 Hz, 1H, H-1''), 4.58 (m 1H, CH-1'''), 4.23 (m, 1H,

H_a-6"), 4.19 (m, 1H, H_b-6"), 3.99 (m, 1H, H-5"), 3.97 (m, 1H, H-5"), 3.96 (m, 1H, H-3"), 3.94 (m, 1H, H-3"), 3.89 (m, 1H, H-2"), 3.81 (m, 1H, H-2"), 3.45 (m, 1H, H-4"), 3.11 (dd, $J = 17.3, 12.7$ Hz, 1H, H_a-3), 3.03 (m, 1H, H-4"), 2.70 (dd, $J = 17.3, 2.8$ Hz, 1H, H_b-3), 1.32 (d, $J = 6.0, 3$ H, CH₃-6"), 1.12 (d, $J = 7.2$ Hz, 6H, CH₃-2" & CH₃-1").

4'-O-Allyl naringin (3e): Gummy solid, yield 82 %. ¹H NMR (400 MHz, CD₃OD): δ 7.23 (d, $J = 8.0$ Hz, 2H, H-2' & H-6'), 6.84 (d, $J = 7.2$ Hz, 2H, H-3' & H-5'), 6.21 (d, $J = 2.2$ Hz, 1H, H-8), 6.18 (d, $J = 2.2$ Hz, 1H, H-6), 5.89 (m, 1H, H-2"), 5.37 (dd, $J = 2.6, 12.8$ Hz, 1H, H-2), 5.33 (dd, $J = 1.6, 17.2$ Hz, 1H, H_b-3"), 5.28 (d, $J = 1.3$ Hz, 1H, H-1"), 5.05 (d, $J = 7.7$ Hz, 1H, H-1"), 5.03 (dd, $J = 1.6, 10$ Hz, 1H, H_a-3"), 4.20 (d, $J = 6.4$ Hz, 2H, CH₂-1"), 4.15 (m, 1H, H_a-6"), 4.09 (m, 1H, H_b-6"), 3.43 (m, 1H, H-5"), 3.40 (m, 1H, H-5"), 3.37 (m, 1H, H-3"), 3.33 (m, 1H, H-3"), 3.30 (m, 1H, H-2"), 3.28 (m, 1H, H-2"), 3.25 (m, 1H, H-4"), 3.14 (dd, $J = 17.2, 12.8$ Hz, 1H, H_a-3), 3.11 (m, 1H, H-4"), 2.64 (dd, $J = 17.2, 2.6$ Hz, 1H, H_b-3), 1.22 (d, $J = 6.3, 3$ H, CH₃-6").

4'-O-(2"-phenylethyl) naringin (3f): Gummy solid, yield 83 %. ¹H NMR (400 MHz, CD₃OD): δ 7.51 (m, 1H, H-4"), 7.50 (m, 2H, H-3" & H-5"), 7.45 (m, 2H, H-2" & H-6"), 7.35 (d, $J = 8.2$ Hz, 2H, H-2' & H-6'), 7.04 (d, $J = 8.5$ Hz, 2H, H-3' & H-5'), 6.21 (d, $J = 2.3$ Hz, 1H, H-8), 6.18 (d, $J = 2.3$ Hz, 1H, H-6), 5.28 (dd, $J = 2.6, 12.8$ Hz, 1H, H-2), 5.19 (d, $J = 1.3$ Hz, 1H, H-1"), 5.02 (d, $J = 7.4$ Hz, 1H, H-1"), 4.23 (m, 1H, H_a-6"), 4.19 (t, $J = 7.3$ Hz, 2H, CH₂-1"), 4.14 (m, 1H, H_b-6"), 3.96 (m, 1H, H-5"), 3.81 (m, 1H, H-5"), 3.47 (m, 1H, H-3"), 3.42 (m, 1H, H-3"), 3.39 (m, 1H, H-2"), 3.35 (m, 1H, H-2"), 3.30 (m, 1H, H-4"), 3.18 (dd, $J = 17.3, 12.8$ Hz, 1H, H_a-3), 3.14 (m, 1H, H-4"), 2.83 (t, $J = 2.3$ Hz, 2H, CH₂-2"), 2.66 (dd, $J = 17.3, 2.6$ Hz, 1H, H_b-3), 1.23 (d, $J = 6.3, 3$ H, CH₃-6").

