Lipoxygenase Inhibiting Constituents from *Indigofera hetrantha*

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Received March 9, 2004; accepted August 11, 2004

A lignan and two acylphloroglucinols have been isolated from the ethyl acetate soluble fraction of the whole plant of *Indigofera hetrantha*. Their structures have been assigned on the basis of spectral analysis including 1D and 2D NMR techniques. Compounds 1—3 displayed promising inhibitory potential against enzyme lipoxygenase in concentration-dependent manner.

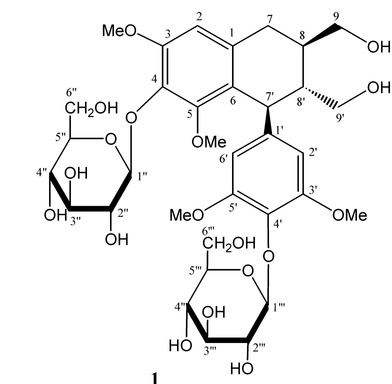
Key words *Indigofera hetrantha*; Leguminosae; lignan; acylphloroglucinols; enzyme inhibition

The genus *Indigofera* (Leguminosae) comprises of 300 species which are herbs or shrubs, distributed throughout the tropical regions of the globe. In Pakistan it is represented by 24 species.[1)] The family Leguminosae is known to be a rich source of flavonoids and most of the phenyl derivatives have been found in this family.[2,3)] The flavonoids exhibit diverse biological activities and recent interest has been focussed on their medicinal and nutritional values.[4)] Recently, it was found that the prenyl groups on the flavonoid skeleton play an important role in anti-HIV activity.[5)] *Indigofera hetrantha* WALL. is widely distributed in northern parts of Pakistan and finds various medicinal uses in the indigenous system of medicine. The sister species of *Indigofera hetrantha* were found to contain toxic nitro compounds of insecticidal activity. Previously triterpenes, steroids and flavonoids have been reported from *Indigofera hetrantha*.[6)] In the previous investigation, a methanolic extract of the whole plant of *Indigofera hetrantha* showed positive activity in the brine shrimp lethality test.[7)] Further biological screening of the methanolic extract and ethyl acetate soluble fraction revealed significant inhibitory activity against the enzyme lipoxygenase. This prompted us to carry out bioassay-guided isolation studies on EtOAc soluble fraction of this plant. Herein we report the isolation and structural elucidation of (+)-lyoniresinol 4,4′-bis-*O*-*β*-D-glucopyranoside (**1**), 1-[(3-methylbutanoyl)phloroglucinyl]-*β*-D-glucopyranoside (**2**) and 1-[(pentanoyl)phloroglucinyl]-*β*-D-glucopyranoside (**3**). The compounds (**1**—**3**) showed promising inhibitory activity against lipoxygenase enzyme.

Results and Discussion

Ethyl acetate soluble fraction of the methanolic extract of the whole plant of *Indigofera hetrantha* was subjected to column chromatography over flash silica eluting with different mobile phases. Compounds **1**—**3** were finally obtained and their structures were established by UV, IR, mass and NMR spectroscopy.

