PART: B

Phytochemical Investigation of *Lespedeza juncea* Pers.
5. 1 General
5. 1.1 Introduction

Fabaceae is a family of herbs/woody/climbing plants. Leaves usually alternate, stipulate, pinnately compound, sometimes 2-3 pinnate, or of a single or 2-3 leaflets; ptyxis of leaflets almost always conduplicate, but supervolute in some species. Inflorescence various, usually a raceme. Flowers usually bisexual, actinomorphic/zygomorphic. Legume indehiscent or lomentum. Lespedeza contains approximately about 60 species which occurs from East Asia to Northeast Australia and North America. The members of this genus are tolerant of arid environments and consequently are often planted to prevent soil erosion. A very large and relatively uniform family of about 600 genera and 13500 species. Many genera are native to Europe or North America and many are cultivated.

Lespedeza juncea is an erect or ascending subshrub standing upto 1 meter tall. The stem is covered with white pubescence, angled upwards. Closely spaced trifoliate leaves alternate along the stem. The blade of the leaflet is cuneate or linear cuneate, about 1-3 cm and 2-5 mm wide, truncate, apex is somewhat retuse with a mucronate tip. The upper surface of the leaf is glabrous or pilose, and the underside is white pubescent. The petiole measures less than 10 mm in length. The stipule is rhomboid and persistent. From July to August, racemes emerge from the leaf axil bearing two to three flowers each. The apetalous flowers occur in clusters having two narrow branchlets, above which the narrowly campanulate calyx, with five lanceolate pubescent lobes. The corolla is light yellow to white, the keel is violet spotted at the base and longer than the standard and the wing, both of which are about 7 mm long. The pubescent pod is broadly ovoid or subglobose, 2.5-3.5 mm long and 2.5 mm wide. Fruits appear from September through
October. *Lespedeza juncea* can be found along roadside, slopes, ridges, stream banks, ravines, and crop field margins at elevation less than 2500 m. *Lespedeza juncea* Pers. is a deciduous undershrub giving off 25-90 cm long, trailing or decumbent, striate, grey-pubescent shoots from a woody stock. Leaflets oblanceolate, mostly 0.7 x 0.25-0.5 cm, mucronate, glabrous above, densely grey-silky beneath, terminal shortly petiolated, lateral sessile or nearly so. Flowers pale purple, 6-7 mm long, in axillary, short-peduncled, 2-4-flowered umbels. Calyx grey-silky, 0.4 mm long; teeth much longer than the tube. Pod silky, ovate; indehiscent, 1-seeded.

5.1.2 Chemical constituents of genus *Lespedeza*

Different species of the genus *Lespedeza* synthesise mostly phenolic natural products during their biogenesis. Prenylated isoflavanones had been isolated from *Lespedeza bicolor*. Isoflavones, prenylated coumarins, pterocarpenes, prenylated pterocarpenes, bipterocarpenes, dihydropterocarpene had been reported from *Lespedeza homoloba*. Flavanones were isolated from *Lespedeza formosa* and *L. davidii*. Isoearlineside, a di-C-glycosylflavone and flavonoids were isolated from *Lespedeza capitata*. Catechins, potassium lespepedezeate and potassium isolespedeazeate were isolated from some species of *Lespedeza*. β-sitosterol, succinic acid, triacontan-1-ol, querceitin, kaempferol, pinitol, avicularin and trifolin were identified from *Lespedeza cuneata*. Isovitexin, isooreientin, succinic acid, lucenin were isolated from leaves of *Lespedeza juncea*. Cinnamic, ferulic, gallic, gentisic and syringic acids were isolated from root exudate of *Lespedeza striata*. Rutin, luteolin 7-O-glucoside, β-sitosterol and its glycoside, genistein, isooreientin, kaempferol, isovitexin, quercetin were reported to be
isolated from *Lespedeza striata*. Isorhamnetin-3-O-neohesperidoside, isorhamnetin-3-O-rutinoside, quercetin-3-O-glucoside, kaempferol-3-O-ribinoside and kaempferol-3-O-rhamnosyl (1-2) galactoside were isolated from aerial parts of *Lespedeza tomentosa*.

5. 1. 3 Medicinal and pharmacological importance of genus *Lespedeza*

*Lespedeza* species show prominent biological activity. Sericea *Lespedeza* hay as a natural deworming agent against gastrointestinal nematode infections in goats. Many isolated flavonoids from *Lespedeza homoloba* are known to be well known antioxidants. Bioorganic nyctinastic activities were shown by *Lespedeza cuneata*. Potassium lespedezate and potassium isolespedezate, bioactive substances concerned with the circadian rhythm in nyctinastic plants.

5. 2 Results and Discussion

Section C: Phytochemical investigation of aerial part of *Lespedeza juncea* Pers.

Recolumn chromatography of Fr-3 results in the isolation of Lesjunceol (7)

5. 2. 1 Lesjunceol (7)

2, 5, 7, 9-tetrahydroxy-6-methoxy-3-(4′-hydroxybenzyl)-4-chromanone

![Chemical Structure](image)
Lesjunceol (7) was isolated (Rf:0.50; CHCl₃:MeOH, 8:2 v/v; 63 mg) as a light yellow amorphous powder, recrystallised from methanol, m.p. 240.2 °C after column chromatography of fraction Fr-3. The Mg/HCl negative test ruling out its possibility to be flavone moiety.

The ESI-MS of the compound 7 showed the molecular ion at m/z 371 [M+Na] in agreement with the molecular formula C₁₇H₁₆O₈. This C-17 carbon skeleton was again supported by ¹³C NMR and DEPT experiments. The specific rotation [α]₀ showed the [α]₀° + 102° (c 0.30, MeOH).

UV spectrum contained λ_max at 242.3, 281.4 and 302.2 were suggestive of flavonoid structure. UV spectrum was measured by adding AlCl₃, AlCl₃/HCl, NaOAc and NaOAc/H₃BO₃. Bathochromic shift with NaOAc (25 nm) and AlCl₃ (15 nm) indicated the –OH groups at C-5 and C-7 or C-4'. The presence of chelated 5-hydroxyl was further confirmed by the singlet at 12.4 ppm in the proton NMR spectrum. Here the pronounced shift with NaOAc/H₃BO₃ is not so indicative but it was later supported by HMBC experiment that one of the OH is at C-7.

The IR spectrum revealed characteristic -OH absorption at 3450.5 cm⁻¹, aromatic -C-H absorption at 2954.3 cm⁻¹ and carbonyl absorption at 1678.3 cm⁻¹. The lower values were at 1310.2, 702.2, and 668.0.