4'-O-(3"-phenylpropyl) naringin (3g): Gummy solid, yield 83 %. ¹H NMR (400 MHz, CD₃OD): δ 7.51 (m, 1H, H-4"), 7.50 (m, 1H, H-3" & H-5"), 7.49 (m, 1H, H-2" & H-6"), 7.24 (d, $J = 8.2$ Hz, 2H, H-2' & H-6'), 6.74 (d, $J = 8.5$ Hz, 2H, H-3' & H-5'), 6.19 (d, $J = 2.3$ Hz, 1H, H-8), 6.15 (d, $J = 2.3$ Hz, 1H, H-6), 5.38 (dd, $J = 2.4, 12.7$ Hz, 1H, H-2), 5.31 (d, $J = 1.2$ Hz, 1H, H-1"), 5.12 (d, $J = 7.2$ Hz, 1H, H-1"), 4.29 (m, 1H, H_a-6"), 4.24 (m, 1H, H_b-6"), 3.97 (m, 1H, H-5"), 3.90 (t, $J = 7.0$ Hz, 2H, CH₂-1"), 3.86 (m, 1H, H-5"), 3.38 (m, 1H, H-3"), 3.33 (m, 1H, H-3"), 3.31 (m, 1H, H-2"), 3.28 (m, 1H, H-2"), 3.21 (m, 1H, H-4"), 3.17 (dd, $J = 17.3, 12.7$ Hz, 1H, H_a-3), 3.07 (m, 1H, H-4"), 2.75 (dd, $J = 17.3, 2.4$ Hz, 1H, H_b-3), 2.65 (m, 2H, CH₂-2"), 1.68 (t, $J = 7.1$ Hz, 2H, CH₂-3"), 1.28 (d, $J = 6.3, 3$ H, CH₃-6").

4'-O-(*p*-Bromobenzyl) naringin (3 h): Gummy solid, yield 81 %. ¹H NMR (400 MHz, CD₃OD): δ 7.67 (d, $J = 8.4$ Hz, 4H, H-2" & H-6"), 7.24 (d, $J = 8.3$ Hz, 2H, H-2' & H-6'), 6.96 (d, $J = 8.8$ Hz, 2H, H-3' & H-5'), 6.21 (d, $J = 2.2$ Hz, 1H, H-8), 6.17 (d, $J = 2.2$ Hz, 1H, H-6), 5.27 (dd, $J = 2.5, 12.8$ Hz, 1H, H-2), 5.22 (d, $J = 1.3$ Hz, 1H, H-1"), 5.09 (d, $J = 7.2$ Hz, 1H, H-1"), 5.02 (br s, 2H, CH₂-1"), 4.19 (m, 1H, H_a-6"), 4.08 (m, 1H, H_b-6"), 3.95 (m, 1H, H-5"), 3.84 (m, 1H, H-5"), 3.48 (m, 1H, H-3"), 3.37 (m, 1H, H-3"), 3.35 (m, 1H, H-2"), 3.33 (m, 1H, H-2"), 3.30 (m, 1H, H-4"), 3.23 (dd, $J = 17.2, 12.8$ Hz, 1H, H_a-3), 3.18 (m, 1H, H-4"), 2.81 (dd, $J = 17.2, 2.5$ Hz, 1H, H_b-3), 1.23 (d, $J = 6.1, 3$ H, CH₃-6").

4'-O-(*p*-Fluorobenzyl) naringin (3i): Gummy solid, yield 80 %. ¹H NMR (400 MHz, CD₃OD): δ 7.67 (d, $J = 7.2$ Hz, 2H, H-3" & H-5"), 7.49 (d, $J = 7.2$ Hz, 2H, H-2" & H-6"), 7.24 (d, $J = 8.3$ Hz, 2H, H-2' & H-6'), 6.81 (d, $J = 8.8$ Hz, 2H, H-3' & H-5'), 6.19 (d, $J = 2.2$ Hz, 1H, H-8), 6.15 (d, $J = 2.2$ Hz, 1H, H-6), 5.41 (dd, $J = 2.6, 12.7$ Hz, 1H, H-2), 5.32 (d, $J = 1.4$ Hz, 1H, H-1"), 5.20 (br s, 2H, CH₂-1"), 5.13 (d, $J = 7.0$ Hz, 1H, H-1"), 4.23 (m, 1H, H_a-6"), 4.10 (m, 1H, H_b-6"), 3.96 (m, 1H, H-5"), 3.83 (m, 1H, H-5"), 3.38 (m, 1H, H-3"), 3.35 (m, 1H, H-3"), 3.33 (m, 1H, H-2"), 3.29 (m, 1H, H-2"), 3.26 (m, 1H, H-4"), 3.18 (dd, $J = 17.2, 12.7$ Hz, 1H, H_a-3), 3.14 (m, 1H, H-4"), 2.82 (dd, $J = 17.2, 2.6$ Hz, 1H, H_b-3), 1.40 (d, $J = 6.4, 3$ H, CH₃-6").

