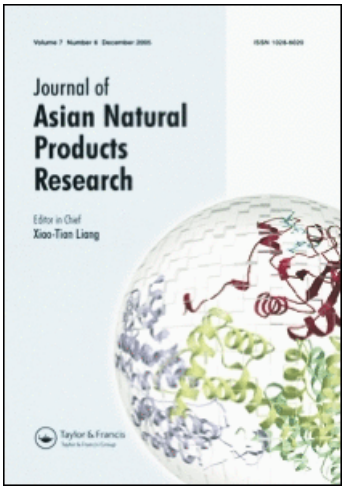

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Samar Ali[a]; Shazia Yasmeen[a]; Nighat Afza[a]; Abdul Malik[b]; Lubna Iqbal[a]; Mehreen Lateef[a]; Naheed Riaz[c]; Muhammad Ashraf[c]
[a] PCSIR Laboratories Complex Karachi, Pharmaceutical Research Centre, Karachi, Pakistan [b] HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan [c] The Islamia University of Bahawalpur, Bahawalpur, Pakistan

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Mutiniside, new antioxidant phenolic glucoside from *Abutilon muticum*

Samar Ali[a], Shazia Yasmeen[a], Nighat Afza[a]*, Abdul Malik[b], Lubna Iqbal[a], Mehreen Lateef[a], Naheed Riaz[c] and Muhammad Ashraf[c]

[a]*Pharmaceutical Research Centre, PCSIR Laboratories Complex Karachi, Karachi 75280, Pakistan;* [b]*HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan;* [c]*The Islamia University of Bahawalpur, Bahawalpur, Pakistan*

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Mutiniside (**1**), new phenolic glucoside, and the flavonoidal glucoside cephacoside (**2**) have been isolated from the *n*-BuOH soluble fraction, along with lupeol (**3**), β-sitosterol (**4**), stigmasterol (**5**), methyl-4-hydroxybenzoate (**6**), taraxacin (**7**), ursolic acid (**8**), and β-sitosterol-3-*O*-β-D-glucopyranoside (**9**), have been isolated from the EtOAc soluble fraction of *Abutilon muticum*. Compounds **2**–**9** are reported for the first time from this species. Compound **1** showed significant antioxidant activity while moderate inhibitory activity was observed against the enzyme lipoxygenase.

Keywords: *Abutilon muticum*; Malvaceae; phenolic glucoside; antioxidant

1. Introduction

Abutilon muticum belongs to the family Malvaceae. It is distributed in tropical and subtropical regions particularly in India, Pakistan, Afghanistan, and Africa [1,2]. In Pakistan, it is found in Sindh (Karachi, Hyderabad, and Thatta), Baluchistan (Lasbela and Nasirabad), and Punjab (Multan and Bahawalpur) [3].

The leaves of *A. muticum* are used as a remedy for piles and as demulcent tonic. A decoction of *A. muticum* is used in bronchitis, catarrhal bilious diarrhea, gonorrhea, inflammation of the bladder, and fever. The seeds are reckoned aphrodisiac and are used in the treatment of cough. These are burnt on charcoal and the rectum of the children affected with thread-worms is exposed to the smoke. In Hong Kong, the seeds are employed as an emollient and demulcent, and the roots are used as a diuretic and pulmonary sedative. The flowers and leaves are used as a local application to boils and ulcers [4].

In our continuing research for biologically active metabolites from medicinal plants [5–7], phytochemical studies have been undertaken on *A. muticum*. The methanolic extract of this plant showed strong toxicity in brine shrimp lethality test [8]. Further, biological screening of the extract and its subsequent *n*-BuOH soluble fraction revealed significant antioxidant activity. This prompted us to carry out phytochemical studies on this plant. Herein, we report the isolation and structure elucidation of a new phenolic glucoside named as mutiniside (**1**) and the flavonoidal glucoside cephacoside (**2**) [9] from the *n*-BuOH soluble fraction. Seven known compounds namely, lupeol (**3**) [10], β-sitosterol (**4**) [11], stigmasterol (**5**) [12], methyl-4-hydroxybenzoate (**6**) [13], taraxacin (**7**) [14],

*Corresponding author. Email: klcdirectorp_d@yahoo.com; shazrasheed@yahoo.com

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Table 1. ^{1}H and ^{13}C NMR spectral data and important HMBC correlations of compound **1** (CD_3OD).

