Three New Flavonoid Glycosides from the Fruits of *Luffa echinata* Roxb.- a Hepatoprotective Plant

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ABSTRACT: Phytochemical investigation of bioactive polar extracts of *Luffa echinata* Roxb. fruits led to the isolation and characterization of three new flavone glycosides *viz.,* 3,5,7,3′,4′-pentahydroxyflavone-3-[-O-β-D-glucopyranosyl-7-O-β-D-glucopyranoside (**1**),3,5,7-trihydroxy-(7′,8′- dioxo,9′,10′,-dihydro[a]cyclohex $\Delta^{13'(14')}$, $\Delta^{11'(12')}$-12′,13′,-dimethyl)-flavone-7-[-O-β-D-galactopyranosyl-(1→2)]-O-β-D-glucopyranoside (**2**), and 5,7,8,4′-tetrahydroxy flavone-7-[-O-β-D-xylosyl-(1→2)]-O-β-D-glucopyranoside (**3**). The structures of the newly isolated compounds were elucidated on the basis of spectral data and chemical evidences.The isolated compounds are found to be new analogs of tricetin, quercetin and isoscutellarin.

Key words: *Luffa echinata*, Cucurbitaceae, Hepatoprotective, Isoscutellarin, Tricetin, Quercetin

INTRODUCTION

Luffa echinata Roxb. is a spreading climber herb, with bifid bristly or smooth tendrils and extremely bitter taste. It grows in several Asian countries such as Pakistan, India,Bangladesh and in Northern Tropical Africa. In India, it is mainly found in Gujarat, Bihar, Rajasthan and Madhya Pradesh. It is known by various names *viz*., English Bristly Luffa; Sanskrit: Koshataki and in Hindi: Bindaal, Bidali, Kukurlata and Ghagerbel. Traditional healers use infusion of the fruit orally to treat biliary and intestinal colic while recommend local application to the body in case of putrid fever. Roots of the plant are also used as traditional medicine for the treatment of bronchitis, piles, jaundice, vaginal discharge, laxative and analgesic. Apart from that, entire herb is used as stomachic, emetic, antihelmintic, nephritis, chronic bronchitis and abortifacient.[1-3] Scientific studies done elsewhere have indicated that aqueous extract of fruits is beneficial in jaundice as it significantly lowers serum bilirubin level in chlorpromazine induced jaundice in rats and human patients.[4] Clinical studies revealed that fruits have significant therapeutic action against viral hepatitis.[5] Practitioners of the indigenous system of medicine, affirm to obtain beneficial results with the fruits of their plant in the treatment of liver ailments.[6] Liver protective plants like *L.echinata*, reported to contain a variety of phytochemicals like phenols, flavonoids, coumarins, monoterpenes, glycosides, alkaloids and xanthenes.[7]

We have previously reported occurrence of two new lanostane type triterpenes*viz*., lanost-6(7),23(24)-diene-3,11,22-trione-20,25-diacetoxy-2-*β*-16-*α*-diol and lanost1(2),6(7),23(24)-triene-3,11,22-trione-20,25-diacetoxy-2,16-*α*-diol from the acetone extract of *L. echinata* Roxb. fruits.[8]

In the present study, the two active polar hepatoprotective extracts (methanol and acetone) of

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L. echinata Roxb. fruits were further subjected to phytochemical investigation which resulted in isolation of three new flavonoid glycosides namely, 3,5,7,3′,4′-pentahydroxyflavone-3-[-O-β-D-glucopyranosyl-7-O-β-D-glucopyranoside(**1**),3,5,7-trihy-droxy-(7′8′-dioxo, 9′,10′-dihydro[a]cyclohex $\Delta^{13'(14')}$, $\Delta^{11'(12')}$-12′13′-dimethyl)-flavone-7-[-O-β-D-galacto-pyranosyl-(1→2)]-O-β-D-glucopyranoside (**2**) and 5,7,8,4′-tetrahydroxyflavone-7-[-O-β-D-xylosyl-(1→2)]-O-β-D-glucopyranoside (**3**).

MATERIAL AND METHODS

General. Melting points were determined by open capillary method and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 MHz (Bruker model DRX-300) NMR spectrometer in $CDCl_3$ using TMS as internal reference. Chemical shifts are expressed in δ (ppm) and the coupling constants (J) are given in Hz. The mass spectra were recorded on Jeol-JMS DX-303 spectrometer and IR spectra on Hitachi IR 270-30 spectrometer in KBr pellets. Column chromatography was carried out using silica gel (60-120 mesh) and TLC was performed on silica gel G. Visualization of the TLC plates was done under UV light at 254 and 366 nm.