Compound **1** was isolated as colorless gummy solid. The high resolution and fast atomic bombardment mass spectrometry (HR-FAB-MS) established the molecular formula to be $C_{34}H_{48}O_{18}$, showing a $[M-H]^+$ peak at *m/z* 743.2768 (Calcd for $C_{34}H_{47}O_{18}$, 743.2762) having eleven degree of unsaturation. The infrared (IR) spectrum showed the absorption band due to benzene rings (1603—1517 cm^{-1}) and hydroxyl groups (3534—3363 cm^{-1}). The ultraviolet (UV) absorption showed λ_{max} at 206, 242 and 278 nm. The ^{1}H- and ^{13}C-NMR spectra of compound **1** exhibited signals characteristic of an aryl-tetralin type lignan-glycoside (Table 1).[8,9)] The signals for the sugar moieties appeared at δ 4.82 (1H, d, J=7.9 Hz, H-1‴), 4.74 (1H, d, J=8.1 Hz, H-1″), 3.51 (2H, m, H-2″, H-2‴), 3.24—3.46 (6H, m, H-3″, H-3‴, H-4″, H-4‴, H-5″ and H-5‴), and methylene protons at δ 3.85 (1H, dd, J=11.9, 1.9 Hz, H-6″A), 3.65 (1H, dd, J=11.9, 5.3 Hz, H-6″B), 3.79 (1H, dd, J=11.8, 2.1 Hz, H-6‴A) and 3.61 (1H, dd, J=11.8, 4.8 Hz, H-6‴B). The ^{1}H-NMR spectrum showed the presence of aliphatic methylene protons at δ 2.61 (1H, dd, J=15.0, 4.7 Hz), 2.43 (1H, dd, J=15.0, 11.5 Hz), two oxygenated methylenes at δ 3.63 (1H, m), 3.48 (1H, m), 3.31 (2H, m) and one methine at δ 4.28 (1H, d, J=5.6 Hz). The signals for four methoxyl group appeared at δ 3.82 (3H, s), 3.72 (6H, s), and 3.36 (3H, s) and the downfield region of the spectrum



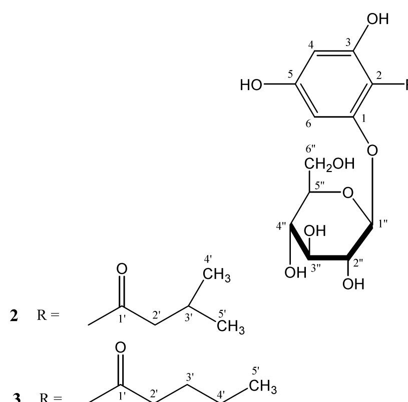


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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data and HMBC Correlations of Compound **1** (100 MHz, 400 MHz and 400 MHz in CD_3OD)

No.	δ_C	$\delta_H$$^{a)}$	HMBC (H to C)
1	134.5 (s)		
2	107.9 (d)	6.57 (s)	1, 3, 4
3	150.6 (s)		
4	136.4 (s)		
5	151.2 (s)		
6	125.8 (s)		
7	32.5 (t)	2.61 (dd, 15.0, 4.7)	1, 6, 8
		2.43 (dd, 15.0, 11.5)	1, 6, 8
8	40.1 (d)	1.48 (m)	7, 9, 8′
9	63.5 (t)	3.63 (m)	7, 8, 8′
		3.48 (m)	7, 8, 8′
1′	137.5 (s)		
2′,6′	107.1 (d)	6.37 (s)	1′, 7′, 3′, 5
3′,5′	152.9 (s)		
4′	134.2 (s)		
7′	40.7 (d)	4.28 (d, 5.6)	6, 1′, 8′
8′	47.1 (d)	1.88 (m)	8, 7′, 9′
9′	64.3 (t)	3.31 (m)	8, 7′, 8′
1″	102.3 (d)	4.74 (d, 8.1)	8, 7′, 8′
2″	75.2 (d)	3.51 (m)	4, 2″, 3″
3″	77.8 (d)	3.24—3.46 (m)	1″
4″	71.4 (d)	3.24—3.46 (m)	2″, 5″
5″	78.1 (d)	3.24—3.46 (m)	1″, 6″
6″	62.7 (t)	3.85 (dd, 11.9, 1.9)	5
		3.65 (dd, 11.9, 5.3)	
1‴	102.0 (d)	4.82 (d, 7.9)	4′, 2‴
2‴	74.9 (d)	3.51 (m)	1‴, 2‴
3‴	77.7 (d)	3.24—3.46 (m)	2‴, 5‴
4‴	71.2 (d)	3.24—3.46 (m)	2‴, 5‴
5‴	77.9 (d)	3.24—3.46 (m)	1‴, 6‴
6‴	62.6 (t)	3.79 (dd, 11.8, 2.1)	5‴
		3.61 (dd, 11.8, 4.8)	
3-OMe	56.2 (q)	3.82 (s)	3
5-OMe	60.2 (q)	3.36 (s)	5
3′,5′-Me	56.9 (q)	3.72 (s)	3′, 5′

a) Coupling constants (*J* in Hz) are in parentheses.