The ¹H NMR (500 MHz) of 7 showed the typical splitting pattern for 3-hydroxy-3-benzyl-4-chromanone-type homoisoflavonoid. Proton NMR showed the presence of two aromatic moieties. The four proton doublets at δ 7.41 J = 8.5, 7.34 J = 8.5, 7.32 J = 8.6, 7.38 J = 8.6 and a one proton singlet at δ 6.10, the presence of four doublets indicated that C-4' is substituted in aromatic ring B and the singlet at δ 6.10 was typical
of methine proton of polysubstituted ring A. The aliphatic proton doublets at \( \delta 4.14 \ J = 7.7, 3.25 \ J = 7.7, 4.3 \) and \( 5.11 \ J = 7.8 \) were indicative of the presence of \(-\text{CH-CH-CH-}\) in the structure \(^{299}\). The singlet at \( \delta 3.82 \) was assigned to the protons of \(-\text{OCH}_3\) attached to the aromatic ring. Based on spectroscopic data, a homoisoflavanone skeleton with penta-substituted ring A and a singly substituted ring B was proposed. The number of hydroxyls was confirmed by preparing acetylated derivative of compound 7 in \( \text{Ac}_2\text{O}/\text{pyridine}\). Formation of pentaacetate confirmed the presence of five hydroxyl groups. There is prominent shift towards downfield region for ortho/para carbon resonance frequencies and upfield shift for hydroxylated carbons.

\[
\begin{align*}
\text{HO} & \quad \text{O} & \quad \text{OH} & \quad \text{OH} & \quad \text{HO} \\
\text{MeO} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{MeO} \\
\text{7} & \quad \text{Ac}_2\text{O}/\text{pyridine} & \quad \text{AcO} & \quad \text{OAc} & \quad \text{OAc} & \quad \text{OAc} \\
\end{align*}
\]

\(^{13}\text{C}\) NMR showed the presence of seventeen carbons. The most downfield signal was assigned to carbonyl carbon at \( \delta 198.2 \), typical of homoisoflavanone, with C-5 hydroxylated. This assignment was confirmed by the downfield shift of C-4 resonance. When there is a hydroxyl group at the C-5, the carbonyl resonance occurs downfield, close to the \( \delta 200 \), due to hydrogen bonding between hydroxyl group proton and carbonyl oxygen, whereas when a methoxyl group is present at C-5, the carbonyl carbon resonance occurs typically around \( < \delta 190 \) \(^{300,301}\). The other downfield signals were given to those carbons which are attached to electronegative carbon at \( \delta 154.2, 158.3, 157.5 \) and 155.2 of C-5, C-7, C-8a and C-4'. The fully substituted resonances at \( \delta 67.1, 51.2, \) and 72.2 were assigned to carbons C-2, C-3 and C-9 with all hydroxyl substituted and \(-\text{OCH}_3\)-6 at frequency \( \delta 60.1 \). The aromatic carbon C-8 absorbs at lower frequency shielded on both
sides by electron donating oxygen (δ 94.5). The rest of the signals were assigned to carbons of C-4a, C-1’, C-2’, C-3’, C-5’ and C-6’ at frequencies δ 102.2, 139.2, 129.5, 128.4, 127.5 and 135.5.

All chemical shift assignments were reconfirmed by HMBC (heteronuclear multiple bond correlation), HSQC (heteronuclear single quantum coherence), HMQC (heteronuclear multiple quantum coherence), COSY (correlated spectroscopy) and NOESY (nuclear overhauser enhancement spectroscopy) techniques. The significant HMBC and HSQC correlation showed the connectivity of C-2 hydroxyl with C-2 (δ 67.1) and C-3 (δ 51.3). The connectivity of C-9 hydroxyl had been observed with C-9 (δ 72.2) and C-3 (δ 51.3). The other C-5 hydroxyl 2D correlation had been comprehensively detected with C-5 (δ 154.4), C-4a (δ 102.2) and C-6 (δ 128.1). The HSQC connectivity of C-8 (δ 94.5) has been observed with C-7 (δ 158.3) and C-8a (δ 157.5). The connectivity in 2D of C-4’ hydroxyl is efficiently observed with C-3’ (128.4) and C-5’ (127.5).

[Image of a chemical structure with significant 1H-13C long range correlations observed in HMBC spectra of Lesjunceol (7)]

114
Table-11: $^{13}$C-NMR (125 MHz, DMSO, δ, ppm) of compound 7:

<table>
<thead>
<tr>
<th>Position</th>
<th>Lesjunceol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>67.1</td>
</tr>
<tr>
<td>3</td>
<td>51.2</td>
</tr>
<tr>
<td>4</td>
<td>198.2</td>
</tr>
<tr>
<td>4a</td>
<td>102.2</td>
</tr>
<tr>
<td>5</td>
<td>154.2</td>
</tr>
<tr>
<td>6</td>
<td>128.1</td>
</tr>
<tr>
<td>7</td>
<td>158.3</td>
</tr>
<tr>
<td>8</td>
<td>94.5</td>
</tr>
<tr>
<td>8a</td>
<td>157.5</td>
</tr>
<tr>
<td>9</td>
<td>72.2</td>
</tr>
<tr>
<td>1'</td>
<td>139.2</td>
</tr>
<tr>
<td>2'</td>
<td>129.5</td>
</tr>
<tr>
<td>3'</td>
<td>128.4</td>
</tr>
<tr>
<td>4'</td>
<td>155.2</td>
</tr>
<tr>
<td>5'</td>
<td>127.5</td>
</tr>
<tr>
<td>6'</td>
<td>135.5</td>
</tr>
<tr>
<td>(OCH$_3$-6)</td>
<td>60.1</td>
</tr>
</tbody>
</table>

Recolumn chromatography of Fr-5 results in the isolation of Lesjuncerol (2)

5.2.2 Lesjuncerol (8)

2, 5, 7, 9-tetrahydroxy-6-methoxy-8-methyl-3-(4'-hydroxybenzyl)-4-chromanone

![Chemical Structure of Lesjuncerol (8)]
Lesjuncerol (8) was isolated (Rt: 0.49; EtOAc: MeOH, 7.5:2.5, v/v, 52 mg) as a white amorphous powder, recrystallised from methanol, m.p. 190.4 °C after column chromatography of fraction Fr-5.

The ESI-MS of the compound 8 showed the molecular ion at m/z 385 [M+Na] in agreement with the molecular formula C_{18}H_{18}O_{8}. This C-18 carbon skeleton was again supported by {^{13}}C NMR and DEPT experiments. The specific rotation [\alpha]_D showed the [\alpha]_D^{25} + 114° (c 0.30, MeOH).

UV showed \lambda_{max} at 243.3, 280.4 and 308.5 characteristic of homoisoflavanones. Bathochromic shift with NaOAc (23 nm) and AlCl_3 (10 nm) indicated the OH groups at C-5 and C-7 or C-4'. Again in UV spectrum was measured by adding AlCl_3, AlCl_3/\text{HCl}, NaOAc and NaOAc/\text{H}_3\text{BO}_3. Bathochromic shift with NaOAc (23 nm) and AlCl_3 (10 nm) indicated the -OH groups at C-5 and C-7 or C-4'. The presence of chelated 5-hydroxyl was further confirmed by the singlet at 12.5 ppm in the proton NMR spectrum. Here also the pronounced shift with NaOAc/\text{H}_3\text{BO}_3 is not so indicative but it was later supported by HMBC experiment that one of the OH is at C-7.