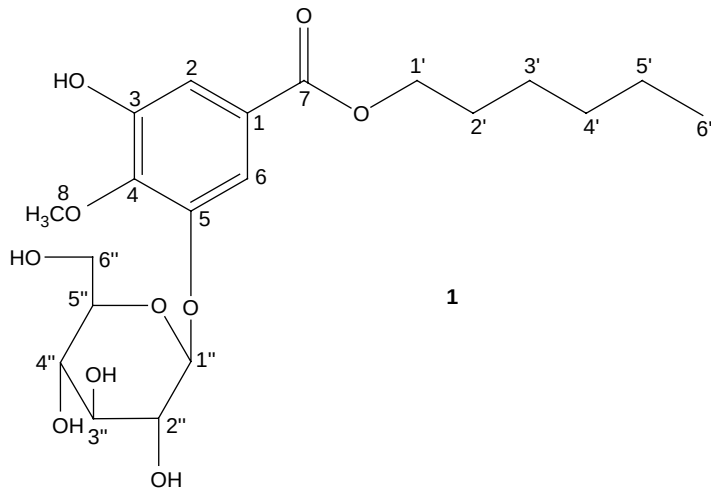
No.	δ_H	δ_C	HMBC (H → C)
1	–	128.7	–
2	7.32 (d, 2.1)	111.6	7, 4, 6
3	–	151.8	–
4	–	132.3	–
5	–	145.1	–
6	7.00 (d, 2.1)	105.5	4, 2, 7
7	–	166.2	–
8	3.82 (s)	50.5	4
1′	4.12 (t, 5.7)	68.1	2′, 3′
2′	1.78–1.81 (m)	30.1	1′, 3′, 4′
3′	1.32–1.36 (m)	29.2	1′, 2′, 4′, 5′
4′	1.18–1.20 (m)	26.7	2′, 3′, 5′, 6′
5′	1.15–1.17 (m)	22.5	3′, 4′, 6′
6′	0.90 (t, 6.7, 3H)	13.9	5′, 4′
1″	5.02 (d, 7.4)	101.5	5, 3″
2″	3.48–3.50 (m)	74.3	1″, 4″
3″	3.39–3.42 (m)	77.0	1″, 5″
4″	3.34–3.37 (m)	70.1	2″, 6″
5″	3.43–3.46 (m)	76.5	3″
6a″	3.60 (dd, 11.5, 3.0)	62.0	4″
6b″	3.70 (dd, 11.5, 4.9)		

ursolic acid (**8**) [15], and β-sitosterol-3-*O*-β-D-glucopyranoside (**9**) [16], are reported for the first time from the EtOAc soluble fraction of *A. muticum.*

2. Results and discussion

The MeOH extract of the whole plant of *A. muticum* was divided into fractions soluble in *n*-hexane, EtOAc, *n*-BuOH, and H_2O. Repeated column chromatography yielded compounds **1** and **2** from the *n*-BuOH soluble fraction, whereas compounds **3**–**9** were obtained from the EtOAc soluble fraction.