showed two singlet at δ 6.57 (1H, s, H-2) and 6.37 (2H, s, H-2′, 6′). The acid hydrolysis of **1** provided the glycone which could be separated and identified as D-glucose through its optical rotation sign and comparison of the retention time of its trimethylsilyl (TMS) ether with that of the standard in gas chromatography (GC). The aglycone could be identified as (+)-lyoniresinol from its spectral features and optical rotation.[10,11] The broad-band and distortionless enhancement by polarization transfer (DEPT) ^{13}C-NMR spectra of **1** (Table 1) disclosed 31 carbon singles for three methyl, five methylene, fifteen methine and eight quaternary carbons. The signals for anomeric carbons appeared at δ 102.3 (C-1″) and 102.0 (C-1‴). The attachment of the glucose moieties was deduced at C-4 and C-4′ due to the upfield shifts of these carbons and downfield shifts of C-3, C-5, C-3′ and C-5′ compared to literature values.[10] It was further confirmed by heteronuclear multiple-bond connectivity (HMBC) experiments; the important correlations are illustrated in Table 1. The relative stereochemistry of **1** was similar to that of (+)-lyoniresinol. On the basis of these evidence, the structure **1** could be assigned to (+)-lyoniresinol 4,4′-bis-*O*-β-D-glucopyranoside.

Compound **2** was isolated as colorless gummy solid. The HR-FAB-MS established the molecular formula to be $C_{17}H_{24}O_9$, showing a $[M+H]^+$ peak at *m/z* 373.1498 (Calcd for $C_{17}H_{23}O_9$, 373.1492) having six degree of unsaturation. The IR spectrum showed the absorption bands due to keto carbonyl (1690 cm^{-1}) and hydroxyl groups (3545—3402 cm^{-1}). The UV absorption showed λ_{max} at 216 and 310 nm. The ^{1}H-NMR spectrum of **2** (Table 2) showed signals for the side chain including two methyl protons at δ 0.95 (3H, d, $J=6.6$ Hz, H-4′), 0.92 (3H, d, $J=6.6$ Hz, H-5′), one methylene at δ 3.16 (1H, dd, $J=15.7$, 6.8 Hz, H-2′), 2.86 (1H, dd, $J=15.7$, 7.4 Hz, H-2′) and one methine at δ 2.23 (1H, m, H-3′). The protons α to carbonyl group resonated at low field, but such a phenomenon is commonly observed in case of acylphloroglucinols when the ^{1}H-NMR spectra are recorded in polar dutrated solvents.[12,13] The aromatic protons appeared at δ 6.15 (1H, d, $J=2.2$ Hz) and 5.94 (1H, d, $J=2.2$ Hz). The upfield shift and smaller coupling constant of these protons showed that these meta coupled protons are between oxygenated quaternary carbons.[12] The signals for the sugar moiety appeared at δ 5.01 (1H, d, $J=7.5$ Hz, H-1″), 3.51 (1H, m, H-2″), 3.42 (2H, m, H-3″, 4″), 3.46 (1H, m, H-5″) and methylene protons at δ 3.89 (1H, br d, $J=12.3$ Hz) and 3.71 (1H, dd, $J=11.9$, 4.9 Hz). The sugar was identified as β-D-glucose by means of its ^{1}H- and ^{13}C-NMR data (Table 2). The acid hydrolysis of **2** provided glycone, which could be separated and identified as D-glucose. The aglycone could be identified as phlorisovalerophenone. The ^{13}C-NMR spectrum of **2** (Table 2) disclosed 17 carbon singles for two methyl, two methylene, eight methine and five quaternary carbons. The signals for anomeric carbon appeared at δ 101.8 (C-1″). The attachment of the glucose moiety was confirmed at C-1 through ^{13}C- and ^{1}H-NMR chemical shifts of C-4 (δ 95.4) and C-6 (δ 98.3) and C_4-H (δ 5.94) and C_6-H (δ 6.15), respectively. It was further confirmed by HMBC experiment; the important interactions are illustrated in Table 2. On the basis of these evidence, the structure of **2** could be assigned as 1-[(3-methylbutanoyl)phloroglucinyl]-β-D-glucopyranoside.