The IR spectrum revealed characteristic -OH absorption at 3410.4 cm^{-1}, aromatic -C-H absorption at 2956.2 cm^{-1} and carbonyl absorption at 1692.3 cm^{-1}. The lower values were at 1303.3, 735.5, and 670.2 cm^{-1}.

The {^1}H NMR spectrum (500 MHz) is almost same as that of 7 except one extra signal of allylic proton. The four proton doublets \delta 7.31 J = 8.5, 7.21 J = 8.5, 7.25 J = 8.6 and 7.32 J = 8.6 were indicative of the protons attached to aromatic ring. The pattern of these signals show that all the protons are aromatic and are attached to the same ring, indicating allylic proton has replaced that proton which comes (aromatic) upfield in 7.
The two singlets at $\delta$ 3.78 and $\delta$ 2.45 indicated one of the methoxyl attached to aromatic ring and other that of allylic proton attached to aromatic ring. The aliphatic proton signals at $\delta$ 4.10 (1H, d, $J = 7.8$, H-2), 3.25 (1H, dd, $J = 7.8$ 4.4, H-3) and at $\delta$ 5.25 (1H, d, $J = 7.7$, H-9) were again indicative of the presence of -CH-CH-CH- in the molecule. Based upon this homoisoflavanone structure with all the positions of aromatic ring A and one of the positions of ring B is substituted. As the spectra pattern of 8 is almost same as that of 7 so the same homoisoflavanone structure with extra methyl group attached to aromatic ring was proposed.

The number of hydroxyls was confirmed by preparing acetylated derivative of compound 8 in Ac$_2$O/pyridine. Formation of pentaacetate confirmed the presence of five hydroxyl groups. Again there is a prominent shift toward downfield region for ortho/para carbon resonance frequencies and upfield shift for hydroxylated carbons.

$^{13}$C NMR showed the presence of eighteen carbons. The most downfield signal was assigned to carbonyl carbon at $\delta$ 191.8. Again corroborated with 7 that C-5 is hydroxylated and due to hydrogen bonding carbonyl comes downfield. The other downfield signals were given to those carbons which are attached to electronegative oxygen at $\delta$ 154.5, 157.3, 150.2 and 154.5 of C-5, C-7, C-8a and C-4'. The most upfield signal was assigned to methyl carbon attached to aromatic ring as allylic carbon at $\delta$ 15.2 (H$_3$C-8). The other upfield signals were assigned to carbons C-2, C-3 and C-9 at
frequencies δ 68.2, 52.2, 75.1. The resonance of typical methoxyl was found at δ 59.4. The aromatic carbon C-8 (δ 98.5) absorbs at lower frequency shielded on both sides by electron donating oxygen and also due to positive inductive effect of methyl group. The rest of the signals were assigned to carbons of C-4a, C-1', C-2', C-3', C-5' and C-6' at frequencies δ 103.2, 139.1, 130.1, 127.7, 129.1 and 134.2 respectively.

All chemical shift assignments were reconfirmed by HMBC (heteronuclear multiple bond correlation), HSQC (heteronuclear single quantum coherence), HMQC (heteronuclear multiple quantum coherence), COSY (correlated spectroscopy) and NOESY (nuclear overhauser enhancement spectroscopy) techniques. The significant HMBC and HSQC correlation showed the connectivity of C-2 hydroxyl proton with C-2 (δ 68.2) and C-3 (δ 52.2). The connectivity of C-9 hydroxyl proton with C-9 (δ 75.1) and C-3 (δ 52.2). The other C-5 hydroxyl 2D correlation had been comprehensively detected with C-5 (154.5), C-4a (δ 103.2) and C-6 (δ 128.2). The HSQC connectivity of C-8 (δ 98.5) has been observed with C-7 (δ 157.3) and C-8a (δ 150.2). The single quantum connectivity of C-8 methyl had been observed with C-8 (δ 98.5). The connectivity in 2D of C-4' hydroxyl is efficiently observed with C-3' (δ 128.4) and C-5' (δ 127.5).

Significant $^1\text{H}-^{13}\text{C}$ long range correlations observed in HMBC spectra of Lesjuncerol (8)
Table-12: $^{13}$C-NMR (125 MHz, DMSO, $\delta$, ppm):

<table>
<thead>
<tr>
<th>Position</th>
<th>Lesjuncerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>68.2</td>
</tr>
<tr>
<td>3</td>
<td>52.2</td>
</tr>
<tr>
<td>4</td>
<td>191.8</td>
</tr>
<tr>
<td>4a</td>
<td>103.2</td>
</tr>
<tr>
<td>5</td>
<td>154.5</td>
</tr>
<tr>
<td>6</td>
<td>128.2</td>
</tr>
<tr>
<td>7</td>
<td>157.3</td>
</tr>
<tr>
<td>8</td>
<td>98.5</td>
</tr>
<tr>
<td>8a</td>
<td>150.2</td>
</tr>
<tr>
<td>9</td>
<td>75.1</td>
</tr>
<tr>
<td>1'</td>
<td>139.1</td>
</tr>
<tr>
<td>2'</td>
<td>130.1</td>
</tr>
<tr>
<td>3'</td>
<td>127.7</td>
</tr>
<tr>
<td>4'</td>
<td>154.5</td>
</tr>
<tr>
<td>5'</td>
<td>129.1</td>
</tr>
<tr>
<td>6'</td>
<td>134.2</td>
</tr>
<tr>
<td>(OCH$_3$-6)</td>
<td>59.4</td>
</tr>
<tr>
<td>(CH$_3$-8)</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Section D: Phytochemical investigation of root of *Lespedeza juncea* Pers.

Recolumn chromatography of Fr-2 results in the isolation of Meoside A

5.2.3 Meoside A (9)

Meoside A was isolated ($R_f$: 0.47; CHCl$_3$: MeOH, 1:1 v/v; 35 mg) as a colourless amorphous powder, recrystallised from methanol, m.p. 183.3 °C after column chromatography of fraction Fr-2.
The ESI-MS of the compound 9 showed the molecular ion at m/z 505 [M+H] in agreement with the molecular formula C_{22}H_{32}O_{13}. This C-22 carbon skeleton was again supported by $^{13}$C NMR and DEPT experiments. The specific rotation $[\alpha]_D$ showed the $[\alpha]_D^{25} -148^\circ$ (c 0.25, EtOH).

The $\lambda_{max}$ at 281.5 nm in UV spectrum revealed substituted benzene moiety. The IR showed band at 3450.3 cm\(^{-1}\) of hydroxyl functionality, 2929.8 cm\(^{-1}\) of aromatic -C-H stretching. The other stretching frequencies are 1599.1, 1580.3, 1523.8, 1453.8 and 983.7 cm\(^{-1}\) indicating substituted benzene system.

