

Bioactive Phenolics from *Launaea intybacea*

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Summary: Phytochemical investigations on the methanolic extract of *Launaea intybacea* yielded eleven compounds namely, octadecyl (*E*)-*p*-coumarate (**1**), 4-hydroxy-3-methoxybenzaldehyde (**2**), 4-hydroxybenzoic acid (**3**), 4-hydroxy-3-methoxybenzoic acid (**4**), coumaric acid (**5**), methyl gallate (**6**), 3,4-dihydroxybenzoic acid (**7**), 4',5,7-trihydroxyflavone (**8**), 3',4',5,7-tetrahydroxyflavone (**9**), 3,3',5,7-tetrahydroxy-4'-methoxyflavone (**10**) and apigenin 7-*O*-(4''-*O*-*p*-coumaroyl)- β -D-glucopyranoside (**11**). Their structures were determined by using 1D-NMR (¹H, ¹³C), mass spectrometry (EIMS, HREIMS, FABMS, HRFABMS) and comparison with the reported data. These compounds (**1-11**) showed DPPH radical scavenging and enzyme inhibitory activities.

Introduction

Launaea is a small genus of family Compositae comprised of about 40 species, of which 20 species are available in Pakistan. *Launaea intybacea* is a biennial herb commonly found in North and Central America, West Indies, tropical parts of Asia and commonly found in Subcontinent [1]. The herb is cosmopolitan in nature and has adapted to the dry conditions, commonly found in the coastal areas [2]. Root and the aerial parts of the plants have been used by the local people as a folk remedy to treat jaundice, as blood purifier, galactagogue, hepatomegaly, dyspepsia, skin diseases, dry cough and galactoriya [1]. *Launaea intybacea* has also been used in folk medicine for the treatment of liver disorders [4]. The extract of the aerial parts of *L. intybacea* has been reported to possess hepatoprotection against CCl₄-induced hepatoprotective injury in albino rats [4]. Literature survey revealed that no phytochemical investigations have so far been carried out on this plant. Herein we report the isolation and characterization of octadecyl (*E*)-*p*-coumarate (**1**), 4-hydroxy-3-methoxybenzaldehyde (**2**), 4-hydroxybenzoic acid (**3**), 4-hydroxy-3-methoxybenzoic acid (**4**), coumaric acid (**5**), methyl gallate (**6**), 3,4-dihydroxybenzoic acid (**7**), 4',5,7-trihydroxyflavone (**8**), 3',4',5,7-tetrahydroxyflavone (**9**), 3,3',5,7-tetrahydroxy-4'-methoxyflavone (**10**) and Apigenin 7-*O*-(4''-*O*-*p*-coumaroyl)- β -D-glucopyranoside (**11**), respectively, from methanolic extract of whole plant *L. intybacea*.

Although all the isolates are known natural products but are reported for the first time from this source. These compounds (**1-11**) showed DPPH radical scavenging and enzyme inhibitory activities against enzyme acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX).

DPPH scavengers have been used as important protective agents for human health. These agents are used to preserve foods by retarding discoloration, rancidity, or deterioration due to auto-oxidation [5]. Acetylcholinesterase (EC 3.1.1.7) comprise a family of enzymes which include serine hydrolases [6] and plays a prominent role as it participate in termination of acetylcholine based signal transmission through neurosynaptic cleft [7, 8]. Butyrylcholinesterase (BChE) is a non-specific ester hydrolyzing enzyme [9] whose deficit in CNS leads to conditions such as Alzheimer's disease, a progressive neurological disease of the brain. It has been further suggested that dual inhibition of AChE and BChE enzymes should be one of the objectives in the treatment of cognitive dysfunction associated with Alzheimer's disease [10]. Lipoxygenases (EC 1.13.11.12) are non-heme, non-sulphur, iron cofactor containing dioxygenases that catalyze the oxidation of polyunsaturated fatty acids as substrate such as linoleic, linolenic and arachidonic acid to yield hydroperoxides [11]. Lipoxygenases play a key role

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in the variety of disorders such as bronchial asthma, inflammation [12] and tumor angiogenesis etc [13].