Compound **3** was also isolated as colorless gummy solid. The HR-FAB-MS showed $[M+H]^+$ peak at *m/z* 373.1495, which confirmed the molecular formula $C_{17}H_{24}O_9$. The IR, UV and EI-MS spectra of **3** were similar to those of **2** but some differences were observed in ^{1}H- and ^{13}C-NMR spectra. In the ^{1}H-NMR spectrum (Table 2) of **3**, many structural features were similar to **2** except the differences in the side chain. The signals for the side chain methylene protons now appeared at δ 2.62 (2H, t, $J=7.2$ Hz, H-2′), 1.41 (2H, m, H-3′), 1.62 (2H, m, H-4′) and a methyl protons at δ 0.85 (3H, t, $J=6.6$ Hz, H-5′). The acid hydrolysis provided the aglycone and D-glucose. The ^{13}C-NMR spectrum (Table 2) disclosed 17 carbon signals for one methyl, three methylene, eight methine and five quaternary carbons. The downfield signals at δ 166.2, 167.8 and 161.9 were assigned to the oxygenated quaternary carbons and signals at δ 34.5, 24.8 and 28.2 were assigned to the methylene carbons. The structure was further confirmed by HMBC interactions; important interactions being illustrated in Table 2. On the basis of these evidence the structure of **3** could be assigned as 1-[(pentanoyl)phloroglucinyl]-β-D-glucopyranoside.

Lipoxygenases (EC 1.13.11.12) constitute a family of non-heme iron containing dioxygenases that are widely distributed in animals and plants. In mammalian cells these are key enzymes in the biosynthesis of variety of bioregulatory compounds such as hydroxyl eicosatetraenoic acids (HETEs),

Table 2. ^{1}H- and ^{13}C-NMR Spectra Data and HMBC Correlations of Compounds **2** and **3** (100 MHz, 400 MHz and 400 MHz in CD_3OD)

No.	**2**		**3**		HMBC (H to C)
	δ_C	δ_H[a)]	δ_C	δ_H[a)]	
1	162.1 (s)		161.9 (s)		
2	106.9 (s)		106.6 (s)		
3	165.8 (s)		166.2 (s)		
4	95.4 (d)	5.94 (d, 2.2)	95.5 (d)	5.98 (d, 2.1)	2, 3, 5
5	167.5 (s)		167.8 (s)		
6	98.3 (d)	6.15 (d, 2.2)	98.6 (d)	6.13 (d, 2.1)	1, 2, 4
1′	207.2 (s)		206.9 (s)		
2′	38.4 (t)	3.16 (dd, 15.7, 6.8)	34.5 (t)	2.62 (t, 7.2)	1′, 2, 3′
		2.86 (dd, 15.7, 7.4)			1′, 2, 3′
3′	26.2 (d)	2.23 (m)	24.8 (t)	1.41 (m)	1′, 2′, 4′
4′	23.8 (q)	0.95 (d, 6.6)	28.2 (t)	1.62 (m)	2′, 3′, 5′
5′	22.7 (q)	0.92 (d, 6.6)	12.0 (q)	0.85 (t, 6.6)	2′, 3′, 4′
1″	101.8 (d)	5.01 (d, 7.5)	101.5 (d)	5.02 (d, 7.2)	1, 2″, 3″
2″	74.8 (d)	3.51 (m)	74.8 (d)	3.52 (m)	1″, 3″
3″	78.3 (d)	3.42 (m)	78.2 (d)	3.40 (m)	2, 1′, 4″
4″	71.1 (d)	3.42 (m)	71.1 (d)	3.40 (m)	5″, 6″
5″	78.3 (d)	3.46 (m)	78.2 (d)	3.48 (m)	4″, 6″
6″	62.4 (t)	3.89 (br d, 12.3)	62.3 (t)	3.81 (br d, 12.1)	4″, 6″
		3.71 (dd, 11.9, 4.9)		3.67 (dd, 12.0, 5.1)	4″, 5″

