Inhibitory Effects of Egyptian Folk Medicines on Human Immunodeficiency Virus (HIV) Reverse Transcriptase

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Extracts of 41 medicinal plants used in Egyptian folk medicine were screened for their inhibitory effects on human immunodeficiency virus-1 reverse transcriptase. The extracts of fruits of *Phyllanthus emblica*, *Quercus pedunculata*, *Rumex cyprius*, *Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrida* showed significant inhibitory activity with $IC_{50} \leqq 50\ \mu g/ml$. Through a bioassay guided-fractionation of the methanol extract of the fruit of *P. emblica*, putranjivain A (1) was isolated as a potent inhibitory substance with $IC_{50} = 3.9\ \mu M$, together with 1,6-di-*O*-galloyl-β-D-glucose (2), 1-*O*-galloyl-β-D-glucose (3), kaempferol-3-*O*-β-D-glucoside (4), quercetin-3-*O*-β-D-glucoside (5) and digallic acid (6). The inhibitory mode of action by 1, 2 and 6 was non-competitive with respect to the substrate but competitive with respect to a template-primer. Furthermore, the stereochemistry of 1 was established in this paper by nuclear magnetic resonance spectroscopy.

Key words HIV-1; reverse transcriptase inhibition; Egyptian folk medicine; *Phyllanthus emblica*; tannins; putranjivain A

The fundamental role played by reverse transcriptase (RT) in the replication of retroviruses has made this enzyme a key target in the chemotherapy of human immunodeficiency virus (HIV) infection, the acquired immunodeficiency syndrome (AIDS). Since the replicative cycle of HIV is interrupted by RT inhibitors, the inhibition of HIV RT is currently considered as a useful approach, especially in the prophylaxis and intervention of AIDS. In the last decade, a significant effort in the development of HIV RT inhibitors has focused on the synthetic nucleoside analogs, AZT (3′-azido-3′-dideoxy-thymidine), DDC (2′,3′-dideoxycytidine) and DDI (2′,3′-dideoxy-inosine) which are clinically used in AIDS patients.[2] Furthermore, a number of natural (flavonoids,[3] tannins,[4] alkaloids[5]) and synthetic (TIBO,[6] and piperazine derivatives[7]) compounds with diverse molecular structures have been reported as HIV RT inhibitors. However, no experimental regimen has been proven to restore the underlying immunodeficiency of the disease and most of the drugs under study may have substantial side effects. Thus, there is a crucial need to develop new drugs which are effective for retroviral diseases and have fewer side effects. We have initiated an extensive screening program of various traditional medicines to find substances that interfere with the replicative cycle of retroviruses.[8]

In the present paper we report on the screening of folk medicines used in Egypt for their inhibitory effects on HIV-1 RT and the identification of inhibitory substances from the fruit of *Phyllanthus emblica* that showed a potent inhibitory activity to HIV-1-RT.

Results and Discussion

The MeOH and water extracts of forty-one medicinal plants used in Egyptian folk medicine were evaluated for their HIV-1 RT inhibitory effects. The enzyme activity was determined by the amount of tritium labeled-substrate incorporation into a polymer fraction in the presence of a template-primer, $(rA)_n$-$(dT)_{12-18}$. Of the plant materials tested, the fruits of *Phyllanthus emblica* L. (MeOH extract), *Quercus pedunculata* EHRH. (MeOH and water extracts), *Rumex cyprius* MURB. (MeOH and water extracts), *Terminalia bellerica* ROXB. (MeOH and water extracts), *Terminalia chebula* RETZ. (MeOH and water extracts) and *Terminalia horrida* STEUD. (MeOH extract) showed significant inhibitory effects with IC_{50} of 2—49 μg/ml (Table 1). However, in the presence of bovine serum albumin (BSA), the inhibitory potency of most of the extracts except for *P. emblica* (MeOH extract) and *T. chebula* (water extract) was appreciably reduced by non-specific binding of their ingredients with BSA (Table 2). Accordingly, the MeOH extract of the fruit of *P. emblica* (Euphorbiaceae) was chosen for the isolation of its RT inhibitory principles. The powdered fruit was successively extracted with $CHCl_3$ and MeOH followed by subsequent fractionation (Chart 1), and each extract was tested for HIV-1 RT inhibitory effect. This effect was significant in both EtOH-soluble and EtOH-insoluble fractions. Since the EtOH-insoluble fraction was found to be a mixture of inorganic salts and sugars, the EtOH-soluble fraction was subjected to isolation of its inhibitory substances. The bioactivity guided-fractionation of the EtOH-soluble fraction ($IC_{50} = 95\ \mu g/ml$) using column chromatography on Sephadex LH-20 afforded some fractions with potent inhibitory activity against HIV-1-RT. Repeated column chromatography of these fractions on Sephadex LH-20 and preparative-TLC led to the isolation of six compounds and their structures were determined as follows:

Compound **1** was obtained as an off-white amorphous powder; $[\alpha]_D$ −89.0° (MeOH). The negative ion FAB-MS and high resolution (HR) FAB-MS showed an ion peak at m/z 1083 $(M-H)^-$, indicating the molecular formula $C_{46}H_{36}O_{31}$. The ^{1}H-NMR spectrum showed the presence of two methylene groups at δ 1.40 and 2.57, and at δ 3.73 and 4.10, two hydroxyl bearing protons at δ 3.90 and 3.95, two allylic protons at δ 4.50 and 4.80, and a glucose unit with an anomeric proton signal at δ 6.20 ($J = 3.5$ Hz). Appreciable downfield shifts of the sugar protons and

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Table 1. HIV-1 RT Inhibitory Effects of Plants Used in Egyptian Folk Medicine