$^1$H NMR (500 MHz) showed two singlets of aromatic region at $\delta$ 7.12 and $\delta$ 6.99, for two proton, one of them being obviously shielded by two oxygen functions of two glycosidic oxygen moieties. The frequencies at $\delta$ 6.32 (1H, dd, J=2.1, 14.1) and $\delta$ 6.28 are of olefinic protons of side chain. The splitting pattern of these signals showed that all are a part -C-C-C- side chain\(^{302}\). The signals at $\delta$ 1.92 and $\delta$ 3.82 can be attributed to methyl and methoxyl groups respectively. The series of resonance frequencies at $\delta$ 4.91 (1H, d, J=7.4), 3.47 (1H, m), 3.46 (1H, m), 3.42 (1H, m), 3.65 (1H, m), 4.10 (1H, dd, J=2.1, 12.3) and 3.78 (1H, m) are diagnostic signals of sugar moiety attached at position-1. The remaining signals at frequencies $\delta$ 4.73 (1H, d, J=7.1), 3.52 (1H, m), 3.45 (1H, m),
3.59 (1H, m), 3.81 (1H, dd, J=3.2, 12.1) and 3.43 (1H, m) agreed with another glucose moiety attached to benzene ring at position-5.

$^{13}$C NMR (125 MHz) showed the presence of C-22 carbon skeleton. DEPT showed the presence of fourteen methines, two methylenes of two sugar moieties and two methyls, one of methyl and other of methoxyl. The most downfield signals were assigned to aromatic carbons which are attached to oxygen function at $\delta$ 146.1, 149.4 and 148.2 of carbons C-1, C-4 and C-5 respectively. The olefinic carbons of side chain were assigned values $\delta$ 132.1 and 125.2 (C-7 and C-8). The C-6 carbon absorbs at lower frequencies at $\delta$ 100.1, flanked by two sugar moieties. The allylic carbon of side chain comes at $\delta$ 19.2. The typical methoxyl signal at $\delta$ 55.6 is attributed to C-10. The resonance frequencies at $\delta$ 101.1, 74.3, 77.7, 70.1, 78.4 and 65.2 were assigned to carbons C-1', C-2', C-3', C-4', C-5' and C-6' of sugar moiety attached to benzene ring at position-1. Another absorbing frequencies at $\delta$ 102.8, 71.2, 73.2, 69.6, 74.3 and 65.4 were assigned to carbons C-1'', C-2'', C-3'', C-4'', C-5'' and C-6'' respectively of another sugar moiety attached to benzene ring at position-5.

Based on the above data compound 9 has the following structure.

![Chemical Structure](image)

The chemical shift assignments were reconfirmed by HMBC (heteronuclear multiple bond correlation), HSQC (heteronuclear single quantum coherence), HMQC
(heteronuclear multiple quantum coherence). COSY (correlated spectroscopy) and
NOESY (nuclear overhauser enhancement spectroscopy) techniques. The COSY
connectivity of C-7 (δ 132.1) has been observed with H-8 (δ 6.28). Significant correlation
experiment has shown correlation of C-8 (δ 125.2) with H-7 (δ 6.32) and H-9 (δ 1.90).
The HMBC connectivity is efficient, C-3 (δ 119.2) with H-7 (δ 6.32). The connectivity
shown by H-10 is significantly observed with C-4 (δ 149.4) and C-5 (δ 148.2). The
connectivity of H-6 has been observed with C-1 (δ 146.1) and C-5 (δ 148.2). The
COSY correlation is being efficiently observed H-1' (δ 4.91) with C-2' (δ 74.3), H-3' (δ 3.46)
with C-2' (δ 74.3) and C-4' (δ 70.1). The H-6' (δ 4.10) showed significant COSY and
HMBC correlation with C-5' (δ 78.4) and C-4' (δ 70.1) respectively. Same type of COSY
correlation is being efficiently observed from another sugar moiety, H-1'' (δ 4.73) with
C-2'' (δ 71.2), H-3'' (δ 3.45) with C-2'' (δ 71.2) and C-4'' (δ 69.9). The H-6'' (δ 3.81)
showed significant COSY and HMBC correlation with C-5'' (74.3) and C-4'' (δ 65.4)
respectively. Assignments are shown in Table-14.

Significant $^1H-^{13}C$ long range correlations observed in HMBC spectra of Meoside A (9)
Table-13: $^1$H and $^{13}$C NMR (500 MHz, CD$_3$OD, $\delta$, ppm, J/Hz) data of Meoside A (9).

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_C$</th>
<th>$\delta_H$</th>
<th>COSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>146.1</td>
<td>7.12 (1H, s)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>130.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>119.2</td>
<td>6.99 (1H, s)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>149.4</td>
<td>6.32 (1H, dd, J= 2.1,14.1)</td>
<td>6.28</td>
</tr>
<tr>
<td>5</td>
<td>148.2</td>
<td>6.28 (1H, m)</td>
<td>6.32, 1.90</td>
</tr>
<tr>
<td>6</td>
<td>100.1</td>
<td>1.90 (3H, dd, J= 1.5,7.8)</td>
<td>6.28</td>
</tr>
<tr>
<td>7</td>
<td>132.1</td>
<td>3.82 (3H, s)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>125.2</td>
<td>3.47 (1H, m)</td>
<td>4.91, 3.46</td>
</tr>
<tr>
<td>9</td>
<td>19.2</td>
<td>3.46 (1H, m)</td>
<td>3.47, 3.42</td>
</tr>
<tr>
<td>10</td>
<td>55.6</td>
<td>3.42 (1H, m)</td>
<td>3.46, 3.65</td>
</tr>
<tr>
<td>1'</td>
<td>101.6</td>
<td>3.65 (1H, m)</td>
<td>4.10, 3.78, 3.42</td>
</tr>
<tr>
<td>2'</td>
<td>74.3</td>
<td>4.10 (1H, dd, J=2.1, 12.3, H-6'α)</td>
<td>3.65</td>
</tr>
<tr>
<td>3'</td>
<td>77.7</td>
<td>3.78 (1H, m, H-6'β)</td>
<td>3.65</td>
</tr>
<tr>
<td>4'</td>
<td>70.1</td>
<td>4.73 (1H, d, J=7.1)</td>
<td>3.52</td>
</tr>
<tr>
<td>5'</td>
<td>78.4</td>
<td>3.52 (1H, m)</td>
<td>4.73, 3.45</td>
</tr>
<tr>
<td>6'</td>
<td>65.2</td>
<td>3.45 (1H, m)</td>
<td>3.76, 3.52</td>
</tr>
<tr>
<td>1&quot;</td>
<td>102.8</td>
<td>3.76 (1H, m)</td>
<td>3.59, 3.45</td>
</tr>
<tr>
<td>2&quot;</td>
<td>71.2</td>
<td>3.59 (1H, m)</td>
<td>3.81, 3.76, 3.43</td>
</tr>
<tr>
<td>3&quot;</td>
<td>73.2</td>
<td>3.81 (1H, dd, J=3.2,12.1 H-6&quot;α)</td>
<td>3.59</td>
</tr>
<tr>
<td>4&quot;</td>
<td>69.6</td>
<td>3.43 (1H, m, H-6&quot;β)</td>
<td>3.59</td>
</tr>
</tbody>
</table>

Recolumn chromatography of Fr-3 results in the isolation of Meoside B

**5.4.4 Meoside B (10)**

Meoside B was isolated (Rf:0.49; CHCl$_3$: MeOH, 4:6 v/v; 25 mg) as a white amorphous powder, recrystallised from methanol, m.p. 192.4 °C after column chromatography of fraction Fr-3.
The ESI-MS of the compound 10 showed the molecular ion at m/z 521 [M+H] in agreement with the molecular formula C_{22}H_{32}O_{14}. This C-22 carbon skeleton was again supported by $^{13}$C NMR and DEPT experiments. The specific rotation $[\alpha]_D$ showed the $[\alpha]_D^{25} +124^\circ$ (c 0.25, EtOH).