Mutiniside (**1**) was isolated as colorless gummy solid and gave violet coloration with $FeCl_3$ for a phenol. The HR-FAB-MS (positive mode) established the molecular formula to be $C_{20}H_{30}O_{10}$ showing a $[M+H]^+$ peak at *m/z* 431.1920 having six degrees of unsaturation. The IR spectrum of **1** showed the absorption bands due to ester carbonyl (1730 cm^{-1}) and hydroxyl (3460 cm^{-1}) functions. The appearance of peak at *m/z* 166 in EI-MS was attributed to the loss of a $C_6H_{13}O$ alkyl chain along with the sugar moiety. The ^{1}H NMR spectrum of **1** (Table 1) showed signals for the hexyl chain including methyl protons at δ 0.90 (t, $J = 6.7$ Hz, 3H, H-6′), eight methylene protons at δ 1.78–1.81 (m, 2H, H-2′), 1.32–1.36 (m, 2H, H-3′), 1.18–1.20 (m, 2H, H-4′), 1.15–1.17 (m, 2H, H-5′), while the signal for oxymethylene protons appeared at δ 4.12 (t, $J = 5.7$ Hz, 2H, H-1′). The signal for the methoxyl group was observed as singlet at δ 3.82. The *meta*-coupled aromatic protons appeared at δ 7.00 (d, $J = 2.1$ Hz, 1H, H-6) and 7.32 (d, $J = 2.1$ Hz, 1H, H-2). The signals of a hexose moiety appeared at δ 5.02 (d, $J = 7.4$ Hz, 1H, H-1″), 3.48–3.50 (m, 1H, H-2″), 3.39–3.42 (m, 1H, H-3″), 3.34–3.37 (m, 1H, H-4″), 3.43–3.46 (m, 1H, H-5″) and oxymethylene protons at δ 3.60 (dd, $J = 11.5$, 3.0 Hz, 1H, H-6a″) and 3.70 (dd, $J = 11.5$, 4.9 Hz, 1H, H-6b″). The ^{13}C NMR spectrum of **1** (Table 1) showed 20 carbon signals for two methyl, six methylene, seven methine, and five



1

Figure 1. Structure of mutiniside (**1**).

quarternary carbons. The most downfield signal appearing at δ 166.2 was attributed to the carbonyl, whereas the quaternary carbons appearing at δ 151.8, 145.1, and 132.3 were assigned to the oxygenated aromatic carbons. The signal at δ 128.7 could be assigned to the aromatic carbon containing ester moiety. The signal for the anomeric carbon appeared at δ 101.5. Assignments of all the signals were made through HMQC and HMBC correlations. The NMR data of **1** particularly those of aromatic ring and its substituents showed close agreement to those of methyl-3-hydroxy-4-methoxy-5-(*O*-β-D-glucopyranosyl)benzoate [17]. The two compounds therefore differ in the side chain. Acid hydrolysis of **1** provided a mixture of aglycones from which the major compound could be obtained pure through column chromatography using $CHCl_3$–MeOH as eluant and identified as 3,5-dihydroxy-4-methoxybenzoic acid through comparison of its physical and spectral data with the literature [18]. The sugar moiety could be identified as D-glucose through the sign of its optical rotation and comparison of the retention time of trimethyl silyl ether with that of standard in GC. The attachments of the glucose and methoxyl moieties were also confirmed through HMBC experiment showing ^3J correlation of the anomeric proton at δ 5.02 with C-5 (δ 145.1) and ^3J correlations of methoxy protons at δ 3.82 with C-4 (δ 132.3). On the basis of these evidences, the structure of mutiniside (**1**) could be assigned as hexyl-3-hydroxy-4-methoxy-5-*O*-(β-D-glucopyranosyl)-benzoate (Figure 1).

Compound **1** was screened for its antioxidant potential using different assays

Table 2. Antioxidant and lipoxygenase (LOX) inhibition activities of compound **1**.

Compound	DPPH scavenging activity (IC_{50}[a]; μm)	LOX inhibition activity (IC_{50}[a]; μm)
1	16.3 ± 0.21	125 ± 0.15
BHA[b]	44.3 ± 0.09	–
Baicalein[c]	–	22.6 ± 0.08

[a] Values ± SEM (standard error of the mean of three assays).
[b] Standard DPPH scavenging activity.
[c] Standard LOX inhibition activities.