Plant material. The fruits of *L. echinata* Roxb. were procured from HerbaIndica, Chandigarh, India and were identified by the taxonomist of Dept. of Botany, Jamia Hamdard, New Delhi, where a voucher specimen has been kept for future reference.

Extraction and isolation. The air dried plant material (6.0 kg) was crushed to a coarse powder and was exhaustively extracted with ethanol by cold percolation method. The crude alcoholic extract was concentrated to small volume under reduced pressure to obtain a viscous mass (1.0 kg). It was then fractionated into petroleum ether (60-80°C), acetone and methanol to give 150 g, 400 g and 100 g of extracts, respectively. The concentrated acetone extract (400 g) was chromatographed over silica gel column prepared in petroleum ether (60-80°C). The column was eluted successively with petroleum ether and mixture of petroleum ether, chloroform and methanol in increasing order of polarities. The eluentchloro form-MeOH (4:1) afforded compound **1** (200 mg), chloroform-MeOH (3 : 2) afforded Compound **2** (500 mg) and the concentrated methanol extract (100 g) eluted with chloroform-MeOH (9:1) afforded compound **3** (250 mg).

3,5,7,3′,4′-pentahydroxyflavone-3-[-O-β-D-glucopyranosyl-7-O-β-D-glucopyranoside(1): Yellow solid, yield: 200 mg, R_f = 0.803 (1:1, $CHCl_3$-MeOH), mp. 180°C; IR υ_{max} (KBr): 3440-3500 (OH), 2950, 2850 (CH_3, CH_2), 1680 (C=O, α,β-unsaturated ketone), 1640 (C=C), 1460, 1060 (C-O alcoholic) 980, 960, 840 and 800 cm^{-1}; UV: (Table 1a); EIMS (rel. int.) (Aglycone) *m/z*: 302 [$M^{+\cdot}$, $C_{15}H_{10}O_7$, (90%)], 285 [M^+- OH (4.0)], 274 [M^+- CO(11)], 152 [M^+ - $C_7H_4O_4$, (12)], 152 [M^+ - $C_8H_8O_3$, (12)] 124 [152 - CO (20)], 134 [$C_8H_8O_3$-$2H_2O$ (15)], 103 [134-CO].

3,5,7-trihydroxy-(7′8′-dioxo,9′,10′-dihydro-[a]cyclohex$\Delta^{13'(14')}$,$\Delta^{11'(12')}$-12′13′-dimethyl)-flavone-7-[-O-β-D-galactopyranosyl-(1→2)]-O-β-D-glucopyranoside (2): Yellow solid, yield: 500 mg, R_f= 0.678 (1:1, $CHCl_3$-MeOH), mp. 140°C; IR υ_{max} (KBr): 3450-3500 (OH), 2950, 2850 (CH_3, CH_2), 1680 (C=O, α,β-unsaturated ketone), 1620 (C=C), 1500, 1340, 1080 (-O-), 1020 (C-O alcoholic), 820 and 800 cm^{-1}; IR υ_{max} (KBr) (acetate): 2950, 2850 (CH_3, CH_2), 1750 (C=O), 1680 (C=O, α, β-unsaturated ketone), 1620 (C=C), 1260-1240 (C-O, ester), 1020 (C-O alcoholic), 800 cm^{-1}; UV: (Table 2a); EIMS (rel. int.) (Aglycone) *m/z*: 407 [$M^{+\cdot}$, $C_{23}H_{19}O_7$, (5)], 392 [M^+- CH_3 (10)], 389 [M^+- H_2O (28)], 379 [M^+- CO (25)], 326 [M^+ - C_6H_9 (25)], 300 [M^+ - C_8H_{11} (40)], 268 [M^+ - 139 $C_6H_{11}O_2$ (35)], 273 [300 - CO (28)], 255 [M^+ - $C_7H_4O_4$ (30)], 149 [$C_{16}H_{15}O_3$ - C_8H_{10} (5)], 135 [$C_7H_4O_4$-H_2O (10)], 129 [149 - H_2O (20)], 124 [$C_7H_4O_4$ - CO (20)].