The $\lambda_{max}$ at 285.1 nm in UV spectrum revealed substituted benzene moiety. The IR showed band at 3429.4 cm$^{-1}$ of hydroxyl functionality, 2952.5 cm$^{-1}$ of aromatic -C-H stretching. The remaining stretching values at 1567.6, 1506.7, 1453.3, and 922.4 cm$^{-1}$ indicating substituted benzene system.

$^1$H NMR (500 MHz) showed two aromatic signals of benzene ring at $\delta$ 6.88 and $\delta$ 6.98 at position 7 and 10 respectively. The signals triplet, multiplet and triplet at $\delta$ 3.00, 1.68 and 1.01 showed the side chain having –CH$_2$-CH$_2$-CH$_3$ pattern. The remaining signals at $\delta$ 4.89 (d), 3.41 (m), 3.39 (m), 3.42 (m), 3.32 (m), 4.11 (dd) and 3.98 (m) are of glycoside attached at position-6. Another series of signals at frequencies 4.96 (d), 3.32 (m), 3.29 (m), 3.33 (m), 3.18 (m), 4.60 (dd) and 3.98 (m) are diagnostic signals of another glycoside attached at position-8.
Based on the above data the following structure was proposed to compound **10** which was further supported by 2D experiments.

\[
\text{\includegraphics[width=\textwidth]{structure.png}}
\]

\(^{13}\text{C} \text{NMR (125 MHz)} \text{ showed the presence of C-22 carbon skeleton. DEPT showed the presence of twelve methines, four methylene and one methyl. The most downfield signal was assigned to carbonyl carbon of side chain } \delta \text{ 205.6 (C-1). The other downfield signals were assigned to aromatic carbons which are attached to oxygen function at } \delta \text{ 155.8, 154.2 and 156.6 of carbons C-6, C-8 and C-9 respectively. The aliphatic carbons of side chain were assigned values } \delta \text{ 47.2, 18.2 and 15.4 of carbons C-2, C-3 and C-4. The C-7 carbons absorbs at lower frequencies at } \delta \text{ 98.4, flanked by two oxygen functions of sugar moieties. The C-10 carbon of aromatic ring absorbs at } \delta \text{ 125.6. The frequencies at values } \delta \text{ 102.6, 73.3, 77.9, 70.5, 78.7 and 62.2 were assigned carbons C-1', C-2', C-3', C-4', C-5' and C-6' of sugar moiety attached to benzene ring at position-6. Another absorbing frequencies at } \delta \text{ 100.8, 72.2, 75.2, 70.6, 76.3 and 61.8 were assigned to carbons C-1'', C-2'', C-3'', C-4'', C-5'' and C-6'' respectively of another sugar moiety attached to benzene ring at position-8.}

The chemical shift assignments were reconfirmed by HMBC (heteronuclear multiple bond correlation), HSQC (heteronuclear single quantum coherence), HMQC (heteronuclear multiple quantum coherence), COSY and NOESY (nuclear overhauser
enhancement spectroscopy) techniques. The COSY and HMBC showed connectivity of H-4 (δ 1.01) with C-3 (δ 18.2) and C-2 (47.2). Significant correlation had been shown by H-10 (δ 6.98) with C-1 (δ 205.6). The COSY and HMBC connectivity is efficient of C-9 hydroxyl proton with C-9 (δ 156.6) and C-8 (δ 154.2). The HMBC connectivity shown by H-7 is significantly observed with C-6 (δ 155.8) and C-5 (δ 154.2). The COSY correlation is being efficiently observed H-1' (δ 4.89) with C-2' (δ 73.3), H-3' (δ 3.39) with C-2' (δ 73.3) and C-4' (δ 70.5). The H-6' (δ 4.11) showed significant COSY and HMBC correlation with C-5' (δ 78.7) and C-4' (δ 70.5) respectively. Same type of COSY correlation is being efficiently observed from another sugar moiety. H-1'' (δ 4.96) with C-2'' (δ 72.2), H-3'' (δ 3.29) with C-2'' (δ 72.2) and C-4'' (δ 70.6). The H-6'' (δ 4.60) showed significant COSY and HMBC correlation with C-5'' (76.3) and C-4'' (δ 61.8) respectively. Assignments are shown in Table-14.

Significant \(^1H\)\(^{13}C\) long range correlations observed in HMBC spectra of Meoside B (10)
Table-14: $^1$H and $^{13}$C NMR (500 MHz, CD$_3$OD, $\delta$, ppm, J/Hz) data of Meoside B (10).

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_c$</th>
<th>$\delta_h$</th>
<th>COSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>205.6</td>
<td>3.00 (2H, t)</td>
<td>1.68</td>
</tr>
<tr>
<td>2</td>
<td>47.2</td>
<td>1.68 (2H, m)</td>
<td>3.00, 1.01</td>
</tr>
<tr>
<td>3</td>
<td>18.2</td>
<td>1.01 (3H, t)</td>
<td>1.68</td>
</tr>
<tr>
<td>4</td>
<td>15.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>115.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>155.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>98.4</td>
<td>6.88 (1H, s)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>154.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>156.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>125.6</td>
<td>6.98 (1H, s)</td>
<td>3.41</td>
</tr>
<tr>
<td>$1'$</td>
<td>102.6</td>
<td>4.89 (1H, d, J= 7.3)</td>
<td>3.41</td>
</tr>
<tr>
<td>$2'$</td>
<td>73.3</td>
<td>3.41 (1H, m)</td>
<td>4.89, 3.39</td>
</tr>
<tr>
<td>$3'$</td>
<td>77.9</td>
<td>3.39 (1H, m)</td>
<td>3.42, 3.41</td>
</tr>
<tr>
<td>$4'$</td>
<td>70.5</td>
<td>3.42 (1H, m)</td>
<td>3.39, 3.32</td>
</tr>
<tr>
<td>$5'$</td>
<td>78.7</td>
<td>3.32 (1H, m)</td>
<td>4.113.983.42</td>
</tr>
<tr>
<td>$6'$</td>
<td>62.2</td>
<td>4.11 (1H, dd, J=3.0, 11.3, H-6'(\alpha))</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.98 (1H, m, H-6'(\beta))</td>
<td>3.32</td>
</tr>
<tr>
<td>$1''$</td>
<td>100.8</td>
<td>4.96 (1H, d, J=7.2)</td>
<td>3.32</td>
</tr>
<tr>
<td>$2''$</td>
<td>72.2</td>
<td>3.32 (1H, m)</td>
<td>4.96, 3.29</td>
</tr>
<tr>
<td>$3''$</td>
<td>75.2</td>
<td>3.29 (1H, m)</td>
<td>3.33, 3.32</td>
</tr>
<tr>
<td>$4''$</td>
<td>70.6</td>
<td>3.33 (1H, m)</td>
<td>3.29, 3.18</td>
</tr>
<tr>
<td>$5''$</td>
<td>76.3</td>
<td>3.18 (1H, m)</td>
<td>4.603.983.33</td>
</tr>
<tr>
<td>$6''$</td>
<td>61.8</td>
<td>4.60 (1H, dd, J=3.2, 11.1 H-6''(\alpha))</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.98 (1H, m, H-6''(\beta))</td>
<td>3.18</td>
</tr>
</tbody>
</table>

Antioxidant activity of extracts of *Lespedeza juncea* Pers.