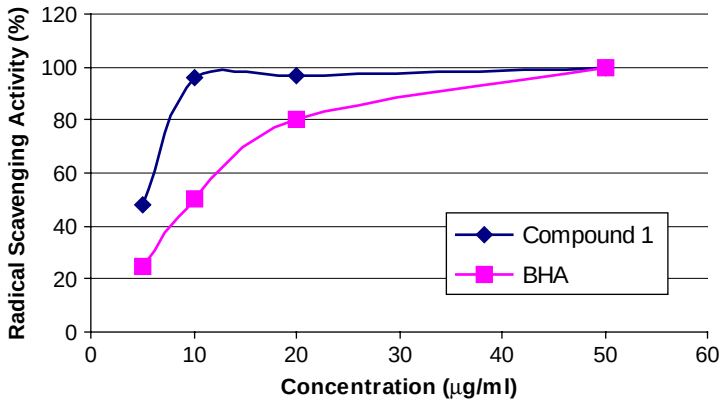


Figure 2. Free radical scavenging activity of compound **1** and BHA at different concentrations (5, 10, 20, and 50 μg/ml) by the DPPH radical.

including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity as well as reduction capability. It showed significant antioxidant activity. On the other hand, moderate inhibitory activity was also observed against the LOX enzyme (Table 2).

2.1 DPPH radical scavenging activity

The scavenging of stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time when compared with other methods. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [19]. The reduction capability of DPPH radicals is determined by decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical is due to a reaction with antioxidant molecules that is the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow.

Compound **1** showed higher radical scavenging activity than butylated hydroxyanisole (BHA). The radical scavenging activity of compound **1** at 5, 10, and 20 μg/ml concentrations was 48, 96, and 97%, respectively. On the other hand, the percentage radical scavenging activity of BHA at the same concentrations was 25, 50, and 80, respectively (Figure 2).

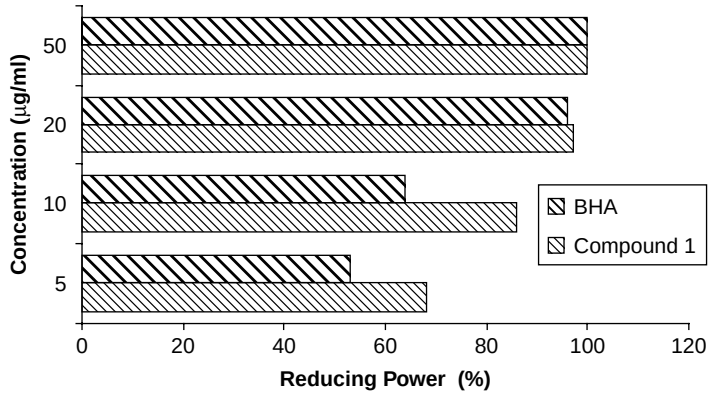


Figure 3. Reducing power of compound **1** and BHA at different concentrations (5, 10, 20, and 50 μg/ml) using the potassium ferricyanide reduction method.

2.2 Total reductive capability using the potassium ferricyanide reduction method

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [20]. The reductive capabilities of compounds are assessed by the extent of conversion of the Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form. The reducing power of compound **1** was observed at different concentrations (5, 10, 20, and 50 μg/ml), and the results were compared with BHA as shown in Figure 3. Compound **1** showed 68, 86, and 90% reduction capability, while the percentage reduction capability of BHA was 53, 64, and 96% at the concentration of 5, 10, and 20 μg/ml, respectively. These results indicate that the reducing capacity of compound **1** is increased with increasing concentration and exhibits more powerful reduction ability than BHA at all concentrations.

2.3 LOX inhibition activity

LOXs are the family of the key enzyme in the biosynthesis of leukotrienes that are postulated to play an important role in the pathophysiology of several inflammatory diseases [21].