5,7,8,4’-tetrahydroxyflavones-7-[-O-β-D-xylosyl-(1→2)]-O-β-D-glucopyranoside (3): Yellow solid, 100 mg, R_f = 0.386 (8:2, C_6H_6-EtOAc), mp. 150°C; IR υ_{max} (KBr): 3450-3500 (OH), 2950, 2850 (CH_3, CH_2), 1670 (C=O, α,β-unsaturated ketone), 1610 (C=C), 1450, 1390, 1260, 1030 (C-O alcoholic) and 820 cm^{-1}; UV: (Table 3a); EIMS (rel. int.) (Aglycone) *m/z*: 286 [$M^{+\cdot}$, $C_{15}H_{10}O_6$, (2.5)], 258 [M^+

- CO (2.0)], 269 [M^+ - OH (2.3)], 168 [M^+ - 118 (15.5), fission via 1(2) - 3(4)], 140 [168 - CO (13.6)], 147 [M^+ - 139, fission via 1(2) - 4(5) (7.9)], 150 [168 - H_2O (37)], 132 [150 - H_2O (21.6)], 122 [150 - CO (4.4)], 94 [M^+ - 192, fission via 1(2) - 3(4) (67)], 77 [94-OH (85)], 44 [77-23 (100)].

RESULTS AND DISCUSSION

All the three compounds **1**, **2** and **3** were obtained as yellowish brown powder and gave positive Shinoda test for flavones and Molisch's test for carbohydrates, indicating them to be flavonoid glycosides.[9] The IR spectra of all three compounds clearly showed absorption bands in the range of 3450-3500 cm^{-1} due to hydroxyl group, 1670-1690 cm^{-1} due to α,β-unsaturated ketone, 1610 cm^{-1} due to double bond and 1030 cm^{-1} due to C-O alcoholic groups. The UV spectrum of compound **1** in MeOH/NaOMe exhibited a bathochromic shift of 64 nm in band I, indicating a free 4'-hydroxyl group, $AlCl_3$/HCl displayed a bathochromic shift of 47 nm, indicating a free 5-hydroxyl group, MeOH/NaOAc did not show significant bathochromic shift in band II, due to the absence of 7-hydroxyl group and NaOAc/H_3BO_3 spectrum revealed 57 nm bathochromic shift due to free 3'- and 4'- hydroxyl groups.[10]

The flavone glycoside **1** on hydrolysis with dilute HCl afforded an aglycone which exhibited a 58 nmbathochromic shift of band I in UV spectrum in the presence of $AlCl_3$/HCl, indicating free 3- and 5-hydroxyl groups which clearly confirmed that one sugar was linked with the aglycone at position-3. The MeOH/NaOAc spectrum of aglycone also displayed a bathochromic shift of 7 nm in band II indicating a free 7-hydroxyl group in the aglycone confirming that another sugar was linked at 7 position (Table 1a).

The ^{1}H NMR spectrum of **1** exhibited five signals at δ 6.46 (1H, *d*, *J*= 1.8 Hz, H-6), 6.87 (1H, *d*, *J*= 1.8Hz, H-8), 7.6 (1H, *d*, *J*= 2.5Hz, H-2'), 6.99 (1H, *d*, *J*= 2.5Hz, H-5') and 7.59 (1H, *dd*, *J*= 2.5, 8.5Hz, H-6'). The peaks at δ 12.96 were observed due to ahydroxyl group at position-5, which is downfield due to hydrogen bonding with C-4 carbonyl function. The ^{1}H NMR spectrum also showed signals for 14 sugar protons in the range of δ 3.71-5.43 which suggested the presence of two sugar units in the molecule (Table 1b). It was also confirmed on hydrolysis of glycoside which afforded only 40% aglycone, indicating two sugar units in the molecule.[11] The proposed structure was further supported by its ^{13}C NMR spectrum that exhibited 27 carbon signals corresponding to one aglycone moiety (15 carbon atoms) and two sugar units (12 carbon atoms) (Table 1b).

The sugars were identified as glucose by co-paper chromatography with authentic sample of glucose. The glucose moiety was linked with the aglycone through a β-linkage as evident by a doublet (1H, *J*=6.0 Hz) of the signal at δ 5.35 of the anomeric proton of glucose. The other glucose moiety was also linked through a β-linkage with the glucose moiety as evidenced by doublet (*J*= 6.1Hz) of the signal at δ5.43 of the anomeric proton of the second glucose.