Study activity

The antioxidant activity was evaluated using DPPH (2,2-diphenyl-picryl-hydrazil) test$^{304}$, 5-lipoxygenase assay$^{305-307}$ and xanthine oxidase$^{308-310}$.

Tested material

Ethanolic and ethylacetate extracts (aerial part and root) of said plant were tested for activity. 5 ml 50 mM Na-phosphate buffer (pH-7.5) and 5 ml ethylacetate were added
to the sample (3 g) and the resulting material was shaken and centrifuged subsequently at 14000 rev./min. The different phases were collected to measure antioxidant activity.

**Table-15**: Antioxidant activity of extracts of *Lespedeza juncea* Pers.

<table>
<thead>
<tr>
<th></th>
<th>DPPH</th>
<th>Lipid peroxidation</th>
<th>Superoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanolic</td>
<td>12.023 ± 1.223</td>
<td>11.022 ± 1.213</td>
<td>28.330 ± 0.500</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>14.051 ± 0.112</td>
<td>16.345 ± 1.131</td>
<td>50.338 ± 11.61</td>
</tr>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanolic</td>
<td>0.998 ± 0.280</td>
<td>0.091 ± 0.02</td>
<td>0.982 ± 0.510</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>0.053 ± 0.012</td>
<td>7.305 ± 0.441</td>
<td>5.218 ± 0.779</td>
</tr>
</tbody>
</table>

DPPH (mg DW/ml); Lipid peroxidation (mg DW/ml); Superoxide (mg DW/ml)

*Data are referred to DW (dry weight) and the values are the average of five determinations (± S.D.).

**Conclusion**: The preparations of various extracts of *Lespedeza juncea* posses a significant antioxidant activity in all tests performed. The use of extracts of *Lespedeza juncea* can be recommended for antioxidant profile qualities. In short both aerial part as well as roots of *Lespedeza juncea* is an enriched source of antioxidant compounds in human diet.
Table-16: Antioxidant activity of natural isolates Lesjunceol (7) and Lesjuncerol (8)

<table>
<thead>
<tr>
<th></th>
<th>DPPH</th>
<th>Lipid peroxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesjunceol</td>
<td>11.213 ± 1.431</td>
<td>11.022 ± 1.213</td>
</tr>
<tr>
<td>Lesjuncerol</td>
<td>10.053 ± 3.552</td>
<td>12.365 ± 2.671</td>
</tr>
</tbody>
</table>

DPPH (mg DW/ml); Lipid peroxidation (mg DW/ml); Superoxide (mg DW/ml)

*Data are referred to DW (dry weight) and the values are the average of five determinations (± S.D.)
6. Experimental

6.1 General Experimental Conditions

6.1.1 Physical Constants

Melting points (corrected and uncorrected) were determined in glass capillary tubes using Buchi B-545 melting point apparatus. Optical rotations were measured in Jasco DIP-360 digital polarimeter.

6.1.2 Spectroscopy

Ultraviolet (UV) spectra's were recorded in methanol in nm on Specard S 100. Infrared (IR) were recorded on a Bruker Vector 22 spectrometer as KBr pellets with absorption given in cm$^{-1}$. $^1$H NMR and $^{13}$C NMR, COSY, HMBC, HMQC, HSQC were run on 200 and 500 MHz Bruker Daltonics spectrometers respectively. The chemical shifts ($\delta$) are reported in ppm relative to tetramethylsilane (TMS) as internal standard and coupling constants were measured in Hz. Mass spectra were recorded by using Bruker Daltonics electrospray ionization and on a Finnigan MAT 311 mass spectrometer with MASPEC data system. Peak matching and field desorption (FD MS) experiments were performed on a Finnigan MAT 312 mass spectrometer. High-resolution mass measurements were carried out on Jeol JMS HX 110 mass spectrometer.

6.1.3 Chromatography

Column chromatography was carried out on silica gel (Qualigens, 60-120 & 120-240 mesh). Precoated silica gel preparative plates (20 x 20, 0.5 mm thick, Qualigens) were used for isolation. The purity of samples was also checked on the same precoated plates. High performance liquid chromatography (HPLC) was performed on Shimadzu instrument using RP-18 column. Solvents LR grade (Qualigens) were used for isolation.
HPLC solvents (Ranbaxy) were used for qualitative and quantitative analysis. Acetic anhydride and pyridine. DMP (Ranbaxy) were used for acetylation. Drying agents used CaCl$_2$, K$_2$CO$_3$, CaCO$_3$, CaO, Na$_2$SO$_4$, KOH (Qualigens). Sulphuric and hydrochloric acid (Qualigens) were used for hydrolysis.

6.1.4 Detection of Compounds on Chromatograms

Thin layer chromatographic (TLC) plates were viewed with ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots. Ceric sulphate, sulphuric acid and FeCl$_3$ (Qualigens) were used as spraying reagents to visualize the spots. Iodine (Merck) was used to detect the spots.

6.2 Plant Material

The aerial part and stem bark of *Euonymus hamiltonianus* Wall. was collected from Naranag (Ganderbal), Srinagar in October 2004 and the aerial part and roots of the plant *Lespedeza juncea* Pers. (Fabaceae) collected from hilly areas of Shopian (July 2005), Pulwama (3000-4500 m), Kashmir, India. Voucher specimens were deposited in the herbarium of the institute (IIIM).