Botanical name	Local name	Part used	IC_{50} (μg/ml)	
			MeOH ext.	H_2O ext.
Abrus precatorius L. (Leguminosae)	Ain-Afreet	Seed	240	60
Aloe vera L. (Liliaceae)	Saabr	Resin	>1000	>1000
Ambrosia maritima L. (Compositae)	Damaseisa	Aerial part	>1000	>1000
Ammi majus L. (Umbelliferae)	Khella shitani	Fruit	>1000	190
Anagallis arvensis L. (Primulaceae)	Saboon-gheit	Whole plant	1000	n.d.
Artemisia herba-alba Asso. (Compositae)	Sheeh-gabali	Aerial part	>1000	310
Artemisia absinthium L. (Compositae)	Afsanteen	Aerial part	460	65
Balanites aegyptiaca (L.) Delile (Balanitaceae)	Higleeg	Fruit	890	>1000
Bassia muricata (L.) Murr. (Chenopodiaceae)	Haythaam	Whole plant	>1000	810
Boswellia carterii Birdw. (Burseraceae)	Cander	Resin	870	780
Bryonia cretica L. (Cucurbitaceae)	Le'eba-murra	Resin	180	240
Cassia acutifolia Del. (Leguminosae)	Senna	Leaf	860	1000
Catharanthus roseus G. Don (Apocynaceae)	Winca	Leaf	>1000	230
Centaurea scoparia L. (Compositae)	El-burkan	Aerial part	800	750
		Root	640	760
Citrullus colocynthis (L.) Schrad (Cucurbitaceae)	Hanzal	Pericarp	>1000	>1000
		Seed	>1000	840
Cleome droserifolia (Forssk.) Del. (Cleomaceae)	Sammo	Bark	>1000	>1000
Colchicum ritchii R. Br. (Liliaceae)	Khameerit-el-arab	Seed	>1000	70
Commiphora molmol L. (Burseraceae)	Mour	Resin	750	220
Croton tiglium L. (Euphorbiaceae)	Habb-el-muluk	Seed	>1000	>1000
Datura stramonium L. (Solanaceae)	Datura	Seed	195	530
Digitalis purpurea L. (Scrophulariaceae)	Digitala	Leaf	1000	>1000
Ferula foetida Regel (Umbelliferae)	Halteet	Resin	>1000	>1000
Gymnocarpos decandrum Forssk. (Caryophyllaceae)	Garad	Whole plant	375	n.d.
Hibiscus sabdariffa L. (Malvaceae)	Karkadeh	Calyx	>1000	320
Juniperus phoenicea L. (Cupressaceae)	Arar	Fruit	>1000	887
Lepidium sativum L. (Cruciferae)	Hab-er-rashad	Seed	>1000	>1000
Lupinus termis Forssk. (Leguminosae)	Tirmis	Seed	>1000	1000
Maerua crassifolia Forssk. (Capparaceae)	Margaam	Leaf	840	266
Nigella sativa L. (Ranunculaceae)	Habba soda	Seed	>1000	550
Petroselinum sativum L. (Umbelliferae)	Bagdonis	Fruit	>1000	1000
Phyllanthus emblica L. (Euphorbiaceae)	Sananir	Fruit	9	10
Polycarpea repens Forssk. (Caryophyllaceae)	Qameyla	Whole plant	n.d.	581
Quercus pedunculata Ehrh. (Fagaceae)	Ballot	Fruit	9	49
Rumex cyprius Murb. (Polygonaceae)	Homed	Fruit	378	40
Solanum nigrum L. (Solanaceae)	Enabed-deeb	Fruit	1000	>1000
Solenostemma argel (Del.) Hayne (Asclepiadaceae)	Hargal	Leaf	850	>1000
Terminalia bellerica Roxb. (Combretaceae)	Bellileg	Fruit	9.5	10
Terminalia chebula Retz. (Combretaceae)	Kabuli	Fruit	2	6
Terminalia horrida Steud. (Combretaceae)	Hind sheiri	Fruit	3	39
Trigonella foenum-graecum L. (Leguminosae)	Helba	Seed	>1000	>1000
Zygophyllum dumosum Boiss (Zygophyllaceae)	Kammon-karamaani	Seed	710	275

The assay was carried out in the presence of $r(A)_n$-$(dT)_{12-18}$ as a template-primer. n.d.: not determined.

Table 2. Inhibitory Effects of the Plant Extracts on HIV-1 RT in the Presence of Albumin

Botanical name	Extract	Inhibition (%)	
		BSA (−)	BSA (+)
Phyllanthus emblica	MeOH	76.1±3.76	52.1±4.63
	H_2O	75.2±8.30	15.6±0.76
Quercus pedunculata	MeOH	71.0±2.60	8.49±10.2
	H_2O	83.1±4.20	7.60±3.30
Rumex cyprius	H_2O	97.6±0.52	17.6±0.52
Terminalia bellerica	MeOH	82.5±0.97	36.6±1.03
	H_2O	69.4±4.30	18.9±0.83
Terminalia chebula	MeOH	85.4±1.60	19.4±1.70
	H_2O	91.9±3.20	76.1±8.90
Terminalia horrida	MeOH	90.9±1.70	18.0±0.79
	H_2O	63.5±5.80	31.3±1.32

Inhibitory effects of the above extracts (0.1 mg/ml) were measured in the presence (+) or absence (−) of 0.5 mg/ml BSA. The results are the mean±S.E. of 4 experiments.

absence of sugar hydroxy protons suggested that all the sugar hydroxyl groups were acylated. In addition, most of the sugar protons were observed as broad singlets or with small coupling patterns, characteristic for a $^1C_4$ glucopyranose core or a related boat conformation which is similar to that reported for geraniin.[9,10] Since no long-range *W*-type couplings were observed between $\mathbf{G}_1$-H and $\mathbf{G}_3$-H and between $\mathbf{G}_3$-H and $\mathbf{G}_5$-H in the ^1H–^1H correlated spectroscopy (COSY) spectrum, the $^1C_4$ form was eliminated and the related boat form was deduced for the sugar core.[9] In an aromatic region of the ^1H-NMR spectrum, two singlets were observed at δ 6.46 and 6.76 (1H each), attributable to $\mathbf{C}_3$-H and $\mathbf{B}_3$-H of a hexahydroxydiphenoyl (HHDP) group, respectively, together with a galloyl proton signal at δ 7.03 (2H, s) and another singlet signal at δ 7.19 ($\mathbf{D}_3$-H) (Chart 2).