Result and Discussion

Methanolic extract of whole plant of *L. intybacea* was subjected to the silica gel and flash column chromatography. After repeated column chromatography eleven compounds were isolated which have been characterized on the basis of 1D-NMR (^1H , ^{13}C), mass spectrometry (EIMS, HREIMS, FABMS, HRFABMS) and comparison with the reported data. Compound **1** was obtained as white amorphous powder. Its UV absorbance at 227 and 312 nm showed the presence of substituted benzene ring where as its ^1H -NMR spectrum showed signals for olefinic protons at δ 7.60 (1H, d, J = 18.0 Hz), 7.43 (2H, d, J = 7.8 Hz), 6.81 (2H, d, J = 7.8 Hz), 6.25 (1H, d, J = 18.0 Hz) and aliphatic chain at δ 4.21 (2H, t, J = 6.5 Hz), 1.70 (2H, m), 1.25 (30H, br s), 0.88 (3H, t, J = 6.0 Hz) indicated **1** could be alkyl (*E*)-*p*-coumarate. The molecular formula $\text{C}_{27}\text{H}_{44}\text{O}_3$ and the ^{13}C -NMR spectrum confirmed **1** as octadecyl (*E*)-*p*-coumarate [14]. Compound **2** was obtained as white needles and its molecular formula $\text{C}_8\text{H}_8\text{O}_3$ was determined by HREIMS. The ^1H -NMR spectrum showed the presence of an aldehyde at δ 9.74 (1H, s), three aromatic protons at δ 7.42 (1H, dd, J = 8.4, 1.6 Hz), 7.29 (1H, d, J = 1.6 Hz), 7.09 (1H, d, J = 8.4 Hz) and a methoxy signal at δ 3.94. The ^{13}C -NMR spectrum showed eight carbon signals including one methyl, three methine and four quaternary carbons. The above data confirmed **2** as 4-hydroxy-3-methoxybenzaldehyde [15]. Compound **3** was obtained as crystalline solid having molecular formula $\text{C}_7\text{H}_6\text{O}_3$. The ^1H -NMR showed two doublets at δ 7.86 (2H, d, J = 8.8 Hz) and 6.80 (2H, d, J = 8.8 Hz) where as its ^{13}C -NMR showed five carbon signals for two methine and the quaternary carbons at δ 169.5, 163.7, 132.9, 123.0 and 115.9. The above data was completely matched with the data reported for 4-hydroxybenzoic acid [16]. Compound **4** was purified as crystalline solid having UV bands at 258 and 289 nm indicated the presence of phenolic chromophore. The ^1H -NMR spectrum showed three signals in the aromatic region at δ 7.55 (1H, d, J = 2.0 Hz), 7.53 (1H, dd, J = 8.5, 2.0 Hz), 6.82 (1H, d, J = 8.5 Hz) and a methoxy group at δ 3.88. The ^{13}C -NMR spectrum showed eight carbon signals for one methyl, three methine and four quaternary carbons at δ 169.8, 152.5, 148.6, 127.2, 125.2, 115.7, 113.8 and 56.3. The above data confirmed **4** as 4-hydroxy-3-methoxybenzoic acid [17]. Compound **5** was isolated as yellow powder. Its ^1H -NMR spectrum showed signals at δ 7.44 (2H, d, J = 8.6 Hz), 7.22 (1H, d, J =