4'-O-(2"-Bromoethyl) naringin (3j): Gummy solid, yield 82 %. ¹H NMR (400 MHz, CD₃OD): δ 7.31 (d, $J = 8.4$ Hz, 2H, H-2' & H-6'), 6.87 (d, $J = 8.4$ Hz, 2H, H-3' & H-5'), 6.05 (d, $J = 2.0$ Hz, 1H, H-8), 6.02 (d, $J = 2.0$ Hz, 1H, H-6), 5.37 (dd, $J = 2.5, 12.6$ Hz, 1H, H-2), 5.32 (d, $J = 1.2$ Hz, 1H, H-1"), 5.11 (d, $J = 7.2$ Hz, 1H, H-1"), 4.30 (m, 1H, H_a-6"), 4.25 (m, 1H, H_b-6"), 4.21 (t, $J = 6.0$ Hz, 2H, CH₂-1"), 3.95 (m, 1H, H-5"), 3.82 (m, 1H, H-5"), 3.60 (t, $J = 6.0$ Hz, 2H, CH₂-2"), 3.37 (m, 1H, H-3"), 3.35 (m, 1H, H-3"), 3.33 (m, 1H, H-2"), 3.31 (m, 1H, H-2"), 3.28 (m, 1H, H-4"), 3.15 (dd, $J = 17.4, 12.6$ Hz, 1H, H_a-3), 3.12 (m, 1H, H-4"), 2.68 (dd, $J = 17.4, 2.5$ Hz, 1H, H_b-3), 1.35 (d, $J = 6.8, 3$ H, CH₃-6").

4'-O-(Ethylacetato) naringin (3k): Gummy solid, yield 83 %. ¹H NMR (400 MHz, CD₃OD): δ 7.32 (d, $J = 8.2$ Hz, 2H, H-2' & H-6'), 6.86 (d, $J = 8.6$ Hz, 2H, H-3' & H-5'), 6.17 (d, $J = 2.3$ Hz, 1H, H-8), 6.13 (d, $J = 2.3$ Hz, 1H, H-6), 5.36 (dd, $J = 2.6, 12.5$ Hz, 1H, H-2), 5.22 (d, $J = 1.4$ Hz, 1H, H-1"), 5.11 (d, $J = 7.4$ Hz, 1H, H-1"), 4.59 (s, 2H, CH₂-1"), 4.26 (m, 1H, H_a-6"), 4.21 (q, $J = 7.2$ Hz, 2H, CH₂-4"), 4.17 (m, 1H, H_b-6"), 3.93 (m, 1H, H-5"), 3.82 (m, 1H, H-5"), 3.45 (m, 1H, H-3"), 3.36 (m, 1H, H-3"), 3.34 (m, 1H, H-2"), 3.32 (m, 1H, H-2"), 3.29 (m, 1H, H-4"), 3.17 (dd, $J = 17.4, 12.5$ Hz, 1H, H_a-3), 3.14 (m, 1H, H-4"), 2.74 (dd, $J = 17.4, 2.6$ Hz, 1H, H_b-3), 1.27 (t, $J = 7.2$ Hz, 3H, CH₃-5"), 0.89 (d, $J = 6.7, 3$ H, CH₃-6").

4'-O-(N-3"-, 5"-Dimethylphenyl-C-acetamido) naringin (3l): Gummy solid, yield 82 %. ¹H NMR (400 MHz, CD₃OD): δ 7.41 (d, $J = 8.4$ Hz, 2H, H-2' & H-6'), 6.94 (br d, $J = 2.4$ Hz, 1H, H-4"), 6.75 (d, $J = 8.8$ Hz, 2H, H-3' & H-5'), 6.29 (d, $J = 2.3$ Hz, 1H, H-8), 6.25 (d, $J = 2.3$ Hz, 1H, H-6), 6.15 (br d, $J = 2.4$ Hz, 2H, H-2" & H-6"), 5.35 (dd, $J = 2.4, 12.7$ Hz, 1H, H-2), 5.34 (d, $J = 1.4$ Hz, 1H, H-1"), 5.13 (d, $J = 7.0$ Hz, 1H, H-1"), 4.45 (s, 2H, CH₂-1"), 4.27 (m, 1H, H_a-6"), 4.19 (m, 1H, H_b-6"), 3.94 (m, 1H, H-5"), 3.81 (m, 1H, H-5"), 3.39 (m, 1H, H-3"), 3.37 (m, 1H, H-3"), 3.34 (m, 1H, H-2"), 3.32 (m, 1H, H-2"), 3.28 (m, 1H, H-4"), 3.19 (dd, $J = 17.3, 12.7$ Hz, 1H, H_a-3), 3.14 (m, 1H, H-4"), 2.99 (dd, $J = 17.3, 2.4$ Hz, 1H, H_b-3), 2.28 (s, 6H, CH₃-7" & CH₃-8"), 1.23 (d, $J = 6.2, 3$ H, CH₃-6").

Enzyme inhibition assays: Enzyme inhibition activities of all the derivatives as well as that of naringin was checked by following four assays.