The ^{13}C-NMR spectral data analyzed with the aid of ^1H–^{13}C COSY showed the presence of methylene carbons

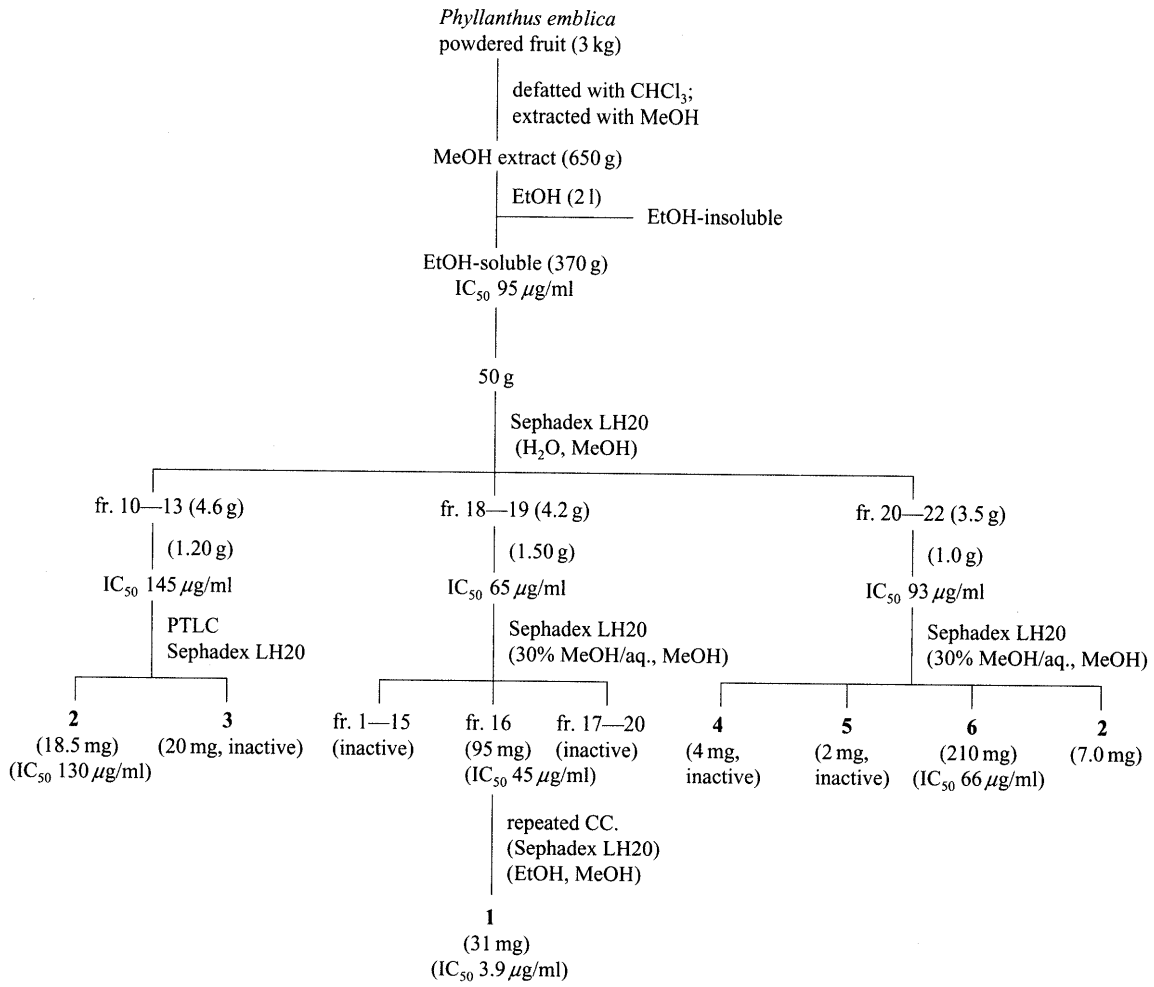

Chart 1. Flow Chart for the Isolation of Active Constituents from the Fruit of *Phyllanthus emblica*

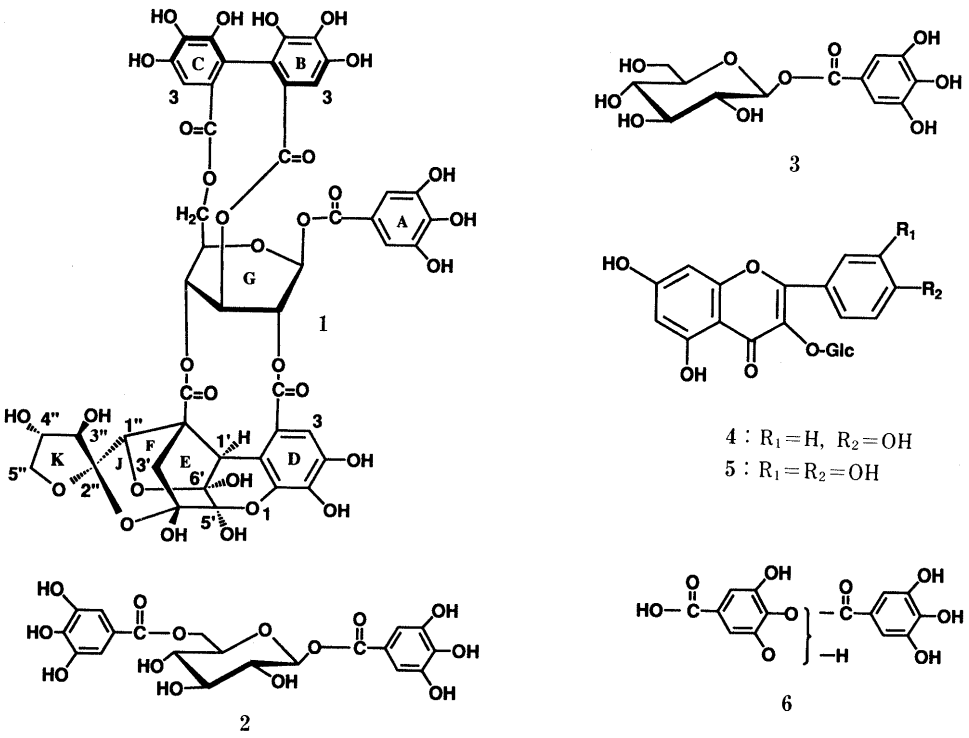

Chart 2. Structures of the Compounds Isolated from the Fruit of *Phyllanthus emblica*

at δ 31.9 (C-3′), 63.3 (C-$\mathbf{G}_6$) and 73.8 (C-5″), two allylic carbons at δ 51.2 (C-1′) and 75.6 (C-1″), seven carbinol carbons at δ 63.0 (C-$\mathbf{G}_3$), 65.8 (C-$\mathbf{G}_4$), 71.2 (C-$\mathbf{G}_2$), 73.7 (C-$\mathbf{G}_5$), 76.8 (C-4″), 81.4 (C-3″) and 91.2 (C-$\mathbf{G}_1$), five sp^3 quaternary carbons at δ 51.8 (C-2′), 95.5 (C-4′), 97.4 (C-6′), 98.2 (C-2″) and 98.3 (C-5′) and five carbonyl carbons at δ 164.2, 164.9, 165.4, 167.6 and 170.4.