a) Coupling constants (*J* in Hz) are in parentheses.

leukotrienes, lipoxins and hepoxylines. It has been found that these lipoxygenase products play a role in a variety of disorders such as bronchial asthma, inflammation[14)] and also profound influence on the development of several human cancers.[15)] Lipoxygenases are therefore potential target for the rational drug design and discovery of mechanism-based inhibitors for the treatment of bronchial asthma, inflammation, cancer and autoimmune diseases.

Baicalein was used as a positive control in lipoxygenase inhibiting assay. From the results it is apparent (Table 3) that lipoxygenase inhibitory potentials of compounds **2** and **3** are almost comparable, but compound **1** has slightly higher inhibitory potential than **2** and **3**. These compounds were also screened against chymotrypsine, urease and cholinesterase but no activity could be observed.

Table 3. IC_{50} Values and Inhibition (%) of Lipoxygenase at Three Various Conc. of Compounds **1**—**3**

Compounds	$IC_{50}\pm$S.E.M.[a)]	Conc. (μM) 25	50	100
		Inhibition (%) of lipoxygenase		
1	41.5±1.7	44.5	53.0	84.5
2	45.5±0.3	38.0	53.0	86.5
3	44.9±0.5	43.7	51.4	85.5
Baicalein[b)]	22.6±0.05	—	—	—

a) Standard mean error of three assays. *b*) Standard inhibitor of lipoxygenase.

Experimental

General Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. El-MS and HR-FAB-MS were recorded on JMS-HX-110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H- and ^{13}C-NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H- and 100.6 MHz for ^{13}C-NMR, respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. Aluminium sheets precoated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck) were used for TLC and flash silica (230—400 mesh) was used for column chromatography. Visualization of the TLC plates was carried out under UV at λ_{max} 254 and 366 nm and by spraying with ceric sulfate reagent solution (with heating). For enzyme inhibition assay, all chemicals used and lipoxygenase (1.13.11.12) type I-B were purchased from Sigma (St. Louis, MO, U.S.A.). The GC was performed on a Shimadzu gas chromatograph (GC-9A) (3% OV-l silanized chromosorb W, column temperature 180 °C, injection port and detector temperature 275—300 °C, flow rate 35 ml/min, flame-ionization detector).

Plant Material The whole plant material was collected from District Swat Pakistan and identified as *Indigofera hetrantha* by Mr. Habib Ahmad. A voucher specimen is deposited in the herbarium of the Department of Botany, Government, Postgraduate Jahanzeb Degree College, Saidu Sharif, Swat, Pakistan.