Acetylcholinesterase assay: The acetylcholinesterase (AChE) inhibition activity was performed according to the reported method¹⁰ with slight modifications as follows. Total volume of the reaction mixture was 100 μ L. It contained 60 μ L

Na₂HPO₄ buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM well⁻¹) was added, followed by the addition of 10 µL (0.005 unit well⁻¹) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of 10 µL of 0.5 mM well⁻¹ substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM well⁻¹). After 15 min of incubation at 37 °C, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well⁻¹) was used as a positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Butyrylcholinesterase assay: The butyrylcholinesterase (BChE) inhibition activity was performed according to the reported method¹⁰ with slight modifications as follows. Total volume of the reaction mixture was 100 µL containing 60 µL, Na₂H PO₄ buffer, 50 mM and pH 7.7. Ten µL test compound 0.5 mM well⁻¹, followed by the addition of 10 µL (0.5 unit well⁻¹) BChE. The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of 10 µL of 0.5 mM well⁻¹ substrate (butyrylthiocholine bromide) followed by the addition of 10 µL DTNB, 0.5 mM well⁻¹. After 15 min of incubation at 37 °C, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well⁻¹) was used as positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC₅₀ values (concentration at which there is 50 % enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Lipoxygenase assay: Lipoxygenase activity was assayed according to the reported method¹¹⁻¹³ but with slight modifications. A total volume of 200 µL assay mixture contained 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL test compound and 15 µL purified lipoxygenase enzyme (Sigma, USA). The contents were mixed and pre-read at 234 nm and preincubated for 10 min at 25 °C. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Quercetin (0.5 mM well⁻¹) was used as a positive control. The percentage inhibition was calculated by formula given below.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Chymotrypsin assay: The α-chymotrypsin inhibition activity is performed according to slightly modified method of Rehman *et al.*,¹⁴. Total volume of the reaction mixture of

100 µL contained 60 µL, 50 mM *tris*-HCl buffer, pH 7.6, 10 µL (0.5 mM) by the addition of 15 µL (0.9 units) enzyme. The contents were mixed, preincubated for 20 min at 37 °C and pre read at 410 nm. The reaction was initiated by the addition of 15 µL of 1.3 mM substrate (N-succinyl phenyl-alanine-P-nitroanilide). 70 µL of buffer was used as a control. After 0.5 h of incubation at 37 °C, absorbance was measured at 410 nm using synergy HT microplate reader. All experiments were carried out in triplicate. The per cent inhibition was calculated by following equation.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC₅₀ values (concentration at which there is 50 % in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

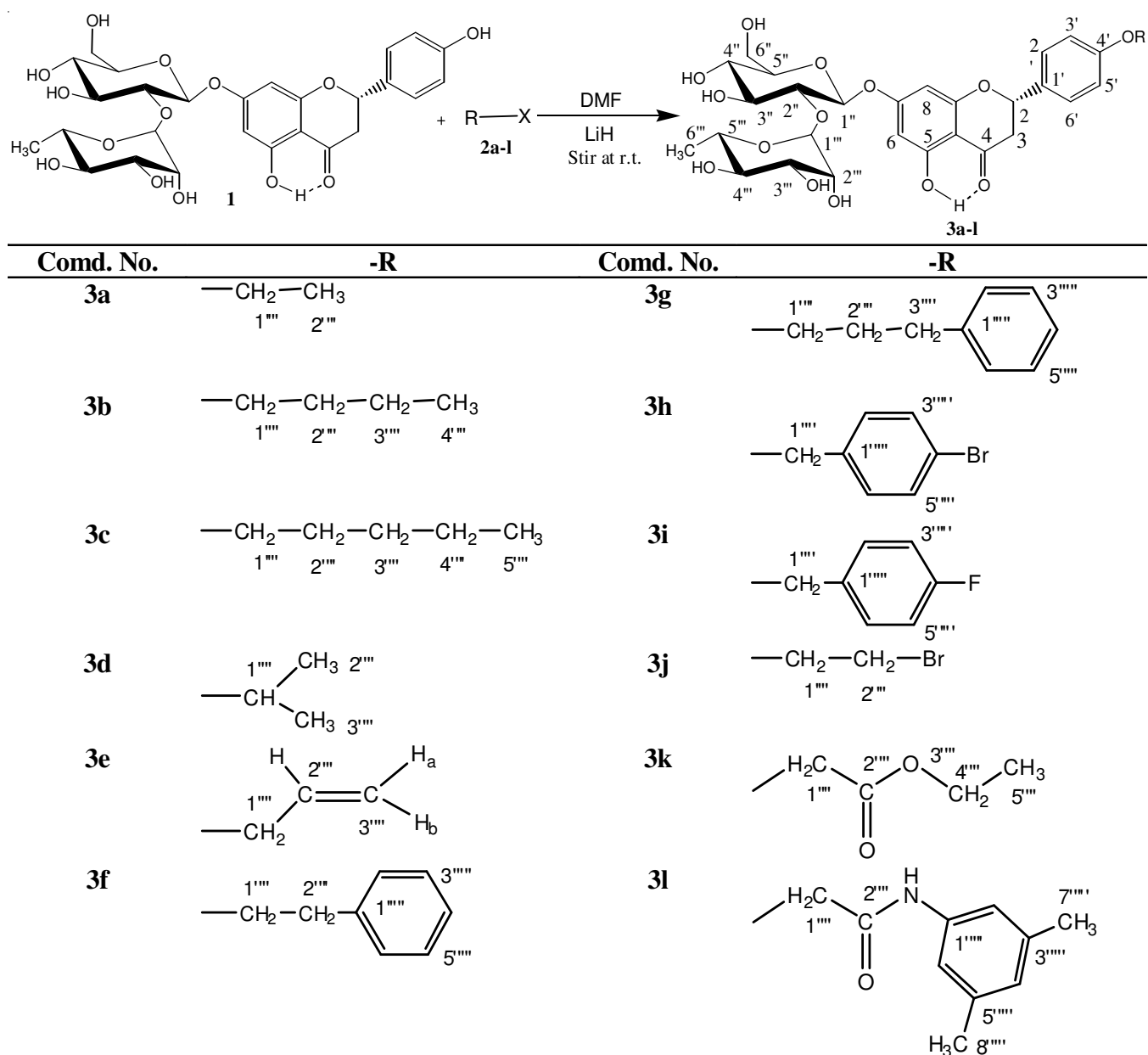
DPPH radical scavenging activity: The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity according to reported method¹⁵. Different concentrations of compounds in respective solvents were added at an equal volume (10 µL) to 90 µL of 100 µM methanolic DPPH in a total volume of 100 µL in 96-well plates. The contents were mixed and incubated at 37 °C for 0.5 h. The absorbance was measured at 517 nm using Synergy HT BioTek® USA microplate reader. Quercetin and L-ascorbic acid were used as standard antioxidants. The experiments were carried out in triplicates. IC₅₀ values were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula.