From the above evidence, we speculated that **1** was composed of a glucose core acylated with HHDP, galloyl, phenyl and cyclohexanyl residues, where proton and carbon chemical shifts of the signals assignable to galloyl and HHDP residues were similar to those reported for geraniin,[9] but those of the phenyl and cyclohexanyl residues were quite different.

The connectivity of these residues was determined by analysis of the ^{1}H–^{13}C long range COSY and HMBC spectra which showed correlations between a carbonyl carbon signal at δ 164.2 and two proton signals at δ 7.03 ($\mathbf{A}_2$-H) and 6.27 ($\mathbf{G}_1$-H) connecting the galloyl group with C-$\mathbf{G}_1$. A clear correlation was also observed between a carbonyl carbon signal at δ 164.9 and two proton signals at δ 7.19 ($\mathbf{D}_3$-H) and 5.37 ($\mathbf{G}_2$-H) connecting ring **D** with C-**G**. sp^2 quaternary carbon signals at δ 110.9 (C-$\mathbf{D}_1$) and 117.4 (C-$\mathbf{D}_2$) were correlated with a proton signal at δ 4.50 (1′-H), connecting C-$\mathbf{E}_1$, (in the cyclohexanyl residue) with C-$\mathbf{D}_1$. A carbonyl carbon signal at δ 170.4 was correlated with proton signals at δ 4.50 (1′-H) and 4.80 (1″-H) and 5.07 ($\mathbf{G}_4$-H), linking the cyclohexanyl residue with C-$\mathbf{G}_4$ and with C-$\mathbf{D}_1$. Moreover, correlations were observed between a carbonyl carbon signal at δ 165.4 and proton signals at δ 5.37 ($\mathbf{G}_3$-H) and 6.76 ($\mathbf{B}_3$-H), connecting HHDP with C-$\mathbf{G}_3$ through C-$\mathbf{B}_7$ on one side, and between a C-$\mathbf{C}_7$ signal at δ 167.6 and a proton signal at 4.24 ($\mathbf{G}_6$-H), connecting HHDP with C-$\mathbf{G}_6$ on the other side through C-$\mathbf{C}_7$. Significant two and three bond correlations were also observed between signals of 1″-H (δ 4.80) and C-6′ (δ 97.4), C-2″ (δ 98.2), C-2′ (δ 51.8) and C-3″ (δ 81.4), and between those of 4″-H (δ 3.95) and C-2′, together with other pertinent correlations between a carbinol carbon signal (δ 76.8, C-4″) and a proton signal at δ 3.90 (3″-H), which suggested a tricyclic cage-like partial structure formed with three rings **E**, **F** and **J**, connecting ring **K** with a spiro carbon (C-2″) (Chart 3). These findings revealed that the plane structure of **1** was identical with that proposed for putranjivain A, an ellagitannin isolated from *Putranjiva matsumurae*.[11] Since the reported structure of putranjivain A was still ambiguous in stereochemistry, we investigated it in more detail by 2D NMR.

The stereochemistry around the spiro carbon (C-2″) was determined by the coupling constants of each proton and nuclear Overhauser enhancing effect (NOE effect) experiments. As shown in Fig. 1, negative NOE effects of 3′-H_b, 3″-H, 1′-H and 1″-H were observed on irradiation at δ 1.40 (3′-H_a), while those of 3′-H_a, 1′-H and 1″-H were seen on irradiation at δ 2.57 (3′-H_b). Furthermore, irradiation at δ 3.90 (3″-H) and 4.80 (1″-H) induced negative NOE effects of 3′-H_a, 3′-H_b, 5″-H, 1′-H and 1″-H, and of 3′-H_a, 3″-H and 1′-H, respectively. The configuration at C-2″ was assigned as *R*, which led us to determine the stereostructure of **1**, as well as the complete assignment of all the carbon signals.

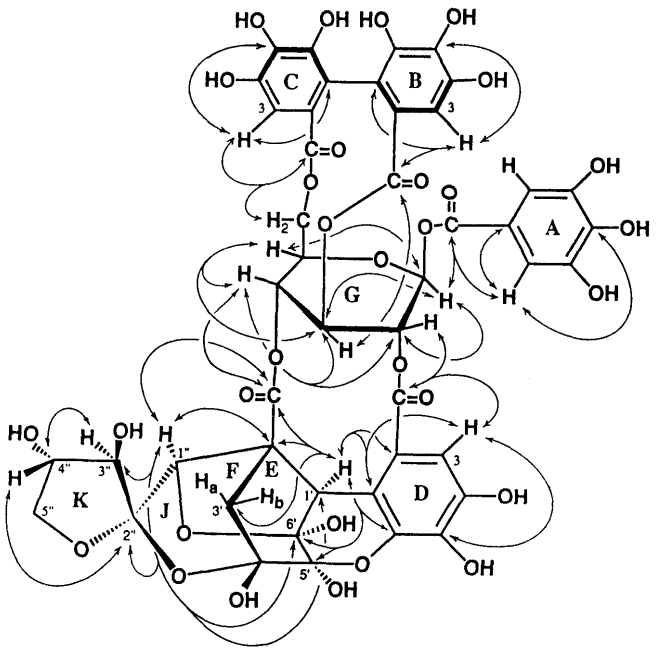

Chart 3. Significant Correlations Observed in ^{13}C–^{1}H Long-Range COSY and HMBC Experiments of Putranjivan A (**1**)

Compound **2** was obtained as colourless needles, mp 192—193 °C, $[\alpha]_D$ −23.0° (MeOH), MS *m/z* 485 $(M+H)^+$. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a glucose unit with an anomeric proton signal at δ 5.70 (d, J=7.5 Hz, 1′-H) and two galloyl groups. Since signals of 1′-H and 6-H_2 were observed relatively downfield at δ 4.35 and 4.55, respectively, the glucose unit was deduced to be acylated at C-1′ and C-6′ with the two galloyl groups. Finally, **2** was identified as 1,6-di-*O*-galloyl-β-D-glucose by comparing the ^{1}H- and ^{13}C-NMR spectra with those of reported data.[12]

Compound **3** was obtained as colourless needles, mp 215—216 °C, $[\alpha]_D$ −5.0° (MeOH), MS *m/z* 332 $(M+H)^+$. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a glucose unit acylated at C-1′ with a galloyl group, and **3** was finally determined as 1-*O*-galloyl-β-D-glucose.

Compounds **4** and **5** were obtained as yellow amorphous powder and yellow needles, respectively. These compounds were identified as kaempferol-3-*O*-glucoside and quercetin-3-*O*-glucoside, respectively, by comparing their ^{1}H- and ^{13}C-NMR spectra with those of authentic samples.