Extraction and Isolation The shade dried whole plant material (25 kg) was extracted thrice with MeOH at room temperature. The combined methanolic extract (600 g) was partitioned between *n*-hexane and water. The water soluble fraction was further extracted out with $CHCl_3$ and EtOAc. The EtOAc fraction (65 g) was subjected to VLC (vacuum liquid chromatography) over silica gel and eluted with $CHCl_3$, $CHCl_3$–MeOH and MeOH in increasing order of polarity to obtain six fractions (A—F). The fraction C obtained from $CHCl_3$–MeOH (9.5 : 0.5) was further subjected to medium pressure liquid chromatography over flash silica eluted with $CHCl_3$–MeOH in increasing order of polarity. The fraction obtained from $CHCl_3$–MeOH (9 : 1) was a mixture of two components which were subjected to preparative TLC ($CHCl_3$: MeOH : H_2O; 85 : 14.5 : 0.5) to afford **2** (15 mg) and **3** (12 mg), respectively. The fraction D obtained from $CHCl_3$–MeOH (8 : 2) was rechromatographed over flash silica and eluted with $CHCl_3$–MeOH (8.5 : 1.5) to afford **1** (18 mg).

Compound **1**: Colorless gummy solid. HR-FAB-MS *m*/*z* 743.2768 (Calcd for $C_{34}H_{47}O_{18}$, 743.2762) $[M-H]^+$. IR ν_{max} cm^{-1} (KBr): 3534, 3363, 1603, 1517. UV λ_{max} (MeOH) nm (log ε): 206 (4.15), 242 (2.41), 278 (2.61). El-MS *m*/*z*: 420 (18), 401 (48), 369 (11), 249 (20), 217 (23) 183 (22), 167 (100), 151 (18), 137 (53), 91 (20). $[\alpha]_D^{25}$ +18.2° (c=0.21, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD) data of **1** are shown in Table 1.

Compound **2**: Colorless gummy solid. HR-FAB-MS *m*/*z* 373.1498 (Calcd for $C_{17}H_{25}O_9$, 373.1492) $[M+H]^+$. IR ν_{max} cm^{-1} (KBr): 3545, 3402, 1690. UV λ_{max} (MeOH) nm (log ε): 216 (3.81) 310 (4.22). El-MS *m*/*z*: 210 (28), 195 (15), 154 (8), 153 (100), 85 (17), 73 (14), 69 (24), 57 (19). $[\alpha]_D^{25}$ −62.2° (c=0.11, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD) data of **2** are shown in Table 2.

Compound **3**: Colorless gummy solid. HR-FAB-MS *m*/*z* 373.1495 (Calcd for $C_{17}H_{25}O_9$, 373.1492) $[M+H]^+$. IR ν_{max} cm^{-1} (KBr): 3547, 3404, 1690. UV λ_{max} (MeOH) nm (log ε): 215 (2.81) 312 (4.18). El-MS *m*/*z*: 210 (24), 195 (12), 154 (10), 153 (100), 85 (19), 73 (12), 69 (24), 57 (19). $[\alpha]_D^{25}$ −58.6° (c=0.14, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD) data of **3** are shown in Table 2.

Acid Hydrolysis of Compounds 1—3 A solution of compounds (**1**—**3**) (each 8 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) was refluxed for 4 h,

concentrated under reduced pressure, and diluted with H_2O (8 ml). It was extracted with EtOAc. The aqueous phase in each case was concentrated to obtain the glycone while the organic phase provided the corresponding aglycones. In all the cases the glycone could be identified as D-glucose by the sign of optical rotation $[\alpha]_D^{20}$ +52° from **1**; ($[\alpha]_D^{20}$ +51° from **2** and $[\alpha]_D^{20}$ +51.3° from **3**, respectively). It was also confirmed based on the retention time of its TMS ether (α-anomer 4.1 min, β-anomer 7.8 min) with a standard.

In case of **1** the aglycone was obtained as a gummy solid which showed $[\alpha]_D^{25}$ +16.5° (c=0.92, MeOH). Its spectral data and optical rotation showed complete agreement to those reported for (+)-lyoniresinol.[8]

The aglycone from **2** was obtained as a crystalline solid, mp 143—145 °C. The ^{1}H-NMR (DMSO) spectrum showed signals at δ: 0.90 (6H, d, J=6.4 Hz, H-4′, H-5′), 2.12 (1H, m, H-3′), 2.84 (2H, d, J=6.4 Hz, H-2′) and 5.83 (2H, s, H-4, H-6). It could be identified as phlorisovalerophenone by the comparison of physical and spectral data in literature.[13]