15.8 Hz), 6.80 (2H, d, J = 8.6 Hz), 6.23 (1H, d, J = 15.8 Hz) typical for coumaric acid which was also confirmed by its ^{13}C -NMR having total seven carbon signals at δ 170.6, 158.7, 138.2, 129.4, 126.8, 115.5 and 114.6 [18]. Compound **6** was purified as crystalline solid. Its molecular formula $\text{C}_8\text{H}_8\text{O}_5$ was determined by HREIMS through a peak at 184.0345. The ^1H -NMR showed only two singlets at δ 7.03 and 3.80 where as six signals at δ 169.0, 146.5, 139.7, 121.5, 110.0 and 56.3 in its ^{13}C -NMR spectrum confirmed it as methyl gallate [19]. The molecular formula of compound **7** was deduced as $\text{C}_7\text{H}_6\text{O}_4$. Its ^1H -NMR spectrum showed only three signals in the aromatic region at δ 7.42 (1H, dd, J = 8.5, 1.5 Hz), 7.41 (1H, d, J = 1.5 Hz), 6.78 (1H, d, J = 8.5 Hz) where as the ^{13}C -NMR spectrum showed seven carbon signals at δ 169.4, 151.1, 145.2, 124.2, 123.8, 117.7 and 115.7. The above data showed close resemblance to the data reported for 3,4-dihydroxybenzoic acid [20]. Compound **8** was isolated as yellow needles having molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_6$ determined by HREIMS. The UV bands at 269 and 340 nm is typical for flavanoid nucleus which is supported by its ^1H -NMR spectrum having resonances at δ 7.85 (2H, d, J = 8.5 Hz), 6.93 (2H, d, J = 8.5 Hz), 6.58 (1H, s), 6.45 (1H, d, J = 2.0 Hz), 6.20 (1H, d, J = 2.0 Hz). The ^{13}C -NMR spectrum showed altogether thirteen carbon signals at δ 183.9, 166.3, 166.1, 163.2, 162.1, 159.4, 129.4, 123.2, 117.0, 105.3, 103.8, 100.1 and 95.0. The above data confirmed **8** as 4',5,7-trihydroxyflavone [21]. The compound **9** have same physical appearance, UV and IR data as **8** with molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_6$. The ^1H -NMR showed the a double doublet, a singlet and a doublet at δ 7.39 (1H, dd, J = 7.5, 2.5 Hz'), 7.37 (1H, br s, H-2'), 6.90 (1H, d, J = 8.5 Hz), respectively instead of two doublets as in **8** indicated the presence of additional hydroxyl group in ring C which is supported by its ^{13}C -NMR spectrum due to signal at δ 147.0 (C-3'). The above data allow us to confirm **9** as 3',4',5,7-tetrahydroxyflavone [22, 23]. The UV and IR spectra of **10** was similar to those for **8,9** whereas the molecular formula $\text{C}_{16}\text{H}_{12}\text{O}_7$ showed the addition of fourteen unit was due a methoxy group appeared both in ^1H - and ^{13}C -NMR spectra (δ_{H} 3.93; δ_{C} 56.8), respectively, confirming **10** as 3,3',5,7-tetrahydroxy-4'-methoxyflavone [24]. Compound **11** was obtained as amorphous powder. The UV spectrum was similar to that for the above flavanoids. The ^1H -NMR spectrum was similar to **8** with the additional signals for *p*-*E*-coumaroyl at δ 7.71 (1H, d, J = 16 Hz), 7.49 (2H, d, J = 8.5 Hz'), 6.82 (2H, d, J = 9.0 Hz), 6.41 (1H, d, J = 15.5 Hz) and glucose moiety at δ 5.16 (1H, d, J = 8.0 Hz), 4.96 (1H, t, J = 9.5 Hz), 3.81 (1H, m), 3.78 (1H, m), 3.68 (1H, m), 3.61 (1H, m) and

their attachments were confirmed by the downfield shifts of C-7 (δ 164.7) and C-4'' (δ 72.1) and confirmed **11** to be apigenin 7-*O*-(4''-*O*-*p*-*E*-coumaroyl)- β -D-glucopyranoside [24]. All the isolates (**1-11**) showed DPPH radical scavenging and enzyme inhibitory activities against enzyme

acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX) with IC₅₀ values ranging from 76.2-211.5, 106.3-143.9, 53.9-230.6 and 42.2-99.1 μ M against DPPH, AChE, BChE and LOX, respectively (Table-1).

Table-1: DPPH scavenging, AChE, BChE and LOX inhibitory activities of compounds **1-11**.