6.3 Extraction and Isolation

The shade dried aerial part of *Euonymus hamiltonianus* Wall. (family Celastraceae; 2 Kg) was chopped and soaked in hexane (5L x 2) at room temperature for 30 hrs. The defatted plant material was extracted with ethylacetate (7.5L x 3) for 40 hrs at room temperature for 40 hrs and the resulting extract was concentrated to a gum (58 g). The remaining plant material (1.9 Kg) was extracted with methanol (10L x 4) at room temperature for 40 hrs, the resulting extract was reduced to a manageable residue to get gummy material about 102 g.
The methanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form slurry. This slurry was loaded onto a silica gel column (60-120 mesh, 500 g). The column was eluted with EtOAc:MeOH (90:10; v/v) to get 20 fractions each 150 ml. These fractions were combined to get a single fraction Fr-1. Polarity of ethylacetate was increased to EtOAc:MeOH (75:25; v/v) to get 15 fractions each 150 ml, all these fractions were combined as Fr-2. The polarity of eluted solvent was then increased to 50% EtOAc:MeOH (50:50; v/v) to get 15 fractions each 150 ml. These fractions were combined as Fr-3. The polarity was then increased to EtOAc:MeOH (25:75; v/v) to get 20 fractions each 100 ml. All these fractions were combined to get Fr-4. The repeated column chromatography of Fr-2 with solvents CHCl₃, EtOAc and MeOH afforded a pinkish amorphous powder of Euonidiol (1), which was again recrystallized from pure methanol. The repeated column chromatography of Fr-4 with solvents CHCl₃, EtOAc and MeOH of increasing polarity afforded a white amorphous powder of Euoniside (2), which was again recrystallized from pure methanol.

6.3.1 Euonidiol (1)

IUPAC Name: (2S, 3R)-3-hydroxy-2-[1-hydroxy-1-methyl ethyl]-2,3-dihydro-8H-furo[3,2-h] chromen-8-One.

Physical State: Amorphous pinkish powder (32 mg), crystallized as needles in methanol.

Melting point: 166-167 °C.

Rf: 0.52 (CHCl₃:MeOH, 9.5:0.5 v/v).

[α]D²⁵ (c 0.25, MeOH): + 88°.

IR (KBr, \( \nu \), cm\(^{-1} \)): 3457.5, 2973.3, 1712.3, 1619.4, 1489.5, 1399.6, 1350.4, 1080.0, 852.4, 770.3.

**ESI-MS:** m/z 285 [M+Na], (calculated for \( C_{14}H_{14}O_5Na \)), 263 [M+H], (calculated for \( C_{14}H_{14}O_3H \)).

**Table-17:** Flow chart showing extraction and isolation of aerial part of *Euonymus hamiltonianus* Wall.

---

*CC: Column Chromatography; RCC: Re-column chromatography*
\(^1\)H-NMR (200 MHz, DMSO, \(\delta\), ppm, J/Hz): 6.24 (1H, d, J = 9.5, H-3), 7.90 (1H, d, J = 9.5, H-4), 7.56 (1H, d, J = 8.4, H-5), 6.99 (1H, d, J = 8.4, H-6), 4.40 (1H, d, J = 6.0, H-2'), 5.68 (1H, d, J = 6.0, H-3'), 1.47 (3H, s, H-5'), 1.52 (3H, s, H-6').

\(^13\)C-NMR (125 MHz, DMSO, \(\delta\), ppm): 163.2 (C-2), 112.4 (C-3), 145.3 (C-4), 131.7 (C-5), 123.0 (C-6), 117.2 (C-7), 160.5 (C-8), 151.4 (C-9), 113.3 (C-10), 71.6 (C-2'), 92.6 (C-3'), 68.6 (C-4'), 26.7 (C-5'), 27.4 (C-6').

\(^13\)C NMR-DEPT (125 MHz, DMSO, \(\delta\), ppm): 112.4 (C-3), 145.3 (C-4), 131.7 (C-5), 123.2 (C-6), 71.1 (C-2'), 92.5 (C-3'), 26.7 (C-5'), 27.4 (C-6').

6.3.2 Euoniside (2)

IUPAC Name: 6,8-dimethoxy-7-[3,4,5-trihydroxy-6-(hydroxy methyl tetrahydro-2H-pyran-2-yl) oxy]-2H-chromen-2-One.

Physical State: White amorphous powder (30 mg), recrystallised from methanol.

Melting point: 198-199 °C.

\(R_f\) : 0.52 (CHCl\(_3\):MeOH, 7:3 v/v).

\([\alpha]_D^{25}\) (c 0.40, MeOH): +212°

UV (MeOH, \(\lambda_{max}, \text{nm}\)): 220.2, 242.1, 291.2, 302.2 (log \(\epsilon\) 4.32, 3.78, 4.17, 4.19).

IR (KBr, \(\nu, \text{cm}^{-1}\)): 3572.2, 2954.4, 1717.1, 1642.6, 1573.7, 1408.5, 1073.4, 805.2, 725.5.

ESI-MS: m/z 407.1 [M+Na], (calculated for C\(_{17}\)H\(_{20}\)O\(_{10}\)Na).

\(^1\)H-NMR (200 MHz, DMSO, \(\delta\), ppm, J/Hz): 6.41 (1H, d, J = 9.5, H-3), 7.97 (1H, d, J = 9.5, H-4), 7.13 (1H, s, H-5), 5.15 (1H, d, J = 7.8, H-1'), 4.03 (1H, m, H-2').
(1H, m, H-3'), 4.16 (1H, m, H-4'), 4.31 (1H, m, H-5'), 3.92 (1H, d, J 7.7, H-6'), 3.82 (3H, s, -OCH$_3$-8), 3.91 (3H, s, -OCH$_3$-6).

$^{13}$C-NMR (125 MHz, DMSO, $\delta$, ppm): 160.2 (C-2), 115.0 (C-3), 144.9 (C-4), 115.9 (C-5), 142.2 (C-6), 142.7 (C-7), 140.9 (C-8), 149.9 (C-9), 115.2 (C-10), 102.6 (C-1'), 74.6 (C-2'), 77.9 (C-3'), 70.4 (C-4'), 76.9 (C-5'), 61.7 (C-6'), 57.1 (-OCH$_3$-8), 61.3 (-OCH$_3$-6).

$^{13}$C NMR-DEPT (125 MHz, DMSO, $\delta$, ppm): 115.3 (C-3), 144.9 (C-4), 115.0 (C-5), 102.6 (C-1'), 74.4 (C-2'), 78.0 (C-3'), 70.4 (C-4'), 77.0 (C-5'), 61.7 (C-6'), 57.1 (OCH$_3$-8), 61.8 (OCH$_3$-6).

The ethylacetate extract was dissolved in minimum amount of ethylacetate and adsorbed on silica gel to form slurry. This slurry was loaded onto silica gel column (60-120 mesh, 500 g). The column was eluted with CH$_2$Cl$_2$:EtOAc (75:25; v/v) to get 25 fractions each 150 ml. These fractions were combined to get a single fraction Fr-1. Polarity of dichloromethane was increased to CH$_2$Cl$_2$:EtOAc (50:50; v/v) to get 15 fractions each 150 ml, all these fractions were combined as Fr-2. The polarity of solvent was then increased to CH$_2$Cl$_2$:EtOAc (25:75; v/v) to get 10 fractions each 150 ml. These fractions were combined as Fr-3. The repeated column chromatography of Fr-3 yielded yellow amorphous powder of Luteolin 7-methyl ether (3).
Table-18: Flow chart showing extraction and isolation of aerial part of *Euonymus hamiltonianus* Wall.