$$\text{Scavenging activity (\%)} = \frac{100 - \text{Absorbance of test compound}}{\text{Absorbance of control}} \times 100$$

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2003. Results are presented as mean ± SEM.

RESULTS AND DISCUSSION

Ethyl acetate soluble fraction of *Rhynchosia pseudo-cajan* (Camb.) yielded a flavanone glycoside, naringin (**1**). Its structures were determined on the basis of ¹D and ²D NMR spectroscopic studies and by comparison with the published data¹⁶. Naringin was purified as white powder through column chromatography and its molecular formula C₂₇H₃₂O₁₄ was established by counting carbons and hydrogens from the data of its ¹H NMR and ¹³C NMR DEPT spectra. The ¹H NMR spectrum of naringin thoroughly supported a flavanone glycoside structure closely resembling to that of reported data¹⁶. The presence of flavanone skeleton was supported by ¹H NMR spectra that showed resonances for one H-2 proton and two H-3 protons [δ 5.33 (dd, *J* = 2.7, 12.9 Hz, 1H, H-2), 3.16 (dd, *J* = 17.1, 12.9 Hz, 1H, H_a-3) and 2.72 (dd, *J* = 17.1, 2.7 Hz, 1H, H_b-3)] of characteristic flavanones¹⁶. Two *ortho* coupled doublets at δ 7.28 (*J* = 8.4 Hz, 2H, H-2' & H-6') and δ 6.79 (*J* = 8.7 Hz, 2H, H-3' & H-5') were in agreement with the presence of hydroxyl group at C-4' in the ring C of the molecule. The doublets at δ 6.16 and 6.14, showing *meta*-coupling (*J* = 2.1

Scheme-I: Synthesis of naringin derivatives **3a-l**

Hz) were due to H-6 and H-8 protons for ring A respectively and downfield shifted values represented presence of substituted oxygen at C-5, with which glycone part was attached. The chemical shift of C-5 at δ 164.98 in ^{13}C NMR supported the presence of substituted phenolic group at this position. The presence of two anomeric doublets at δ 5.06 (d, $J = 7.2$ Hz, 1H, H-1'') and δ 5.24 (d, $J = 1.2$ Hz, 1H, H-1''') along with another doublet at δ 1.27 (d, $J = 6.3$, 3H, CH₃-6'') for a methyl group revealed the presence of glucose and rhamnose sugars in the molecule and their connectivity of C-1''' $\rightarrow$ C-2'' was thoroughly supported by the HMBC correlations of H-1''' with C-2'' and H-2'' with C-1''. The spectral data of naringin was also in complete agreement with that of its reported data in the literature¹⁷.