The mass spectrum of the aglycone exhibited molecular ion peak at *m/z* 302 corresponding to a molecular formula as $C_{15}H_{10}O_7$. The sharp peak at 152 due to fragment "A" and 153 due to fragment "B", supported the proposed structure of the aglycone. Other peaks in the mass spectrum at *m/z* 284, 273, 136 and 124 were also structurally indicative of the flavone nucleus. Thus, on the basis of above chemical and spectral studies, the structure of aglycone was established as 3,5,7,3',4'-pentahydroxyflavone (quercetin) and the structure of the glycoside was deduced as 3,5,7,3',4'-pentahydroxyflavone-3-[-O-β-D-glucopyranoside-7-O-D-glucopyranoside or quercetin-3,7-O-β-D-diglycoside.[12,13]

The UV spectrum of compound **2** in MeOH/NaOMe did not exhibit a significant bathochromic shift in band I, indicating the lack of free 4'-hydroxyl group, $AlCl_3$/HCl exhibited a bathochromic shift of 55 nm, indicating free 3,5-hydroxyl groups, MeOH/NaOAc did not reveal any significant bathochromic shift in band II, due to the absence of free 7-hydroxyl group and NaOAc/H_3BO_3 spectrum did not show bathochromic shift in band I,

indicating the absence of 3'- and 4'- hydroxyl groups.[10]

The glycoside on hydrolysis with dilute HCl afforded an aglycone which exhibited bathochromic shift of 15 nm in band II in the UV spectrum, which clearly indicated that the sugar was linked with 7-hydroxyl group in the glycoside (Table 2a).

The ^{1}H NMR spectrum exhibited nine signals at δ 6.44 (1H, *d*, *J*= 1.8Hz, H-6), 6.82 (1H, *d*, *J*= 2.1Hz, H-8), 7.97 (1H, *d*, *J*= 8.7Hz, H-2'), 6.99 (1H, *d*, *J*= 7.2Hz, H-5'), 7.62 (1H, *dd*, *J*= 1.8, 7.3Hz, H-6'), 5.35 (1H, *dd*, *J*= 5.1,11.7Hz, H-9'), 5.33 (1H, *dd*, *J*= 5.1, 11.7Hz, H-10'), 6.93 (1H, *dd*, *J*= 2.7, 11.1Hz, H-11') and 6.96 (1H, *dd*, *J*=2.7, 11.1Hz, H-14'). The peak at δ 12.97 was due to the hydroxyl group at position-5, which is downfield due to hydrogen bonding with C-4 carbonyl function.

The ^{1}H NMR spectrum also gave signals for sugar protons in the range of δ 3.70-5.18 for 14 protons indicating the presence of two sugar units in the molecule (Table 2b). It was also confirmed on hydrolysis of the glycoside which afforded only 40% aglycone, indicating two sugar units in the molecule.[11] The sugars were identified as glucose and galactose by co-paper chromatography with authentic samples of glucose and galactose. The partial hydrolysis of the glycoside indicated that terminal sugar was galactose and glucose moiety was linked with the aglycone moiety through a β-linkage as evidenced by a doublet (*J*= 6.3 Hz) of the signal at δ 5.18 due to the anomeric proton of glucose. The galactose moiety was also linked through a β-linkage with the glucose moiety as evident by doublet (*J*=5.1 Hz) of the signal at δ 5.19 of the anomeric proton of galactose. The linkage of galactose to glucose was found to be (1→2) as the^{1}H NMR spectrum of its acetate which did not exhibit the signal at δ 1.72 due to acetoxyl group at position-2 of the glucose moiety indicating that 2"-hydroxyl group was linked with the galactose moiety.[12]

The mass spectrum of the aglyconedisplayed molecular ion peak at *m/z* 407 corresponding to the molecular formula as $C_{23}H_{19}O_7$. The sharp peaks at *m/z*152 and 150 due to fragment "A" and 255 due to fragment "B", which supported the proposed structure of the aglycone. Other peaks in mass spectrum at *m/z* 326, 300, 268 and 379 were also supportive to the structure.

The above chemical and spectral studies led to the structure of the aglycone as 3,5,7-trihydroxy (7',8'-dioxo 9',10'-dihydro(a) cycloh-ex$\Delta^{11'(12')}$, $\Delta^{13'(14')}$ diene-12',13'-dimethyl flavone (tricetin) and the structure of glycoside was found as 3,5,7-trihydroxy (7', 8'-dioxo9', 10' dihydro(a)-cyclohex $\Delta^{11'(12')}$, $\Delta^{13'(14')}$ diene-12',13'- dimethyl-flavone-7-[-O-β-D-galactopyranosyl-(1→2)-O-D-glucopyrano-side and which appears to be a new compound.