Compound **6** was obtained as yellow amorphous powder, FAB-MS *m/z* 323 $(M+H)^+$. The ^{1}H-NMR spectrum showed the presence of two galloyl groups, but no signals for sugar protons, which led us to speculate it was a gallic acid dimer. The ^{13}C-NMR spectrum was in good agreement with those reported for *m*- and *p*-digallic acids.[12,13] Consequently, **6** was determined to be an equilibrium mixture of *m*- and *p*-digallic acids (a ratio of 1 : 1 in DMSO-d_6).

Of the six compounds isolated from the fruit of *P. emblica*, **1** showed the most potent inhibitory activity against HIV-RT with IC_{50} of 3.9 μM, but **2** and **6** weakly inhibited the enzyme with IC_{50} of 270 μM and 200 μM, respectively (Table 3). Compounds **3**, **4** and **5** showed no appreciable inhibition even at a higher concentration (1000 μM).

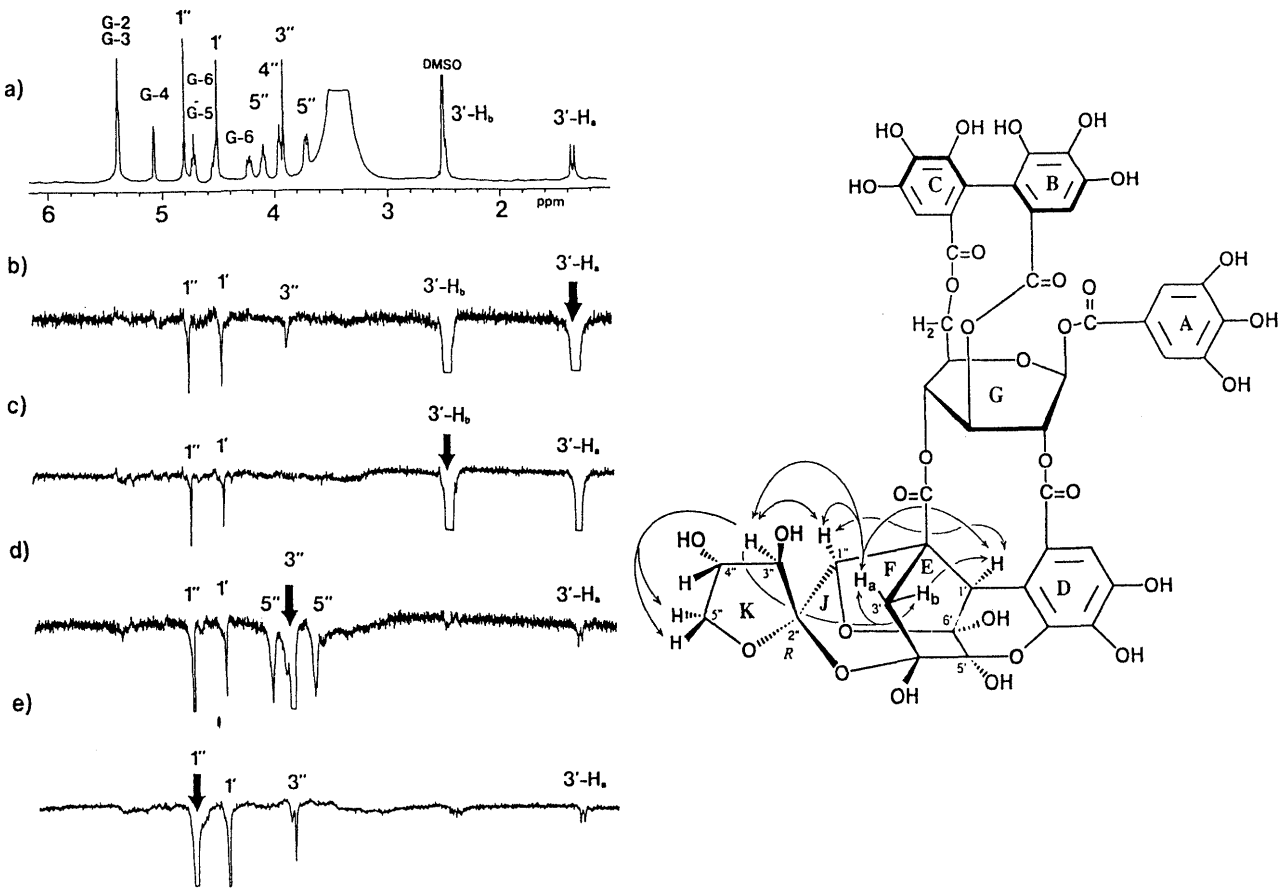

Fig. 1. ^{1}H-NMR (Normal and NOE Difference) Spectra of Putranjivain A (**1**) in DMSO-d_6

a) Normal spectrum. b—e) NOE difference spectra on irradiation at δ 1.40 (3'-H$_a$), 2.75 (3'-H$_b$), 3.90 (3''-H) and 4.80 (1''-H), respectively.

The Lineweaver–Burk plots for **1** showed that the inhibitory mode of action was non-competitive with respect to the substrate (Fig. 2a) but competitive with respect to the template-primer (Fig. 2b). The respective inhibitory constants (K_i) were 0.89 μM for the substrate and 0.25 μM for the template-primer (Table 3). Similarly, the inhibitory mode of action by **2** and **6** was non-competitive with respect to the substrate (K_i=160 and 18.6 μM for **2** and **6**, respectively) but competitive with respect to the template-primer (K_i=96 and 34.5 μM for **2** and **6**) (Table 3).

When **1** was added to a reaction mixture 5 min after the initiaiton of DNA synthesis, the reaction was significantly suppressed depending on its concentration (Fig. 3), suggesting that **1** inhibits not only the initiation of the polymerization, but also the chain elongation.