In this paper, we report the synthesis of 12 new 4'-O-substituted derivatives, **3a-l**, of **1** (Scheme-I) and their structures have been ascertained with the help of their ^1H NMR

spectra. The compound, **3a**, was synthesized as gummy solid. Its molecular formula, C₂₉H₃₆O₁₄, was established by counting the number hydrogens from its ^1H NMR data. It exhibited similar ^1H NMR signals to those of naringin (**1**) but the additional signals at δ 3.85 (q, $J = 7.1$ Hz, 2H, CH₂-1''') and 1.38 (t, $J = 7.1$ Hz, 2H, CH₃-2''') characteristic of ethyl group confirmed the substitution of an ethyl group at the more exposed phenolic group at C-4' in this molecule as the other hydroxyl group at C-5 was sterically hindered and could not be substituted under the conditions. On the basis of above cumulative evidences the structure of **3a** was assigned as 4'-O-ethyl naringin. The **3b** was also a gummy solid with molecular formula C₃₁H₄₀O₁₄. In its ^1H NMR spectrum, the signals at δ 3.45 (t, $J = 8.4$ Hz, 2H, CH₂-1'''), 1.75 (m, 2H, CH₂-2'''), 1.47 (m, 2H, CH₂-3''') and 0.98 (t, $J = 7.2$ Hz, 3H, CH₃-4''') confirmed the substitution of an *n*-butyl group in this molecule so the structure of **3b** was assigned as 4'-O-*n*-butyl naringin.

TABLE-1
DPPH RADICAL SCAVENGING ACTIVITY, BUTYRYLCHOLINESTERASE ASSAY, ACETYLCHOLINESTERASE ASSAY, LIPOXYGENASE ASSAY AND CHYMOTRYPSIN ASSAY

Compd No.	DPPH radical scavenging activity		Butyrylcholinesterase Assay		Acetylcholinesterase Assay		Lipoxygenase assay		Chymotrypsin assay	
	(Inh. %) 0.5 mM	(IC ₅₀) μM	(Inh. %) 0.5 mM	(IC ₅₀) μM	(Inh. %) 0.5 mM	(IC ₅₀) μM	(Inh. %) 0.5 mM	(IC ₅₀) μM	(Inh. %) 0.5 mM	(IC ₅₀) μM
1	10.11 ± 0.33	NIL	33.53 ± 0.22	NIL	45.22 ± 0.74	NIL	78.32 ± 0.14	143.51 ± 0.12	30.16 ± 0.71	NIL
3a	75.58 ± 0.23	36.21 ± 0.32	71.48 ± 0.58	335.81 ± 0.09	50.70 ± 0.74	NIL	56.98 ± 0.71	<500	19.85 ± 0.014	NIL
3b	36.19 ± 0.14	NIL	38.78 ± 0.47	NIL	51.97 ± 0.14	NIL	52.50 ± 0.85	474.51 ± 0.03	40.44 ± 0.24	NIL
3c	27.56 ± 0.51	NIL	29.69 ± 0.65	NIL	40.13 ± 0.64	NIL	1.88 ± 0.25	NIL	39.71 ± 0.62	NIL
3d	11.96 ± 0.65	NIL	31.04 ± 0.14	NIL	44.20 ± 0.85	NIL	73.69 ± 0.65	164.91 ± 0.52	38.24 ± 0.22	NIL
3e	8.16 ± 0.32	NIL	49.13 ± 0.25	NIL	54.90 ± 0.25	NIL	58.26 ± 0.45	<500	19.85 ± 0.45	NIL
3f	18.27 ± 0.14	NIL	79.66 ± 0.22	266.21 ± 0.35	42.04 ± 0.31	NIL	24.85 ± 0.74	NIL	30.88 ± 0.34	NIL
3g	10.47 ± 0.71	NIL	65.14 ± 0.35	212.91 ± 0.56	45.10 ± 0.06	NIL	22.86 ± 0.17	NIL	25.74 ± 0.78	NIL
3h	15.26 ± 0.01	NIL	57.74 ± 0.39	<400	40.38 ± 0.74	NIL	23.56 ± 0.37	NIL	68.38 ± 0.52	159.3 ± 0.05
3i	41.45 ± 0.23	NIL	65.14 ± 0.11	358.11 ± 0.01	52.87 ± 0.01	NIL	16.53 ± 0.81	NIL	41.91 ± 0.34	NIL
3j	13.07 ± 0.74	NIL	40.59 ± 0.75	NIL	55.03 ± 0.32	NIL	21.10 ± 0.73	NIL	34.56 ± 0.22	NIL
3k	8.75 ± 0.65	NIL	35.04 ± 0.88	NIL	44.97 ± 0.15	NIL	31.30 ± 0.16	NIL	34.92 ± 0.81	NIL
3l	10.41 ± 0.45	NIL	50.17 ± 0.09	<400	27.01 ± 0.64	NIL	88.06 ± 0.31	157.91 ± 0.54	31.11 ± 0.64	NIL
Control	Quercetin	16.96 ± 0.14	Eserine	0.85 ± 0.001	Eserine	0.04 ± 0.001	Baicalein	22.4 ± 1.3	Chymostatin	8.24 ± 0.11