The UV spectrum of compound **3** in MeOH/NaOMe demonstrated a bathochromic shift of 59 nm in band I, indicating a free 4'-hydroxyl group, $AlCl_3$/HCl revealed a bathochromic shift of 44 nm, suggesting a free 5-hydroxyl group, MeOH/NaOAc did not exhibit bathochromic shift in band II, revealing the absence of free 7-hydroxyl group and NaOAc/H_3BO_3 spectrum displayed no bathochromic shift, indicating the absence of orthodihydroxy system in ring-B, i.e. 3'- and 4'-hydroxyl groups.[14]

The glycoside on hydrolysis with dilute HCl afforded an aglycone which exhibited a 18 nm hypsochromic shift in band I in the UV spectrum in presence of $AlCl_3$/HCl with respect to $AlCl_3$ indicating an orthodihydroxyl system in either ring A or B. The presence of orthodihydroxylgroup in ring B was ruled out by another spectrum in NaOAc/H_3BO_3 as stated above. Therefore, it must be present in ring A. Further, it was confirmed that the sugar was linked at position-7 as a bathochromic shift of 10 nm was observed in band II in MeOH/NaOAc spectrum (Table 3a).

The ^{1}H NMR spectrum of **3** showed six signals of one proton each at δ 6.42(*s*, H-3), 6.85(*s*, H-6), 7.94 (*d*, *J*= 8.7 Hz, H-2'), 6.96(*d*, *J*= 8.4 Hz, H-3'), 6.99 (*d*, *J*= 6.9 Hz, H-5') and 7.61(*d*, *J*= 7.5 Hz, H-6'). The peak at δ 12.98 due to a hydroxyl group at position-5 was downfield due to hydrogen bonding with C-4 carbonyl function. The ^{1}H NMR spectrum also gave sugar protons in the range of δ 3.64-5.35 for 13 protons indicating the presence of two sugar

units in the molecule (Table 3b). It was also confirmed on hydrolysis of glycoside which afforded only 40% aglycone, suggesting two sugar units in the molecule.[11]

Table 1a. UV absorption values of compound 1.

UV λ_{max} (Glycoside)	UV λ_{max} (Aglycone)
MeOH: 250, 271, 343	MeOH: 258, 270, 305, 372
MeOH/NaOMe: 257, 407	MeOH/NaOMe: 250, 278, 338, 414
$AlCl_3$: 260, 345 lb, 418 la	$AlCl_3$: 270, 307, 335, 418
$AlCl_3$/HCl: 274, 355 1b, 390 1a	$AlCl_3$/HCl: 265, 305, 360, 430
NaOAc: 268, 347, 415	NaOAc: 268, 347, 415
NaOAc/H_3BO_3: 269, 347, 400	NaOAc / H_3BO_3: 269, 347, 400

Table 1b. ^{1}H and ^{13}C- NMR data of compound 1.

Position	Compound **1**		Aglycone of compound **1**	
	^{1}H NMR*	^{13}C NMR	^{1}H NMR*	^{13}C NMR
1	-	-	-	-
2	-	156.5	-	156.5
3	-	133.7	-	133.7
4	-	177.6	-	177.6
5	-	161.3	-	161.3
6	6.46, *d* (1.8)	99.1	6.46, *d* (1.8)	99.1
7	-	165.3	-	165.3
8	6.87, *d* (1.8)	95.8	6.87, *d* (1.8)	95.8
9	-	157.8	-	157.8
10	-	104.2	-	104.2
1’	-	122.4	-	122.4
2’	7.61, *d* (2.5)	116.9	7.61, *d* (2.5)	116.9
3’	-	144.7	-	144.7
4’	-	148.5	-	148.5
5’	6.99, *d* (8.5)	116.5	6.99, *d* (8.5)	116.5
6’	7.59, *dd* (2.5,8.5)	121.6	7.59, *dd* (2.5, 8.5)	121.6
-OH at C-5	12.96, *s*	-	12.96, *s*	-
	Sugar protons			
1″	5.35, *d*(6.0)	101.4		
1‴	5.43, *d* (6. 1)	101.5		
2″, 2‴	4.65, *brs*(2H)	74.3,74.5		
3″, 3‴	5.08, *m* (2H)	77.5,77.7		
4″, 4‴	5.05, *d* (2H,7.2)	70.3,70.2		
5″, 5‴	3.89, *m* (2H)	76.8,76.5		
6″a, 6‴a	3.74, *dd,* (2H, 6.1, 9.2)	61.3,61.2		
6″b, 6‴b	3.71, *dd* (2H, 6.1, 9.2)	-		

* Coupling constants in Hz are given in parentheses.