Compound	DPPH		AChE		BChE		LOX	
	(%) at 0.5 mM	(IC ₅₀) μ M	(%) at 0.5 mM	(IC ₅₀) μ M	(%) at 0.5 mM	(IC ₅₀) μ M	(%) at 0.5 mM	(IC ₅₀) μ M
1	23.9 $\pm$ 0.2	NIL	82.2 $\pm$ 0.1	106.31 $\pm$ 0.31	91.2 $\pm$ 0.2	53.9 $\pm$ 0.05	45.6 $\pm$ 0.1	NIL
2	22.6 $\pm$ 0.6	NIL	52.5 $\pm$ 0.3	<400	59.9 $\pm$ 0.4	241.1 $\pm$ 0.3	82.6 $\pm$ 0.3	99.1 $\pm$ 0.2
3	24.7 $\pm$ 0.8	NIL	49.5 $\pm$ 0.8	<400	59.6 $\pm$ 0.1	230.6 $\pm$ 0.7	57.5 $\pm$ 0.8	<400
4	18.5 $\pm$ 0.3	NIL	36.6 $\pm$ 0.2	NIL	50.3 $\pm$ 0.2	<400	53.7 $\pm$ 0.2	<400
5	14.3 $\pm$ 0.4	NIL	56.9 $\pm$ 0.1	<400	53.4 $\pm$ 0.4	<300	5.4 $\pm$ 0.1	NIL
6	85.6 $\pm$ 0.6	76.2 $\pm$ 0.4	31.9 $\pm$ 0.3	NIL	64.8 $\pm$ 0.5	120.2 $\pm$ 0.3	12.8 $\pm$ 0.5	NIL
7	69.0 $\pm$ 0.1	211.5 $\pm$ 0.1	56.9 $\pm$ 0.5	<400	62.7 $\pm$ 0.6	152.3 $\pm$ 0.2	27.3 $\pm$ 0.7	NIL
8	21.9 $\pm$ 0.1	NIL	50.5 $\pm$ 0.2	<400	83.8 $\pm$ 0.3	93.3 $\pm$ 0.3	83.6 $\pm$ 0.2	83.7 $\pm$ 0.1
9	78.4 $\pm$ 0.2	146.3 $\pm$ 0.04	58.7 $\pm$ 0.6	<400	80.1 $\pm$ 0.3	106.8 $\pm$ 0.2	42.0 $\pm$ 0.6	NIL
10	28.9 $\pm$ 0.8	NIL	75.1 $\pm$ 0.4	143.9 $\pm$ 0.22	93.2 $\pm$ 0.4	34.1 $\pm$ 0.2	17.3 $\pm$ 0.4	NIL
11	24.1 $\pm$ 0.2	NIL	53.5 $\pm$ 0.2	<400	78.6 $\pm$ 0.3	96.5 $\pm$ 0.2	93.8 $\pm$ 0.2	48.2 $\pm$ 0.3
Quercetin	93.21 $\pm$ 0.97	16.96 $\pm$ 0.14	-	-	-	-	-	-
Eserine	-	-	91.29 $\pm$ 1.17	0.04 $\pm$ 0.0001	82.82 $\pm$ 1.09	0.85 $\pm$ 0.0001	-	-
Baicalein	-	-	-	-	-	-	93.79 $\pm$ 1.2	22.4 $\pm$ 1.3

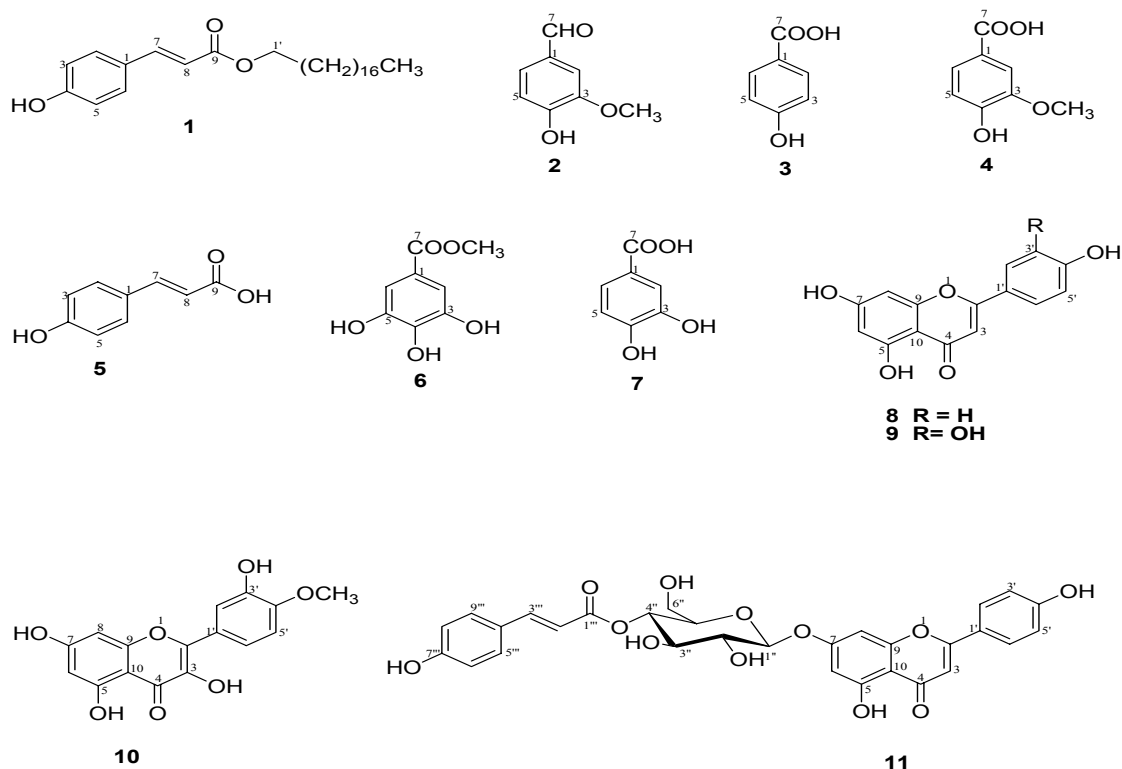


Fig. 1: Compounds isolated from *Launaea intybacea*.