Of the six folk medicines with significant inhibitory effects on HIV-1-RT, *Terminalia chebula* (kabuli) and *Terminalia horrida* (hind sheiri) are tranditionally used in Egypt for the treatment of hypertension, bile congestion and as cholagogue and stomachic.[14]

The *Phyllanthus* plant has been used for the treatment of jaundice, hepatitis and other infectious diseases.[15] Venkateswaran *et al.* reported that an extract of the leaves of *Phyllanthus niruri* had inhibitory effects on endogenous DNA polymerase of hepatitis B virus and on the interaction of the surface antigen of hepatitis B virus with the antibody formed against this virus.[16] Recently, Ogata *et al.* reported the isolation of repandusinic acid from *P. niruri* as an HIV-RT inhibitory substance.[17] Pettit *et al.* reported the isolation of antineoplastic lignans from *P. acuminatus* and *P. niruri*.[18] In the present study, we identified putranjivain A (**1**) from *P. emblica* as an inhibitory substance against HIV-RT though **1** was already reported to be present in *Phyllanthus flexuosus*.[10] and *Putranjiva matsumurae*.[11]

Biological activities of tannins and related polyphenols have been reported as antiviral[19] and antitumor agents, where ellagitannin and its derivatives are generally more active against the S-180 sarcoma[20] than other types of tannins. On the other hand, hydrolysable tannins having galloyl groups and/or HHDP groups are variable in their antiviral potency. The higher RT inhibitory potency in **1** relative to that of **2** and **6** seems to be a characteristic property of HHDP in **1** rather than that of galloyl groups. This speculation is supported by Kakiuchi *et al.*[4a] who reported that ellagitannins such as geraniin and chebulagic acid were more effective inhibitors than gallotannins in the RT reaction, and some dimeric ellagitannins, gemin A and agrimoniin, were more effective than monomeric ones. Two ellagitannins, punicalin and punicacortein C were shown to exhibit potent inhibition on purified HIV RT and HIV replication in cell culture.[4b] A finding of tannins with anti-HIV activity was also reported by Nakashima *et al.*,[21] where several hydrolyzable tannins were shown to be inhibitors of HIV replication and this was mediated, at least in part, by inhibition of HIV adsorption to the cells. Therefore, it is not clear whether

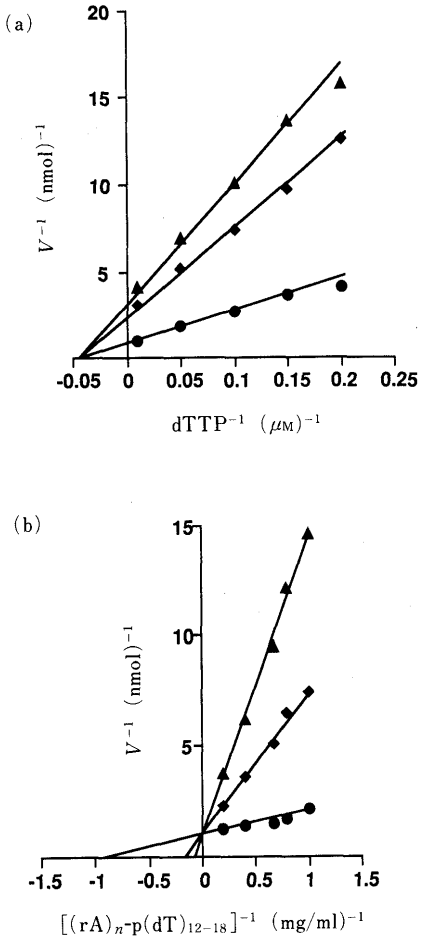

Fig. 2. Lineweaver–Burk Plots of the Inhibition of HIV-1 RT by **1** in the Presence of Various Concentrations of dTTP (a) and r(A)$_n$-(dT)$_{12-18}$ (b)

Concentrations of **1** were 3.9 μM (▲), 1.8 μM (◆), and 0.0 μM (●). The figure is represented as double reciprocal plots.

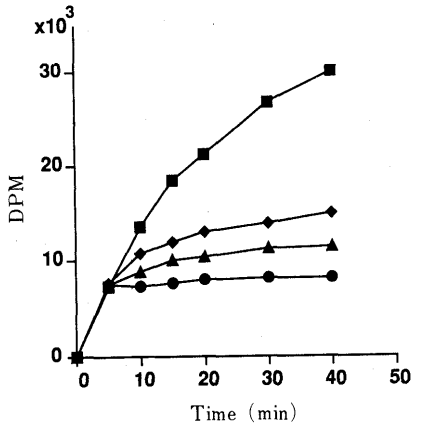

Fig. 3. Time Course of the HIV-1 RT Reactions Where **1** was Added 5 min after the Incubation, at Concentrations of 100 μM (●), 10 μM (▲), 3.9 μM (◆), and 0.0 μM (■)

the tannins present their crucial inhibitory effect on RT activity or during the viral binding to the cell. This paper offers the new complete structure elucidation of a compound with inhibitory effect against HIV-1-RT.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was measured with a Jasco DIP-360 automatic polarimeter at 25 °C. NMR spectra were measured with a JEOL JNM GX-400 spectrometer with tetramethylsilane (TMS) as an internal standard and the chemical shifts were given as δ values. Mass spectra were measured with a JEOL JNM DX-300 mass spectrometer at an ionization voltage of 70 eV. FAB-MS and high-resolution FAB-MS were obtained with a JEOL JMS-SX 102A spectrometer (ionization voltage, 70 eV; accelerating voltage, 5.0 kV) using glycerol + *m*-nitrobenzyl alcohol as an matrix. Merck Kieselgel F_{254} thin layer plates (layer thickness 0.25 mm for TLC and 0.5 mm for p-TLC) were developed with EtOAc–MeOH–H_2O (100 : 16.5 : 13.5, solvent system A), and benzene–ethyl formate–formic acid (1 : 5 : 2, solvent system B), and the spots were detected under UV lamp and after spraying with $FeCl_3$ or $CeSO_4/H_2SO_4$.

Table 3. Inhibitory Mode of the Isolated Compounds

Compound	IC_{50} (μM)	Mode of inhibition (K_i)	
		With respect to rAdT	With respect to dTTP
1	3.9	Competitive (0.25 μM)	Non-competitive (0.89 μM)
2	270	Competitive (96.0 μM)	Non-competitive (160 μM)
6	200	Competitive (34.5 μM)	Non-competitive (18.6 μM)

The K_i values and the mode of inhibition were determined by Lineweaver–Burk plots with respect to dTTP or a template-primer r(A)$_n$-(dT)$_{12-18}$. The experiment was carried out in the standard reaction mixture described under Experimental section with a reaction time of 5 min. Compounds **1** (3.9 and 2.0 μM), **2** (270 and 135 μM) and **6** (200 and 100 μM) were added in the presence of varying concentrations of dTTP (5—100 μM) or (rA)$_n$-(dT)$_{12-18}$ (1—5 μg/ml).