The **3c** having molecular formula C₃₂H₄₂O₁₄ was identified as 4'-O-*n*-pentyl naringin due to the characteristic signals of *n*-pentyl group at δ 3.38 (t, *J* = 8.0 Hz, 2H, CH₂-1'''), 1.73 (m, 2H, CH₂-2'''), 1.52 (m, 4H, CH₂-3''' & CH₂-4''') and 0.65 (t, *J* = 6.2 Hz, 3H, CH₃-5'''). In **3d**, having molecular formula C₃₀H₃₈O₁₄, the signals at δ 4.58 (m, 1H, CH-1''') and 1.12 (d, *J* = 7.2 Hz, 6H, CH₃-2''' & CH₃-1''') were characteristic of isopropyl group and thus its structure was elucidated as 4'-O-iso-propyl naringin. The signals at δ 5.89 (m, 1H, H-2'''), 5.33 (dd, *J* = 1.6, 17.2 Hz, 1H, H_b-3'''), 5.03 (dd, *J* = 1.6, 10 Hz, 1H, H_a-3''') and 4.20 (d, *J* = 6.4 Hz, 2H, CH₂-1''') in **3e** were typical for an allyl group and consequently confirming the structure of this molecule as 4'-O-allyl naringin. In the ¹H NMR spectrum of **3f**, the signals in aromatic region at δ 7.51 (m, 1H, H-4'''), 7.50 (m, 2H, H-3''' & H-5'''), 7.45 (m, 2H, H-2''' & H-6''') indicated a phenyl group while signals at δ 4.19 (t, *J* = 7.3 Hz, 2H, CH₂-1''') and 2.83 (t, *J* = 2.3 Hz, 2H, CH₂-2''') were due to a substituted ethyl group and hence its structure was inferred as 4'-O-(2'''-phenylethyl) naringin. Similarly, on the basis of signals in the aromatic region at δ 7.51 (m, 1H, H-4'''), 7.50 (m, 1H, H-3''' & H-5''') and 7.49 (m, 1H, H-2''' & H-6''') indicative for phenyl group and signals at δ 3.90 (t, *J* = 7.0 Hz, 2H, CH₂-1'''), 2.65 (m, 2H, CH₂-2''') and 1.68 (t, *J* = 7.1 Hz, 2H, CH₂-3''') indicative for propyl group, the structure of **3g** was deduced as 4'-O-(2'''-phenylpropyl) naringin.

In ¹H NMR spectrum of **3h**, the signals of an ortho coupled doublet *i.e.* δ 7.67 (d, *J* = 8.4 Hz, 4H, H-2''', H-3''', H-5''' & H-6''') showed that *p*-Bromo benzyl group has been attached so it thoroughly supported its structure as 4'-O-(*p*-bromo benzyl) naringin. In **3i**, the signals of two ortho coupled doublets *i.e.* δ 7.67 (d, *J* = 7.2 Hz, 2H, H-3''' & H-5'''), 7.49 (d, 7.2 Hz, 2H, H-2''' & H-6''') showed that *p*-fluoro benzyl group has been attached so the structure of **3i** was established as 4'-O-(*p*-fluorobenzyl) naringin. In ¹H NMR spectrum of **3j**, two triplet signals at δ 4.21 (t, *J* = 6.0 Hz, 2H, CH₂-1''') and 3.60 (t, *J* = 6.0 Hz, 2H, CH₂-2''') thoroughly supported its structure as 4'-O-(2'''-bromoethyl) naringin. In **3k**, the signals at δ 4.21 (q, *J* = 7.2 Hz, 2H, CH₂-4''') and 1.27, (t, *J* = 7.2 Hz,

3H, CH₃-5''') were characteristic for an ethoxy group while the one methylene signal at δ 4.59 (s, 2H, CH₂-1''') altogether corroborated the structure of this molecule as 4'-O-(ethylacetato) naringin. For **3l**, only two signals in aromatic region δ 6.94 (br d, *J* = 2.4 Hz, 1H, H-4''') and δ 6.15 (br d, *J* = 2.4 Hz, 2H, H-2''' & H-6''') represented a trisubstituted phenyl ring. Methyl signals at δ 2.28 (s, 6H, CH₃-7''' & CH₃-8''') were also in agreement that two methyl groups were substituted on the phenyl ring while another singlet at δ 4.45 (s, 2H, CH₂-1''') was emblematic for the methylene of acetamido group. Therefore, on the basis of aforesaid evidences the structure of this molecule was established as 4'-O-(N-3''', 5'''-dimethylphenyl-C-acetamido) naringin.