Table 2a. UV absorption data of compound 2.

UV λ_{max} (Glycoside)	UV λ_{max} (Aglycone)
MeOH: 230, 250, 350	MeOH: 230, 255, 355
MeOH/NaOMe: 235, 255, 365	MeOH/NaOMe: 231, 255, 418
$AlCl_3$: 230, 240, 410	$AlCl_3$: 230, 245, 415
$AlCl_3$/HCl: 235, 241, 405	$AlCl_3$/HCl: 234, 245, 405
NaOAc: 231, 250, 418	NaOAc: 245, 250, 418
NaOAc/H_3BO_3: 230, 255, 365	NaOAc / H_3BO_3: 245, 255, 366

Table 2b. ^{1}H and ^{13}C-NMR data of compound 2.

Position	Compound **2** ^{1}H NMR*	Acetate ^{1}H NMR*	Aglycone ^{1}H NMR*	Acetate derivative ^{1}H NMR*
6	6.44, *d* (1.8)	6.66, *d* (1.8)	6.44, *d* (1.8)	6.66, *d* (l.8)
8	6.82, *d* (2.1)	6.59, *d* (2.1)	6.82, *d* (2.1)	6.59, *d* (2.1)
2’	7.97, *d* (8.7)	7.90, *d* (8.7)	7.97, *d* (8.7)	7.90, *d* (8.7)
5’	6.99, *d* (7.2)	7.40, *d* (7.2)	6.99, *d* (7.2)	7.40, *d* (7.2)
6’	7.62, *dd* (1.8, 7.3)	7.49, *dd* (1.8, 7.3)	7.62, *dd* (1.8, 7.3)	7.49, *dd* (1.8, 7,3)
9’	5.35, *dd* (5.1, 1 1.7)	5.47, *dd* (5.1, 1 1.7)	5.35, *dd* (5.1, 11.7)	5.47,*dd* (5.1, 11.7)
10’	5.33, *dd* (5.1, 1 1.7)	5.33, *dd* (5.1, 1 1.7)	5.33, *dd* (5.1, 11.7)	5.33, *dd* (5.l, 11.7)
11’	6.93, *dd* (2.7, 11.1)	6.74, *dd* (2.7, 11.1)	6.93, *dd*(2.7,11.1)	6.74, *dd* (2.7,l1.l)
14’	6.96, *dd* (2.7, 11.1)	7.19, *dd* (2.7, 11.1)	6.96, *dd* (2.7, 1l.l)	7.19, *dd* (2.7, l1.1)
Me at C-12	2.50, *s*, Me	2.44, *s*, Me	2.50, *s*, Me	2.44, *s*, Me
Me at C-13	2.49, *s*, Me	2.35, *s*, Me	2.50, *s*, Me	2.35, *s*, Me
OH	12.97, *brs* (5OH)	2.15, *s* (-O*Ac*)	12.97, *brs* (5 OH)	2.15, *s*(-O*Ac*)
		2.17, *s* (-O*Ac*)		2.16, *s*(-O*Ac*)
				2.17, *s*(-O*Ac*)
	Sugar protons			
1″	5.18, *d* (6.3)	5.30, *d* (6.3)		
2″	4,66, *d* (5.4)	4.49, *d* (5.4)		
3″	5.07, *d* (5.1)	5.17, *d* (5.1)		
4″	4.69, *d* (3.9)	5.10, *d* (3.9)		
5″	3.95, *m* (w½=6.0)	3.99, *m* (w½=6.0)		
6″a	3.72, *dd* (7.5, 5.4)	4.25, *dd* (7.5, 5.4)		
6″b	3.68, *dd* (9.3, 5.4)	4.16, *dd* (9.3, 5.4)		
1‴	5.19, *d* (5.1)	5.27, *d* (5.1)		
2‴	4.53, *d* (2.0)	4.92, *d* (2.0)		
3‴	5.09, *d* (5.7)	5.17, *d* (5.7)		
4‴	4.66, *d* (3.9)	5.07, *d* (3.9)		
5‴	3.89, *m* (w½=6.0)	3.94, *m* (w½=6.0)		
6‴a	3.74, *dd* (7.5, 5.4)	4.28, *dd* (7.5, 5.4)		
6‴b	3.70, *dd* (9.3, 5.4)	4.19, *dd* (9.3, 5.4)		
		Sugar acetoxyls		
		2.05, *s* (3 x O*Ac*)		
		2.05, *s* (2 x O*Ac*)		
		2. 10, *s* (1 x O*Ac*)		
		2.13, *s* (1 x O*Ac*)		

*Coupling constants in Hz are given in parentheses.