Experimental

General

Column chromatography was carried out using silica gel F₂₅₄ (230-400 mesh). Aluminium sheets pre-coated with silica gel 60 F₂₅₄ (20×20 cm, 0.2 mm thick; E. Merck) were used for thin layer chromatography (TLC). TLC plates were visualized under UV lamp at 254 and 366 nm wave length and by spraying with ceric sulphate solution (by heating). IR spectra were recorded on Shimadzu 460 spectrophotometer, and UV spectra were scanned on a Hitachi UV-3200 spectrophotometer (λ_{max} in nm). 1D-NMR data were scanned on Bruker instrument operating at 400, 500 and 100, 125 MHz respectively. Mass spectra were measured on JMS H×110 mass spectrometer with a data system and JMSA 500 mass spectrometers.

Plant Material

The whole plant of *L. intybacea* was collected from Cholistan Desert (District Bahawalpur, Punjab), Pakistan in April 2008 and was identified by Dr. Muhammad Arshad (Late), Ex-Taxonomist, Cholistan Institute for Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan where a voucher specimen (0023-LI/CIDS/08) is deposited.

Extraction and Isolation

The methanolic extract of the *L. intybacea* was subjected to silica gel column chromatography eluting with *n*-hexane, *n*-hexane:dichloromethane (DCM), DCM, DCM:methanol and methanol in increasing order of polarity. Six fractions (1-6) were obtained which on gradient elution using pure DCM yielded octadecyl (*E*)-*p*-coumarate (**1**); 1% MeOH/DCM yielded 4-hydroxy-3-methoxybenzaldehyde (**2**), 2% MeOH/DCM afforded 4-hydroxybenzoic acid (**3**), and 4-hydroxy-3-methoxybenzoic acid (**4**) from head and tail fractions, respectively; 3% MeOH/DCM provided coumaric acid (**5**); 4% MeOH/DCM yielded methyl gallate (**6**); 5% MeOH/DCM afforded 3,4-dihydroxybenzoic acid (**7**); 6% MeOH/DCM 4',5,7-trihydroxyflavone (**8**); 7 % MeOH/DCM provided 3',4',5,7-tetrahydroxyflavone (**9**); 10 % MeOH/DCM afforded 3,3',5,7-tetrahydroxy-4'-methoxyflavone (**10**) and 12 % MeOH/DCM yielded apigenin 7-*O*-(4'-*O*-*p*-*E*-coumaroyl)- β -D-glucopyranoside (**11**), respectively.

Octadecyl (*E*)-*p*-coumarate (**1**)

White amorphous powder (15 mg); m.p. 98-100°C; UV (MeOH) λ_{max} (log ϵ) nm: 204 (3.98), 227

(3.86), 312 (4.11), 425 (2.67); ¹H-NMR (MeOH, 400 MHz) δ : 7.60 (1H, d, *J* = 18.0 Hz, H-7), 7.43 (2H, d, *J* = 7.8 Hz, H-2,6), 6.81 (2H, d, *J* = 7.8 Hz, H-3,5), 6.25 (1H, d, *J* = 18.0 Hz, H-8), 4.21 (2H, t, *J* = 6.5 Hz, H-1'), 1.70 (2H, m, H-2'), 1.25 (30H, br s, H-3'-17'), 0.88 (3H, t, *J* = 6.0 Hz, H-18'); ¹³C-NMR (MeOH, 100 MHz) δ : 167.5 (C-9), 157.5 (C-4), 144.2 (C-7), 129.9 (C-2,6), 115.9 (C-3,5), 115.7 (C-3), 64.6 (C-1'), 22.6-31.9 (C-2'-17'), 14.1 (C-18'); HREIMS *m/z*: 416.3288 (calcd. for C₂₇H₄₄O₃, 416.3290).