Reagents HIV-1 RT was purchased from Eiken Chemical Co., Ltd. (Osaka, Japan), [methyl-^{3}H]thymidine 5′-triphosphate (dTTP, specific activity: 1.55 TBq/mmol) from Amersham-Japan (Tokyo) and scintillation fluid Aquasol-2 from NEN Research Products (Boston, U.S.A.). Adriamycin (doxorubicin hydrochloride) was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). (rA)$_n$-p(dT)$_{12-18}$ was purchased from Pharmacia (Uppsala, Sweden) and DEAE-cellulose discs (Whatman DE 81, 2.3 cm) were obtained from Whatman International, Ltd. (Maidstone, England).

HIV-1 RT Assay HIV-1 RT was adjusted to 0.5 U/μl with a solution of 0.2 M phosphate buffer pH 7.2, 50% of glycerol, 0.002 M dithiothreitol (DTT) and 0.02% of Triton X-100. The reaction mixture consisted of 50 mM Tris–HCl (pH 8.3), 30 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT, 5 μg/ml template-primer (rA)$_n$-P(dT)$_{12-18}$, 0.1 mM methyl-[^{3}H]dTTP (18.5 MBq/ml) and 0.5 U of RT in a final volume of 20 μl. A plant extract was dissolved in dimethylsulfoxide (DMSO) and 1 μl of this solution was added to the reaction mixture. The enzyme was added immediately prior to incubation. The reaction was allowed to proceed for 30 min at 37 °C and terminated by immersion in ice and addition of 0.2 M EDTA (2 μl). A portion (10 μl) of each assay was applied to DEAE-cellulose discs, kept at room temperature for 10 min and washed batchwise (3 ml each) with 5% Na_2HPO_4 (5 times), H_2O (2 times), 99% EtOH (2 times) and ether (once). The discs were dried and their radioactivities were counted in a scintillation fluid (3 ml each).

The HIV-1 RT inhibition was measured as the inhibition of the incorporation of [^{3}H]-labeled substrate (dTTP) into a polymer fraction in the presence of a plant extract or a tested compound as follows:

$$\text{inhibition (\%)} = \{1 - (\text{dpm test/dpm control})\} \times 100$$

The control assay was performed by adding DMSO containing no test sample and its count was *ca.* 19000 dpm. Adriamycin was used as a positive control which inhibited the enzyme activity by 97.0% at a concentration of 0.25 mM.

Plant Materials The plants were purchased at Harraz Herbal Drugstore in Cairo, Egypt. They were identified by Professor El-Sayed E. Aboutabl, Faculty of Pharmacy, Cairo University, Egypt. A voucher specimen of each plant is deposited at the Museum of Materia Medica of Toyama Medical and Pharmaceutical University, Toyama, Japan. The powdered plant material (5 g each) was refluxed separately with MeOH and H_2O (50 ml × 3) for 3 h. The extracts were concentrated,

freeze-dried and tested for their HIV-1 RT inhibitory effects as described above.

Extraction and Isolation *P. emblica* (3 kg powdered fruit) was successively extracted with $CHCl_3$ and MeOH (101 × 3, each). The MeOH extract was evaporated yielding an extract (650 g), from which inorganic salts, sugars and impurities were removed by precipitation in EtOH. The HIV-1 RT inhibitory effect was observed for the EtOH-soluble extract (370 g, $IC_{50}=95\,\mu g/ml$). Fifty grams of this extract was subjected to column chromatography (CC., Sephadex LH-20, 100 × 6.5 i.d.), then eluted with H_2O followed by an increasing amount of MeOH to give 25 fractions. Throughout the isolation procedure all fractions were tested for anti-RT activity. The most potent inhibitory fractions (fr. 18—19, $IC_{50}=65\,\mu g/ml$) were further subjected to repeated CC. (Sephadex LH-20), eluting with EtOH followed by increasing the percentage of MeOH to afford a pure compound (**1**, $IC_{50}=3.9\,\mu g/ml$). The other fractions which showed moderately inhibitory activities (fr. 10—13 and fr. 20—22, $IC_{50}=145\,\mu g/ml$ and 93 $\mu g/ml$, respectively) were rechromatographed on a Sephadex LH-20 column, eluting with 30% MeOH followed by increasing the percentage of MeOH to give **2** (18.5 mg), **3** (20 mg) from fr. 10—13 and **4** (4 mg), **5** (2 mg) and **6** (210 mg) from fr. 20—22.