Compound **1** shows moderate LOX inhibition activity with IC_{50} value of 125 μm as compared to positive control baicalein, which has IC_{50} of 22.6 μm as indicated in Table 2.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco Dip-360 digital polarimeter using a 10-cm tube. The IR spectrum was recorded on Jasco 320-A spectrometer. NMR spectra were recorded in CD_3OD on a Bruker AM-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) using tetramethylsilane (TMS) as the internal standard. Chemical shifts are expressed in δ (ppm) and coupling constant *J* in Hz. The 2D NMR spectra were obtained on a Bruker AM-500 NMR spectrometer. Mass spectra were measured on Finnigan MAT 12 and MAT 312 spectrometers and ions are given in *m/z* (%) and FAB mass measurements were carried out on Jeol JMS HX 110 mass spectrometer; FAB source using glycerol. TLC was performed on precoated silica gel F_{254} plates (E. Merck, Darmstadt, Germany). The detection was done at 254 nm and by spraying with ceric sulfate reagent. Silica gel (230–400 mesh; E. Merck) was used for column and flash chromatography. For antioxidant assay, all the chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA).

3.2 Plant material

The whole plant of *A. muticum* Del. was collected from Cholistan desert (Bahawalpur District) in July 2005 and identified by Prof. Dr Muhammad Arshad, Cholistan Institute of Desert Studies, Islamia University, Bahawalpur, Pakistan, where a voucher specimen (AM-15-06) is deposited.

3.3 Extraction and isolation

The shade dried whole plant of *A. muticum* (30 kg) was extracted with MeOH (3× 50 l) at room temperature. The combined MeOH extract was concentrated and the residue (470 g) was suspended in water and successively extracted with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc soluble fraction (60 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, *n*-hexane–EtOAc, EtOAc, EtOAc–MeOH in the increasing order of polarity. The fractions which were obtained from *n*-hexane–EtOAc (8:2) were combined and chromatographed over silica gel using *n*-hexane–EtOAc (9:1) to provide lupeol (**3**; 23 mg). The fraction which eluted with *n*-hexane–EtOAc (7:3) was subjected to preparative TLC on silver

nitrate impregnated silica gel plates using *n*-hexane–EtOAc (6:4) as the mobile phase affording β-sitosterol (**4**; 25 mg) and stigmasterol (**5**; 20 mg), respectively. The fractions obtained from *n*-hexane–EtOAc (4:6) were combined and chromatographed over silica gel using *n*-hexane–EtOAc (5:5) to afford methyl-4-hydroxybenzoate (**6**; 24 mg). Preparative TLC ($CHCl_3$–MeOH, 9.8:0.2) of the fraction which eluted with *n*-hexane–EtOAc (3:7) furnished taraxacin (**7**; 19 mg). The fractions obtained from *n*-hexane–EtOAc (2:8) were combined and chromatographed over silica gel using the same solvent system to obtain ursolic acid (**8**; 28 mg). Further, chromatography of the fraction obtained from *n*-hexane–EtOAc (1:9) over silica gel using *n*-hexane–EtOAc (1.5:8.5) as eluant afforded β-sitosterol-3-*O*-β-D-glucoside (**9**; 25 mg).

The *n*-BuOH soluble fraction (40 g) was subjected to column chromatography using EtOAc–MeOH as eluant in the increasing order of polarity. The fractions which were obtained from EtOAc–MeOH (8.3:1.7) were chromatographed over silica gel using EtOAc–MeOH (8.5:1.5) as eluant to afford mutiniside (**1**; 17 mg). The fractions which were obtained from EtOAc–MeOH (7.8:2.8) were chromatographed over silica gel using EtOAc–MeOH (8.0:2.0) as eluant to provide cephacoside (**2**; 22 mg).

The compounds **2**–**9** were identified by comparing their physical and spectral data with those of the literature values [9–16].