4-Hydroxy-3-methoxybenzaldehyde (**2**)

White needles (25 mg); m.p. 81-82°C; UV (MeOH) λ_{max} (log ϵ) nm: 234 (4.19), 281 (4.02), 310 (3.01); IR (KBr) ν_{max} cm⁻¹: 3286, 1657, 1608, 1513, 1245, 1150, 1082, 1042, 831; ¹H-NMR (MeOH, 400 MHz) δ : 9.74 (1H, s, H-7), 7.42 (1H, dd, *J* = 8.4, 1.6 Hz, H-6), 7.29 (1H, d, *J* = 1.6 Hz, H-2), 7.09 (1H, d, *J* = 8.4 Hz, H-5), 3.94 (3H, s, OCH₃); ¹³C-NMR (MeOH, 100 MHz) δ : 193.1 (C-7), 155.0 (C-4), 148.4 (C-3), 131.8 (C-1), 126.0 (C-6), 114.8 (C-5), 112.1 (C-2), 56.5 (OCH₃); HREIMS *m/z*: 152.0471 (calcd. for C₈H₈O₃, 152.0473).

4-Hydroxybenzoic acid (**3**)

Crystalline solid (10 mg); m.p. 213-214°C; UV (MeOH) λ_{max} (log ϵ) nm: 222 (3.80); 310 (3.89); IR (KBr) ν_{max} cm⁻¹: 3515, 3335-2730, 1710; ¹H-NMR (MeOH, 400 MHz) δ : 11.1 (1H, s, OH), 7.86 (2H, d, *J* = 8.8 Hz, H-2,6), 6.80 (2H, d, *J* = 8.8 Hz, H-3,5); ¹³C-NMR (MeOH, 100 MHz) δ : 169.5 (C-7), 163.7 (C-4), 132.9 (C-2,6), 123.0 (C-1), 115.9 (C-3,5); HREIMS *m/z*: 138.0321 (calcd. for C₇H₆O₃, 138.0316).

Vanillic acid (**4**)

Crystalline solid (22 mg); m.p. 210°C; UV (MeOH) λ_{max} (log ϵ) nm: 289 (4.5); 258 (4.7), 215 (4.9); IR (KBr) ν_{max} cm⁻¹: 3510, 3335-2730, 1705; ¹H-NMR (MeOH, 500 MHz) δ : 11.3 (1H, s, OH), 7.55 (1H, d, *J* = 2.0 Hz, H-2), 7.53 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 6.82 (1H, d, *J* = 8.5 Hz, H-5), 3.88 (3H, s, OCH₃); ¹³C-NMR: (MeOH 125 MHz) δ : 169.8 (C-7), 152.5 (C-4), 148.6 (C-3), 127.2 (C-1), 125.2 (C-6), 115.7 (C-5), 113.8 (C-2), 56.3 (OCH₃); HREIMS *m/z*: 168.0419 (calcd. for C₈H₈O₄, 168.0422).

4-Hydroxy-trans-cinnamic acid (**5**)

Yellow powder (15 mg); m.p. 210-212°C; UV (MeOH) λ_{max} (log ϵ) nm: 292 (4.69), 308 (4.50);

IR (KBr) $\nu_{\max}$ cm^{-1} : 3400, 3300-2200, 1680, 1620, 1420, 1380; $^1\text{H-NMR}$ (MeOH, 400 MHz) δ : 7.44 (2H, d, $J = 8.6$ Hz, H-2,6), 7.22 (1H, d, $J = 15.8$ Hz, H-1'), 6.80 (2H, d, $J = 8.6$ Hz, H-3,5), 6.23 (1H, d, $J = 15.8$ Hz, H-2'); $^{13}\text{C-NMR}$ (MeOH, 100 MHz) δ : 170.6 (C-3'), 158.7 (C-4), 138.2 (C-2'), 129.4 (C-2,6), 126.8 (C-1), 115.5 (C-3,5), 114.6 (C-1'); HREIMS m/z : 164.04754 (calcd. for $\text{C}_9\text{H}_8\text{O}_3$, 164.047345).

Methyl gallate (6)

Crystalline solid (20 mg); m.p. 157-158°C; UV (MeOH) $\lambda_{\max}$ (log ϵ) nm: 278 (3.78), 283 (4.10); IR (KBr) $\nu_{\max}$ cm^{-1} : 3375, 1698, 1619; $^1\text{H-NMR}$ (MeOH, 500 MHz) δ : 7.03 (2H, s, H-2,5), 3.80 (3H, s, OCH_3); $^{13}\text{C-NMR}$ (MeOH, 125 MHz) δ : 169.0 (C-7), 146.5 (C-3,5), 139.7 (C-4), 121.5 (C-1), 110.0 (C-2,6), 56.3 (OCH_3); HREIMS m/z : 184.0345 (calcd. for $\text{C}_8\text{H}_8\text{O}_5$, 184.0371).