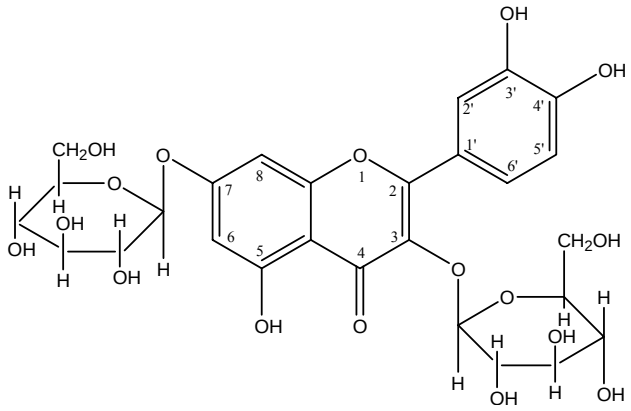
Table 3a. UV absorption data of compound 3.

UV λ_{max} (Glycoside)	UV λ_{max} (Aglycone)
MeOH: 235, 273 (sh), 295, 368	MeOH: 235, 285, 430
MeOH/NaOMe: 235, 285, 427	MeOH/NaOMe: 235, 285, 427
$AlCl_3$: 232, 300(sh), 323, 385, 420	$AlCl_3$: 232, 300 (sh), 385, 430
$AlCl_3$/HCl: 235, 295 (sh), 322, 373, 412	$AlCl_3$/HCl: 235, 295 (sh), 322, 373, 412
NaOAc: 238, 288 (sh), 378, 430	NaOAc: 245, 288 (sh), 378, 430
NaOAc/H_3BO_3: 248, 280 (sh), 295, 375	NaOAc / H_3BO_3: 248, 280 (sh), 295, 385

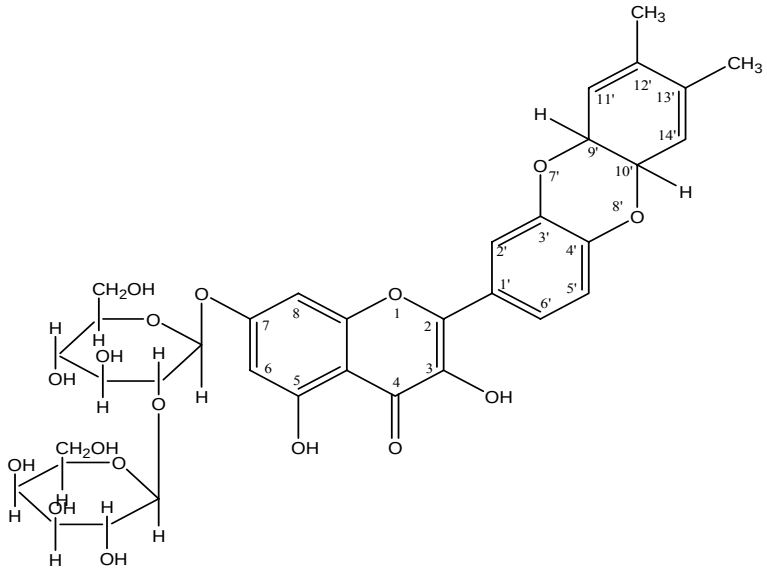
Table 3b. ^{1}Hand ^{13}C- NMR data of compound 3.