*3.3.1 Mutiniside (**1**)*

Gummy solid; $[\alpha]_D^{26} + 3.69$ ($c = 0.03$, MeOH); IR (KBr; cm^{-1}): 3460 and 1730. ^1H and ^{13}C NMR spectral data and the important HMBC correlations are shown in Table 1. HR-FAB-MS (positive): 431.1920 ($[M+H]^+$, calcd for $C_{20}H_{31}O_{10}$: 431.1917), EI-MS: *m/z* 267 (2.5) [M-glucose]$^+$, 166 (52), 101 (2.7), 57 (100).

*3.3.2 Acid hydrolysis of **1***

Compound **1** (4 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) was refluxed for 4 h and diluted with H_2O (8 ml). It was extracted with EtOAc. The organic phase was concentrated and chromatographed over silica gel using gradient solvent system of *n*-hexane–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH in the increasing order of polarity. The fraction obtained from $CHCl_3$–MeOH (9.5:0.5) yielded 3,5-dihydroxy-4-methoxybenzoic acid (mp 244, lit. 246) [22]. The aqueous layer was concentrated and D-glucose was identified by the sign of its optical rotation ($[\alpha]_D^{20} + 51.3$, lit. +52.5). It was also confirmed based on the retention time of its TMS ether (α-anomer 4.1 min and β-anomer 7.8 min) with that of standard in GC.

3.4 Antioxidant assay

3.4.1 Determination of DPPH radical scavenging activity

The free radical scavenging activity was measured by DPPH [23]. The solution of DPPH of 0.3 mM was prepared in ethanol. Five microliters of each sample of different concentration (5–100 μg/ml) in methanol were mixed with 95 μl of DPPH solution in ethanol. The mixture was dispersed in 96 well plates and incubated at 37°C for 30 min. The absorbance at 515 nm was measured by microtiter plate reader (Spectramax plus 384; Molecular Device, Sunnyvale, CA, USA) and the percentage radical scavenging activity was determined in comparison with the methanol treated control

$$\text{DPPH scavenging effect (\%)} = A_c - A_s/A_c \times 100,$$

where A_c is the absorbance of control (DMSO treated) and A_s the absorbance of sample.

3.4.2 Determination of total reduction ability

Total reduction capability of these compounds was estimated according to the method of Oyaizu [24] with some modifications. Different concentrations of the samples (5–100 μg/ml) in methanol were mixed with phosphate buffer (250 μl, 0.2 M, pH 6.6). Then 250 μl potassium ferricyanide [$K_3Fe(CN)_6$] (1%) was added. The mixture was incubated at 50°C for 20 min. After incubation, 250 μl trichloroacetic acid (10%) was added to the mixture and the mixture was centrifuged for 10 min, the upper layer of solution (25 μl) was separated in another set of test tubes and mixed with equal volume of de-ionized water (250 μl). Then 50 μl ferric chloride [$FeCl_3$] (0.1%) was added and the absorbance was measured at 700 nm by using spectrophotometer (Specord 200, Jena, Germany). Higher absorbance of reaction mixture indicated higher reducing power.

3.5 LOX inhibition assay

LOX inhibiting activity was measured by modifying the spectrophotometric method developed by Tappel [25]. LOX enzyme solution was prepared so that the enzyme concentration in reaction mixture was adjusted to give rates of 0.05 absorbance/min. The reaction mixture contained 160 μl (100 mM) sodium phosphate buffer (pH 8), 10 μl of test solution, and 20 μl of LOX solution. The contents were mixed and incubated for 10 min at 25°C. The reaction was then initiated by the addition of 10 μl substrate solution (linoleic acid, 0.5 mM, 0.12% (w/v) Tween 20 in the ratio of 1:2) with the formation of (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate and the change in absorbance at 234 nm was followed for 6 min. The concentration of the test compound that inhibited LOX activity by 50% (IC_{50}) was determined by monitoring the effect of increasing concentrations of these compounds in the assays on the degree of inhibition. The IC_{50} values were calculated by means of EZ-Fit, Enzyme Kinetics Program (Perrella Scientific, Inc., Amherst, NH, USA).