3,4-Dihydroxybenzoic acid (7)

Crystalline solid (15 mg); m.p. 198-200°C; UV (MeOH) $\lambda_{\max}$ (log ϵ) nm: 289 (4.5), 258 (4.7), 215 (4.9); IR (KBr) $\nu_{\max}$ cm^{-1} : 3505, 3235-2857, 1710; $^1\text{H-NMR}$ (MeOH, 500 MHz) δ : 7.42 (1H, dd, $J = 8.5$, 1.5 Hz, H-6), 7.41 (1H, d, $J = 1.5$ Hz, H-2), 6.78 (1H, d, $J = 8.5$ Hz, H-5); $^{13}\text{C-NMR}$ (MeOH, 125 MHz) δ : 169.4 (C-7), 151.1 (C-4), 145.2 (C-3), 124.2 (C-1), 123.8 (C-6), 117.7 (C-2), 115.7 (C-5); HREIMS m/z : 154.0254 (calcd. for $\text{C}_7\text{H}_6\text{O}_4$, 154.0266).

4',5,7-Trihydroxyflavone (8)

Yellow needles (35 mg); m.p. 352°C; UV (MeOH) $\lambda_{\max}$ (log ϵ) nm: 269 (4.2), 340 (4.32); IR (KBr) $\nu_{\max}$ cm^{-1} : 3454, 1650, 1590; $^1\text{H-NMR}$ (MeOH, 500 MHz) δ : 7.85 (2H, d, $J = 8.5$ Hz, H-3',5'), 6.93 (2H, d, $J = 8.5$ Hz, H-2',6'), 6.58 (1H, s, H-3), 6.45 (1H, d, $J = 2.0$ Hz, H-8), 6.20 (1H, d, $J = 2.0$ Hz, H-6); $^{13}\text{C-NMR}$ (MeOH, 125 MHz) δ : 183.9 (C-4), 166.3 (C-2), 166.1 (C-7), 163.2 (C-9), 162.1 (C-4'), 159.4 (C-5), 129.4 (C-3',5'), 123.2 (C-1'), 117.0 (C-2',6'), 105.3 (C-10), 103.8 (C-3), 100.1 (C-6), 95.0 (C-8); HREIMS m/z : 270.0512 (calcd. for $\text{C}_{15}\text{H}_{10}\text{O}_6$, 270.0528).

3',4',5,7-Tetrahydroxyflavone (9)

Yellow needles (18 mg); m.p. 325°C; UV (MeOH) $\lambda_{\max}$ (log ϵ) nm: 253 (4.5), 265 (4.6), 347 (3.7); IR (KBr) $\nu_{\max}$ cm^{-1} : 3455, 1650, 1590; $^1\text{H-NMR}$

(MeOH, 500 MHz) δ : 7.39 (1H, dd, $J = 7.5$, 2.5 Hz, H-6'), 7.37 (1H, br s, H-2'), 6.90 (1H, d, $J = 8.5$ Hz, H-5'), 6.53 (1H, s, H-3), 6.43 (1H, d, $J = 2.5$ Hz, H-8), 6.20 (1H, d, $J = 2.0$ Hz, H-6); $^{13}\text{C-NMR}$ (MeOH, 125 MHz) δ : 183.8 (C-4), 166.3 (C-2), 166.1 (C-7), 163.2 (C-9), 159.4 (C-5), 151.0 (C-4'), 147.0 (C-3'), 123.7 (C-1'), 120.3 (C-6'), 116.8 (C-5'), 114.2 (C-2'), 105.3 (C-10), 103.9 (C-3), 100.1 (C-6), 95.0 (C-8); HREIMS m/z : 286.0435 (calcd. for $\text{C}_{15}\text{H}_{10}\text{O}_6$, 286.0477).