Position	Compound **3**		Aglycone	
	^{1}H NMR*	^{13}C NMR	^{1}H NMR*	^{13}C NMR
1	-	-	-	-
2	-	163.5	-	163.5
3	6.42, *s*	99.2	6.42, *s*	99.2
4	-	183.0	-	183.0
5	-	161.4	-	161.4
6	6.85, *s*	95.9	6.85, *s*	95.9
7	-	165.5	-	165.5
8	-	151.2	6.98, *d* (2.5)	151.2
9	-	157.9	-	157.9
10	-	100.5	-	100.5
1’	-	122.5	-	122.5
2’	7.94, *d* (8.7)	117.0	7.94, *d* (8.7)	117.0
3’	6.96, *d* (8.4)	110.5	6.96, *d* (8.4)	110.5
4’	-	148.8	-	148.8
5’	6.99, *d* (6.9)	110.1	6.99, *d* (6.9)	110.1
6’	7.61, *d* (7.5)	116.7	7.61, *d* (7.5)	116.7
OH at C-5	12.98, *s*		12.98, *s*	
	Sugar protons			
1″	5.35, *m* (w½ =6.0)	104.2		
2″	4.69, *m*	70.4		
3″	5.20, *d* (5.7)	74.7		
4″	5.09, *d* (5.7)	64.5		
5″	3.93, *m* (w½=6.0)	77.5		
6″a	3.75, *dd* (7.2, 6.9)	56.8		
6″b	3.68, *dd* (7.2, 6.9)	-		
1‴	5.33, *d* (5.1)	106.4		
2‴	4.68,*m*	80.3		
3‴	5.15, *d* (7.3)	77.1		
4‴	5.07, *d* (5.7)	64.6		
5‴a	3.74, *dd* (7.2, 6.9)	61.5		
5‴b	3.64, *dd* (7.2, 6.9)	-		

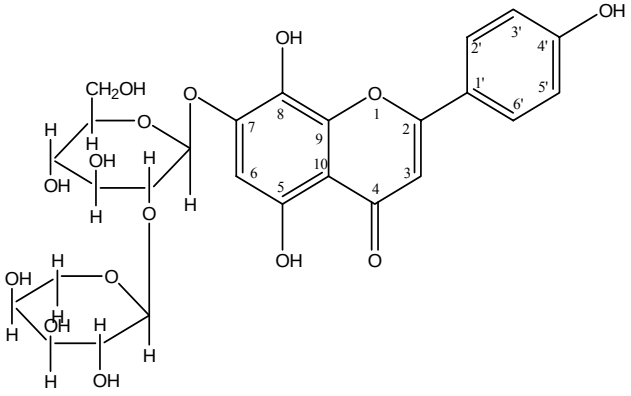
*Coupling constants in Hz are given in parentheses.



1



2



3

The sugars were identified as glucose and xylose by co-paper chromatography with authentic samples of glucose and xylose. The partial hydrolysis of glycoside indicated that the terminal sugar was xylose and the glucose moiety was linked with the aglycone through theβ-linkage as evidenced by a

multiplet (w½=6.0) of the signal at δ 5.35 of the anomeric proton of glucose. The xylose moiety was linked through a β-linkage with the glucose moiety due to the doublet (*J*=5.1 Hz) of the signal at δ 5.33 of the anomeric proton of xylose. The linkage of xylose to glucose was found to be (1→2) as confirmed by ^{1}H NMR of its acetate which did not exhibit the signal at δ 1.72 due to acetoxylproton at position-2 of the glucose indicating that 2' hydroxyl group was linked with the xylose moiety.[12]

The mass spectrum of the aglycone showed the molecular ion peak at *m/z* 286 corresponding to molecular formula as $C_{11}H_{10}O_7$. The sharp peaks at 168 due to fragment "A" and 94 due to fragment "B" supported the proposed structure of the aglycone. Other peaks in the mass spectrum at *m/z*269, 258, 134, 140 and 77 were also characteristics of flavonoid. Thus, the structure of the aglycone was established as 5,7,8,4'-tetrahydroxyflavone (isoscutellarin) and the structure of the glycoside was found as 5,7,8,4'-tetrahydroxyflavone-7-[-O-β-D-xylosyl-(1→2)-O-D-glucopyranoside, which appears to be a new glycosideofisoscutellarin.The spectral values of the isolated compounds were compared with the reported values.[12,13]

CONCLUSIONS

The present work reports, for the first time, the isolation and characterization of three new flavone glycosides from *L. echinata viz.,* 3,5,7,3',4'- penta-hydroxyflavone-3-[-O-β-D-glucopyranosyl-7-O-β-D-glucopyranoside (**1**), 3,5,7-trihydroxy-(7'8'-dioxo-9', 10'-dihydro[a]cyclohex$\Delta^{13'(14')}$, $\Delta^{11'(12')}$-12'13'-dimethyl)flavone-7-[-O-β-D-galactopyranosyl-(1→ 2)]-O-β-D-glucopyranoside (**2**) and 5,7,8,4'-tetra-hydroxyflavone-7-[-O-β-D-xylosyl-(1→2)]-O-β-D-glucopyranoside (**3**).

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