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References

[1] W. George, *A Dictionary of Economic Products of India* (Government of India Printing Office, Calcutta, 1972), Vol. 1, p. 14.
[2] S.M.H. Jafri, *The Flora of Karachi* (The Book Corporation, Karachi, 1966), p. 219.
[3] E. Nasir and S.I. Ali, *The Flora of West Pakistan* (Fakhri Printing Press, Karachi, 1972), p. 476.
[4] K.S. Mhasker, E. Blatter, and J.F. Caius, *The Indian Medicinal Plants* (Sri Satguru Publications, Delhi, 2000), Vol. 2, p. 433.
[5] N. Riaz, S.A. Nawaz, N. Mukhtar, A. Malik, N. Afza, S. Ali, S. Ullah, P. Muhammad, and M.I. Choudhary, *Chem. Biodiv.* **4**, 72 (2007).
[6] I. Ahmad, I. Anis, I. Fatima, A. Malik, S. Khan, N. Afza, R.B. Tareen, M.A. Lodhi, and M.I. Choudhary, *Chem. Biodiv.* **4**, 917 (2007).
[7] A.U. Rehman, A. Malik, N. Riaz, H.R. Nawaz, H. Ahmad, S.A. Nawaz, and M.I. Choudhary, *J. Nat. Prod.* **67**, 1450 (2004).
[8] B.N. Meyer, N.R. Ferringni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.* **45**, 31 (1982).
[9] A.M. Aliev and S.I. Movsumov, *Azerbaidzhanskii Meditsinskii Zhurnal* **58** (12), 36 (1981).
[10] P. Monaco and L. Previteva, *J. Nat. Prod.* **47**, 673 (1984).
[11] I. Rubinstein, L.J. Goad, A.D.H. Clague, and L.J. Mulheirn, *Phytochemistry* **15**, 195 (1976).
[12] H.L. Holland, P.R.P. Diakow, and G.J. Taylor, *Can. J. Chem.* **56**, 3121 (1978).
[13] V.C. da Silva, M.G. Carvalho, and A.N. Alves, *J. Nat. Med.* **62**, 356 (2008).
[14] V.U. Ahmad, S. Yasmeen, Z. Ali, M.A. Khan, M.I. Choudhary, F. Akhtar, G.A. Miana, and M. Zahid, *J. Nat. Prod.* **63**, 1010 (2000).

[15] K. Hiller, H.D. Woitke, and G. Lehmann, *Pharmazie* **28**, 391 (1973).
[16] A.M. Iribarren and A.B. Pomilio, *J. Nat. Prod.* **46**, 752 (1983).
[17] J. Xie, L. Wang, G. Liu, and D. Zhang, *Yaoxue Xuebao* **16** (6), 425 (1981).
[18] K.S. Jeon, H. Na, Y. Kim, and H.J. Kwon, *Biochem. Biophys. Res. Commun.* **330**, 1268 (2005).
[19] J.R. Soares, T.C.P. Dinis, A.P. Cunha, and L.M. Ameida, *Free Radical Res.* **26**, 469 (1997).
[20] S. Meir, J. Kanner, B. Akiri, and S.P. Hadas, *J. Agric. Food Chem.* **43**, 1813 (1995).
[21] S. Yamamoto, *Biochim. Biophys. Acta* **1128**, 117 (1992).
[22] G. Saxena, A.R. McCutcheon, S. Farmer, G.H. Towers, and R.E.W. Hancock, *J. Ethnopharmacol.* **42**, 95 (1994).
[23] N. Cotelle, J.L. Bernier, J.P. Catteau, J. Pommery, J.C. Wallet, and E.M. Gaydou, *Free Radical Biol. Med.* **20**, 35 (1996).
[24] M. Oyaizu, *Jpn. J. Nutr.* **44**, 307 (1998).
[25] A.L. Tappel, *Methods of Enzymology* (Academic Press, New York, 1962), Vol. 5, p. 539.