3,3',5,7-Tetrahydroxy-4'-methoxyflavone (10)

Yellow prism (22 mg); m.p. 259°C; UV (MeOH) $\lambda_{\max}$ (log ϵ) nm: 259 (4.5), 375 (3.63); IR (KBr) $\nu_{\max}$ cm^{-1} : 3450, 1655, 1595; $^1\text{H-NMR}$ (MeOH, 400 MHz) δ : 7.99 (1H, d, $J = 1.6$ Hz, H-2'), 7.63 (1H, dd, $J = 8.4$, 1.6 Hz, H-6'), 6.91 (1H, d, $J = 8.4$ Hz, H-5'), 6.40 (1H, br s, H-8), 6.16 (1H, br s, H-6), 3.93 (3H, s, OCH_3); $^{13}\text{C-NMR}$ (MeOH, 100 MHz) δ : 178.7 (C-4), 166.5 (C-7), 162.8 (C-5), 159.5 (C-2), 158.6 (C-9), 151.5 (C-3'), 148.7 (C-4'), 133.6 (C-3), 123.9 (C-6'), 122.4 (C-1'), 116.2 (C-5'), 114.3 (C-2'), 105.6 (C-10), 100.5 (C-6), 94.9 (C-8), 56.8 (OCH_3); HREIMS m/z : 316.0583 (calcd. for $\text{C}_{16}\text{H}_{12}\text{O}_7$, 316.0583).

Apigenin 7-O-(4'-O-p-E-coumaroyl- β -D-glucopyranoside (11)

White amorphous powder (15 mg); $[\alpha]_{\text{D}}^{25}$ -98.0 (c 0.08, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) nm: 268 (4.5), 320 (4.6); IR (KBr) $\nu_{\max}$ cm^{-1} : 3286, 1657, 1608, 1513, 1245, 1150, 1082, 1042, 831; $^1\text{H-NMR}$ (MeOH, 500 MHz) δ : 7.89 (2H, d, $J = 8.5$ Hz, H-2',3'), 7.71 (1H, d, $J = 16$ Hz, H-3'''), 7.49 (2H, d, $J = 8.5$ Hz, H-5'',9'''), 6.94 (2H, d, $J = 9.0$ Hz, H-3',5'), 6.84 (1H, d, $J = 2.0$ Hz, H-8), 6.82 (2H, d, $J = 9.0$ Hz, H-6'',8'''), 6.66 (1H, s, H-3), 6.53 (1H, d, $J = 2.0$ Hz, H-6), 6.41 (1H, d, $J = 15.5$ Hz, H-2'''), 5.16 (1H, d, $J = 8$ Hz, H-1''), 4.96 (1H, t, $J = 9.5$ Hz, H-2''), 3.81 (1H, m, H-5''), 3.78 (1H, m, H-3''), 3.68-3.61 (2H, m, H-6''); $^{13}\text{C-NMR}$ (MeOH, 125 MHz) δ : 184.1 (C-4), 168.5 (C-1'''), 166.8 (C-2), 164.7 (C-7), 163.0 (C-4'), 163.0 (C-5), 161.5 (C-7'''), 159.0 (C-9), 147.4 (C-3'''), 131.3 (C-5''',9'''), 129.7 (C-2',6'), 127.1 (C-4'''), 123.1 (C-1'), 117.1 (C-3',5'), 116.9 (C-6'',8'''), 114.7 (C-2'''), 104.2 (C-3), 101.5 (C-6), 101.2 (C-1''), 96.1 (C-8), 76.5 (C-5''), 75.6 (C-3''), 74.9 (C-2''), 72.1 (C-4''), 62.2 (C-6''); HREIMS m/z : 579.1499 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{30}\text{H}_{27}\text{O}_{12}$, 579.1505).

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of pure compounds (1-11) were examined by

comparison with that of known antioxidant, quercetin using the method of Lee and Shibamoto [25]. Briefly, various amounts of the compounds (500 µg/mL, 250 µg/mL, 125 µg/mL, 60 µg/mL, 30 µg/mL, 15 µg/mL) were mixed with 3 ml of methanolic solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for one hour. Then absorbance was measured at 517 nm against methanol as a blank in the spectrophotometer. Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Each sample was assayed in triplicate and mean values were calculated.

Acetylcholinesterase Assay

The Acetylcholinesterase (AChE) inhibition activity was performed according to the method used by Ellman [26] with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL 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 30 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 method used by Ellman [27] with slight modifications. 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 mins 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 30 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 with 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 (LOX) activity was assayed according to the reported method [28] but with slight modifications. A total volume of 200 µL assay mixture contained 140 µL sodium phosphate buffer (100 mM, pH 8.0), 20 µL test compound and 15µL(600U) purified lipoxygenase enzyme (Sigma, USA). The contents were mixed and pre-read at 234 nm and pre incubated for 10 minutes 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. Baicalein (0.5 mM well⁻¹) was used as a positive control. The percentage inhibition was calculated by formula given below.

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

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