The aglycone from **3** was obtained as a crystalline solid, mp 155—157 °C. The molecular formula was assigned $C_{11}H_{14}O_4$ by HR-EI-MS showing a $[M]^+$ ion at m/z 210.1079 (Calcd for $C_{11}H_{14}O_4$ 210.1082). The ^{1}H-NMR (DMSO) spectrum showed signals at δ: 0.84 (3H, t, J=6.5 Hz, H-5′), 1.60 (2H, m, H-4′), 1.39 (2H, m, H-3′), 2.59 (2H, t, J=7.1 Hz, H-2′) and 5.82 (2H, s, H-4, H-6).

Assay Lipoxygenase Inhibition Lipoxygenase-inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by Tappel.[16] Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical grade. The reaction mixture contained 160 μl of sodium phosphate buffer (100 mM, pH 8.0), 10 μl of test compound solution and 20 μl of lipoxygenase solution were mixed and incubated for 10 min. at 25 °C. The reaction was then initiated by the addition of 10 μl linoleic acid (substrate) solution, with the formation of (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate. The change of absorbance at 234 nm was followed for 6 min. Test compounds and the control were dissolved in methanol. All the reactions were performed in triplicate in 96-well micro-plate in SpectraMax 384plus (Molecular Devices, U.S.A.). The IC_{50} values were then calculated using the EZ-Fit Enzyme kinetics program (Perrella Scientific Inc., Amherst, U.S.A.). The percentage (%) inhibition was calculated as follows; $(E-S)/E\times100$, where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

References

1) Nasir E., Ali S. I., "Flora of West Pakistan," Vol. 100, National Herbarium Agricultural Research Council, Rawalpindi, 1997, pp. 65—83.
2) Harborne J. B., Williams C. A., *Nat. Prod. Rep.*, **18**, 310—333 (2001).
3) Harborne J. B., Williams C. A., *Nat. Prod. Rep.*, **15**, 631—652 (1998).
4) Harborne J. B., Williams C. A., *Phytochemistry*, **55**, 481—504 (2000).
5) Meragelman K. M., McKee T. C., Boyd M. R., *J. Nat. Prod.*, **64**, 546—548 (2001).
6) Thusoo A., Raina N., Ahmad S. R., *J. Indian Chem. Soc.*, **59**, 1007—1008 (1982).
7) Meyer B. N., Ferrigni J. E., Jacobsen L. B., Nicholas P. E., McLoughlin J. L., *Planta Med.*, **45**, 31—34 (1982).
8) Yuasa K., Lde T., Otsuka H., Ogimi C., Hirata E., Takushi A., Takeda Y., *Phytochemistry*, **45**, 611—615 (1997).
9) Zhang Z., Guo D., Li C., Zheng J., Koike K., Jia Z., Nikaido T., *Phytochemistry*, **51**, 469—472 (1999).
10) Higuchi H., Fukui K., Kinjo J., Nohara T., *Chem. Pharm. Bull.*, **40**, 534—535 (1992).
11) Achenbach H., Benirschke M., Torrenegra R., *Phytochemistry*, **45**, 325—335 (1997).
12) Kosasi S., Sluis W. G. V. G., Labadie R. P., *Phytochemistry*, **28**, 2439—2441 (1989).
13) Fung S., Brussee J., Hoeven R., Niesen W., Scheffer J., Verpoorte R., *J. Nat. Prod.*, **57**, 452—459 (1994).
14) Steinhilber D. A., *Curr. Med. Chem.*, **6**, 71—85 (1999).
15) Ding X. Y., Tong W. G., Adrian T. E., *Pancreatology*, **1**, 291—299 (2001).
16) Tappel A. L., "Methods in Enzymology," Vol. 5, Academic Press, New York, 1962, pp. 539—542.