Putranjivain A (1) Off-white amorphous powder (31 mg, 0.06%); *Rf* 0.18 in solvent system A, and 0.25 in solvent system B; $[\alpha]_D$ −89.0° ($c=0.1$, MeOH); negative ion FAB-MS *m/z*: 1083 $(M-H)^-$, HR-FAB-MS *m/z*: 1083.1172 $(M-H)^-$ (Calcd for $C_{46}H_{35}O_{31}$: 1083.1171); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.40 (1H, br d, $J=14.0$ Hz, 3′-H_a), 2.57 (1H, br d, $J=14.0$ Hz, 3′-H_b), 3.73 (1H, br d, $J=8.0$ Hz, 5″-H), 3.90 (1H, br s, 3″-H), 3.95 (1H, br t, $J=5.0$ Hz, 4″-H), 4.10 (1H, t, $J=8$ Hz, 5″-H), 4.24 (1H, dd, $J=6.0$, 11.0 Hz, $\mathbf{G}_6$-H), 4.50 (1H, s, 1′-H), 4.51 (1H, m, $\mathbf{G}_6$-H), 4.73 (1H, dd, $J=6.0$, 8.0 Hz, $\mathbf{G}_5$-H), 4.80 (1H, s, 1″-H), 5.07 (1H, d, $J=3.0$ Hz, $\mathbf{G}_4$-H), 5.37 (2H, br s, $\mathbf{G}_2$-H, $\mathbf{G}_3$-H), 6.27 (1H, d, $J=3.5$ Hz, $\mathbf{G}_1$-H), 6.46 (1H, s, $\mathbf{C}_3$-H), 6.76 (1H, s, $\mathbf{B}_3$-H), 7.03 (2H, s, $\mathbf{A}_2$-H, $\mathbf{A}_6$-H), 7.19 (1H, s, $\mathbf{D}_3$-H); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.60 (1H, br d, $J=14.0$ Hz, 3′-H_a), 2.70 (1H, dd, $J=1.5$, 14.0 Hz, 3′-H_b), 3.90 (1H, dd, $J=6.0$, 13.0 Hz, 5″-H), 4.15 (1H, s, 4″-H), 4.16 (1H, dd, $J=6.0$, 13.0 Hz, 5″-H), 4.21 (1H, s, 3″-H), 4.44 (1H, m, $\mathbf{G}_6$-H), 4.74 (2H, dd, $J=9.0$, 11.0, $\mathbf{G}_6$-H), 4.76 (2H, d, $J=1.5$ Hz, 1′-H), 4.90 (1H, t, $J=9.0$ Hz, $\mathbf{G}_5$-H), 5.03 (1H, s, 1″-H), 5.34 (1H, br d, $J=4.0$ Hz, $\mathbf{G}_4$-H), 5.60 (1H, br s, $\mathbf{G}_2$-H), 5.66 (1H, br s, $\mathbf{G}_3$-H), 6.51 (1H, br s, $\mathbf{G}_1$-H), 6.64 (1H, s, $\mathbf{C}_3$-H), 7.07 (1H, s, $\mathbf{B}_3$-H), 7.19 (2H, s, $\mathbf{A}_2$-H, $\mathbf{A}_6$-H), 7.32 (1H, s, $\mathbf{D}_3$-H); ^{13}C-NMR (100 MHz DMSO-d_6) δ: 31.9 (t, C-3′), 51.2 (d, C-1′), 51.8 (d, C-2′), 63.0 (d, C-$\mathbf{G}_3$), 63.3 (d, C-$\mathbf{G}_6$), 65.8 (d, C-$\mathbf{G}_4$), 71.0 (d, C-$\mathbf{G}_2$), 73.7 (d, C-$\mathbf{G}_5$), 73.8 (t, C-5″), 75.6 (d, C-1″), 76.8 (d, C-4″), 81.4 (d, C-3″), 91.2 (d, C-$\mathbf{G}_1$), 95.5 (s, C-4′), 97.4 (s, C-6′), 98.2 (s, C-5′), 98.3 (s, C-2″), 106.4 (d, C-$\mathbf{C}_3$), 107.4 (d, C-$\mathbf{B}_3$), 109.3 (2C, d, C-$\mathbf{A}_2$ and C-$\mathbf{A}_6$), 111.0 (s, C-$\mathbf{D}_1$), 112.9 (d, C-$\mathbf{D}_3$), 114.9 (s, C-$\mathbf{C}_1$), 116.3 (s, C-$\mathbf{B}_1$), 117.4 (s, C-$\mathbf{D}_2$), 118.0 (s, C-$\mathbf{A}_1$), 122.5 (s, C-$\mathbf{C}_2$), 123.3 (s, C-$\mathbf{B}_2$), 135.4 (s, C-$\mathbf{C}_5$), 136.3 (s, C-$\mathbf{B}_5$), 138.2 (s, C-$\mathbf{D}_5$), 139.6 (s, C-$\mathbf{A}_4$), 144.1 (s, C-$\mathbf{D}_4$), 144.4 (s, C-$\mathbf{D}_6$), 144.8 (2C, s, C-$\mathbf{C}_4$ and C-$\mathbf{B}_6$), 144.9 (s, C-$\mathbf{B}_4$), 145.0 (s, C-$\mathbf{C}_6$), 145.8 (2C, s, C-$\mathbf{A}_3$ and C-$\mathbf{A}_5$), 164.2 (s, C-$\mathbf{A}_7$), 164.9 (s, C-$\mathbf{D}_7$), 165.4 (s, C-$\mathbf{B}_7$), 167.6 (s, C-$\mathbf{C}_7$), 170.4 (s, C-$\mathbf{E}_7$).

1,6-Di-*O*-galloyl-β-D-glucose (2) Colourless needles from water (18.5 mg, 0.037%); mp 192—193 °C; *Rf* 0.48 in solvent system A, 0.59 in solvent system B; $[\alpha]_D$ −23.0° ($c=0.1$; MeOH); positive ion FAB-MS *m/z*: 485 $(M+H)^+$.

1-*O*-Galloyl-β-D-glucose (3) Colourless needles from water (20 mg, 0.04%); mp 215—216 °C; *Rf* 0.32 in solvent system A; $[\alpha]_D$ −5.0° ($c=0.1$; MeOH); positive ion FAB-MS *m/z*: 333 $(M+H)^+$. ^{1}H-NMR (400 MHz, acetone-d_6) δ: 3.17 (1H, dd, $J=8.5$, 4.0 Hz, 4′-H), 3.23 (3H, m, 5′, 2′, and 3′), 3.45 (1H, dd, $J=12.0$, 6.0 Hz, 6′-H), 3.65 (1H, dd, $J=12.0$, 5.0 Hz, 6′-H), 4.59 (1H, t, $J=5.5$ Hz, 6′-OH), 5.02 (1H, d, $J=5.0$ Hz, 4′-OH), 5.14 (1H, br s, 3′-OH), 5.32 (1H, d, $J=5.0$ Hz, 2′-OH), 5.51 (1H, d, $J=7.5$ Hz, 1′-H), 7.02 (2H, s, galloyl-H); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 60.7 (t, C-6′), 69.6 (d, C-4′), 72.7 (d, C-2′), 76.7 (d, C-5′), 78.0 (d, C-3′), 94.6 (d, C-1′), 109.1 (2C, d, galloyl C-2 and C-6), 118.9 (s, galloyl C-1), 139.0 (s, galloyl C-4), 145.7 (2C, s, galloyl C-3 and C-5), 164.8 (s, galloyl CO).

Kaempferol-3-*O*-glucoside (4) Yellow amorphous powder (4 mg, 0.008%); *Rf* 0.49 in solvent system A; $[\alpha]_D$ −2.8° ($c=0.05$; MeOH); negative ion FAB-MS *m/z*: 447 $(M-H)^-$.

Quercetin-3-*O*-glucoside (5) Yellow needles from MeOH (2 mg, 0.004%); mp 225—227 °C; *Rf* 0.50 in solvent system A; $[\alpha]_D$ −3.7° ($c=0.05$; MeOH); negative ion FAB-MS *m/z*: 463 $(M-H)^-$.

Digallic Acid (6) Yellow amorphous powder (210 mg, 0.42%); *Rf* 0.51 in solvent system A and 0.84 in solvent system B; positive ion FAB-MS *m/z*: 323 $(M+H)^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.02 (2H, s, galloyl-H); 7.15 (2H, s, galloyl-H); ^{13}C-NMR (DMSO-d_6) δ: 108.2, 109.1, 109.2, 113.6, 115.5, 118.2, 118.4, 120.5, 127.3, 130.7, 139.1, 139.2, 139.3, 142.7, 145.6, 145.7, 146.4, 150.5, 163.6, 164.1, 166.8, 167.2.

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