The inhibitory potential of these derivatives, **3a-l**, was studied against four enzymes namely, acetyl cholinesterase, butyryl cholinesterase lipoxygenase and chymotrypsin as well as their antioxidant potential by DPPH radical scavenging was also studied and the results have been shown in Table-1. It was observed that only **3a** had very good antioxidant potential showing IC₅₀ 36.21 ± 0.32 μM as compared to quercetin, a reference standard, having IC₅₀ 16.96 ± 0.14 μM. All the other derivatives as well as naringin showed no antioxidant potential. Only **3a**, **3f**, **3g** and **3i** were moderate inhibitors of butyrylcholinesterase having IC₅₀ values of 335.81 ± 0.09 μM, 266.21 ± 0.35 μM, 212.91 ± 0.56 μM and 358.11 ± 0.01 μM respectively while naringin (**1**) itself, **3b**, **3d**, **3l** and were recognized as moderate inhibitors of lipoxygenase having IC₅₀ values of 143.51 ± 0.12 μM, 474.51 ± 0.03 μM, 164.91 ± 0.52 μM, 157.91 ± 0.54 μM respectively. Only **3h** was found to be moderate inhibitor of chymotrypsin. It showed IC₅₀ value 159.3 ± 0.05 μM as compared to chymostatin, a reference standard having IC₅₀ value 8.24 ± 0.11 μM. It was also obvious from the screening results that the substitution of any group at C-4' of naringin (**1**) has no remarkable effect on its biological activity against studied enzymes.

Conclusion

From the results of antioxidant potential and enzyme inhibition activities it was observed that only 4'-O-ethyl

naringin (**3a**) showed antioxidant potential (IC_{50} 36.21 ± 0.32 μ M). 4'-O-Ethyl naringin (**3a**), 4'-O-(2'''-phenylethyl) naringin (**3f**), 4'-O-(3'''-phenylpropyl) naringin (**3g**) and 4'-O-(*p*-fluorobenzyl) naringin (**3i**) were found to be moderate inhibitors of butyrylcholinesterase while naringin (**1**) itself, 4'-O-*n*-butyl naringin (**3b**), 4'-O-iso-propyl naringin (**3d**) and 4'-O-(N-3''', 5'''-dimethylphenyl-C-acetamido) naringin (**3l**) were identified as moderate inhibitors of lipoxigenase. Against chymotrypsin only 4'-O-(*p*-bromobenzyl) naringin (**3h**) was found to be a moderate inhibitor. So it was concluded that the substitution of any group at C-4' of naringin (**1**) has no remarkable effect on its biological activity against studied enzymes.

ACKNOWLEDGEMENTS

The authors are thankful to Higher Education Commission of Pakistan for financial grant.

REFERENCES

1. M. Ajaib, Z. Khan, N. Khan and M. Wahab, *Pak. J. Bot.*, **42**, 1407 (2010).
2. E.J. Lee and L.K. Soon, Republic Korean, Kongkae Taeho Konjbo KR2006 65, 250, 14 June (2006), Appl. 104, 049, (10 Dec. 2004).
3. A. Dama, G. Duvvuru, R. Pasupulati, S. Otto and S. Hildebebert, *Phytochemistry*, **19**, 478 (1980).
4. A. Dama, G. Duvvuru, R. Pasupulati, S. Otto and S. Hildebebert, *Phytochemistry*, **19**, 483 (1980).
5. A. Dama and R. Pasupulati, *Pharmazie*, **39**, 714 (1984).
6. A. Dama, R. Pasupulati and K.N. Rao, *Experientia*, **41**, 251 (1985).
7. C.V. Rao and D. Gunasikar, *Acta Cienc. Ind. (Chem.)*, **13**, 187 (1987).
8. B.B. Aggarwal, A. Kumar and A.C. Bharti, *Anticancer Res.*, **23**, 363 (2003).
9. G. Nahak and R.K. Sahu, *J. Am. Sci.*, **6**, 123 (2010).
10. G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherstone, *Biochem. Pharmacol.*, **7**, 88 (1961).
11. A.L. Tappel, *Arch. Biochem. Biophys.*, **44**, 378 (1953).
12. A.T. Evans, *Biochem. Pharmacol.*, **36**, 2035 (1987).
13. S. Baylac and P. Racine, *Internat. J. Aromather.*, **13**, 138 (2003).
14. A.M. Khan, S. Anjum, M.I. Choudhary and Atta-ur-Rahman, *World J. Chem.*, **1**, 30 (2006).
15. T.A. Koleva Van Beek, J.P.H. Linssen, A. de Groot and L.N. Evstatieva, *Phytochem. Anal.*, **13**, 8 (2002).
16. M. Ichimaru, M. Moriyasu, Y. Nishiyama and A. Kato, *J. Nat. Prod.*, **5**, 1113 (1996).
17. C.Y. Kim, H.J. Lee, M.K. Lee, M. Ahn and J. Kim, *J. Sep. Sci.*, **30**, 2693 (2007).