![Flow chart](image)

6. 3. 3 Luteolin 7-methyl ether

**IUPAC Name:** 2-(3', 4'-dihydroxy)-5-hydroxy-7-methoxy- 4H-1-benzopyran-4-one

**Physical State:** Yellow amorphous powder (18 mg).

**Melting point:** 242-243 °C.

**R<sub>r</sub>** : 0.48 (EtOAc: Pet.Ether; 9:1, v/v).
UV (MeOH, λ<sub>max</sub>, nm): 218.2, 280.4, 291.6, 351.1 (log ε 4.37, 3.88, 4.02, 4.22).

IR (KBr, v, cm<sup>-1</sup>): 3392.6, 2994.0, 1653.4, 1034.7, 842.2, 765.9, 687.6.

ESI-MS: m/z 301 [M+H].

<sup>1</sup>H NMR (200 MHz, DMSO, δ, ppm, J/Hz): 6.75 (1H, s, H-3), 6.21 (1H, d, J = 1.9, H-6), 6.49 (1H, d, J = 1.9, H-8), 7.43 (1H, m, J=2.8, H-2'), 7.10 (1H, d, J= 8.6, H-5'), 7.53 (1H, m, J= 2.8, H-6'). 3.84 (3H, s, OCH<sub>3</sub> -7).

<sup>13</sup>C NMR (125 MHz, DMSO, δ, ppm): 157.3 (C-2), 108.3 (C-3), 182.4 (C-4), 156.3 (C-5), 98.4 (C-6), 158.6 (C-7), 99.4 (C-8), 145.2 (C-9), 152.3 (C-10), 119.3 (C-1'), 117.3 (C-2'), 141.2 (C-3'), 145.3 (C-4'), 115.3 (C-5'), 127.3 (C-6'), 56.2 (OCH<sub>3</sub>-7).

The stem bark (5 Kg) of *Euonymus hamiltonianus* Wall. was collected, shade air dried and finely powdered plant material was extracted exhaustively with hexane (5L x 3) for 28 hrs. The remaining plant material was dried and extracted with ethylacetate (10L x 4) for 48 hrs. The ethylacetate extract was concentrated under reduced pressure to give a crude extract 124 g. The ethylacetate extract (124 g) was dissolved in minimum amount of chloroform and adsorbed on silica gel to form slurry. The air dried slurry was subjected to silica gel column chromatography. The column was fractionated with different percentage of dichloroform and ethylacetate to get fractions Fr-1 to Fr-6.

Fr-1 was obtained by eluting the column with CH<sub>2</sub>Cl<sub>2</sub>:EtOAc (75:25; v/v) by combining 15 fractions each 150 ml. Another 20 fractions each eluted with CH<sub>2</sub>Cl<sub>2</sub>:EtOAc (50:50; v/v) to get Fr-2. Another four fractions (Fr-3 to Fr-4) were obtained by eluting column with CH<sub>2</sub>Cl<sub>2</sub>:EtOAc (25:75; v/v), CHCl<sub>3</sub>:EtOAc (75:25; v/v), CHCl<sub>3</sub>:EtOAc (50:50; v/v) and CHCl<sub>3</sub>:EtOAc (25:75; v/v) each 150 ml respectively.
Table-19: Flow chart showing extraction and isolation of stem bark of *Euonymus hamiltonianus* Wall.

![Flow chart showing extraction and isolation of stem bark of Euonymus hamiltonianus Wall.](chart)

Recolumn chromatography of fraction Fr-3 with CH$_2$Cl$_2$:EtOAc (7:3; v/v) afforded a white crystalline powder of 19α-glutin-5-ene-19-ol (4).

**6. 3. 4 19α-glutin-5-ene-19-ol (4)**

**IUPAC Name:** 19α-glutin-5-ene-19-ol.

**Physical State:** White crystalline powder (63 mg).
Melting point: 178-79 °C.

Rf: 0.51 (CH₂Cl₂:EtOAc, 8:2 v/v).

[α]D²² (c 0.20, CHCl₃): +119.4°

IR (KBr, v, cm⁻¹): 3429.9, 2960.0, 1596.1, 1465.3, 1382.5, 1242.4, 1192.5, 1050.5, 957.5, 838.4.

¹H NMR (500 MHz, CDCl₃, δ, ppm, J/Hz): 5.38 (1H, d, J= 3.8, H-6), 3.58 (1H, d, J=3.8, H-19), 2.32 (2H, m, H-7), 2.30 (2H, m, H-10), 1.65 (1H, d, J=3.8, H-18), 1.62 (1H, s, H-8), 1.51 (1H, d, J= 12.1, H-12), 1.48 (1H, dd, J=11.2, 5.0, H-16), 1.46 (2H, dd, J=11.2, 5.0, H-15), 1.37 (2H, d, J=12.1, H-22), 1.29 (2H, d, J=12.1, H-21), 1.26 (2H, m, H-1), 1.22 (2H, m, H-2), 1.20 (2H, m, H-3), 1.18 (2H, d, J=10.5, H-11), 0.94 (3H, s, H-23), 0.92 (3H, s, H-26), 0.89 (3H, s, H-29), 0.88 (3H, s, H-30), 0.86 (3H, s, H-28), 0.85 (3H, s, H-25), 0.83 (3H, s, H-27), 0.71 (3H, s, H-24).

¹³C NMR (125 MHz, CDCl₃, δ, ppm): 42.1 (C-1), 38.2 (C-2), 33.3 (C-3), 39.9 (C-4), 142.2 (C-5), 120.0 (C-6), 55.2 (C-7), 35.1 (C-8), 33.1 (C-9), 50.1 (C-10), 29.1 (C-11), 33.3 (C-12), 42.4 (C-13), 40.4 (C-14), 28.1 (C-15), 24.2 (C-16), 36.1 (C-17), 39.4 (C-18), 70.5 (C-19), 32.2 (C-20), 22.2 (C-21), 25.1 (C-22), 28.1 (C-23), 18.2 (C-24), 17.1 (C-25), 18.5 (C-26), 19.1 (C-27), 20.2 (C-28), 28.4 (C-29), 22.4 (C-30).

Recolumn chromatography of fraction Fr-5 with CHCl₃:EtOAc (7.5:2.5; v/v) afforded a white amorphous powder of 2β,15α,21β -glutin -11-ene -2,15,21-triol (5).

6.3.5 2β,15α,21β -glutin -11-ene -2,15,21-triol (5)

IUPAC Name: 2β,15α,21β -glutin -11-ene -2,15,21-triol

Physical State: White amorphous powder (54 mg).
Melting point: 180-181 °C.

$[\alpha]_D^{22}$ (c 0.20, CHCl$_3$): -68.2°

$R_f$: 0.53 (CHCl$_3$:EtOAc 8:2 v/v).

IR (KBr, $\nu$, cm$^{-1}$): 3421.2, 2948.0, 1576.6, 1460.2, 1346.8, 1233.3, 1132.4, 1072.2, 930.8, 852.2.

$^1$H NMR (500 MHz, CDCl$_3$, $\delta$, ppm, J/Hz): 5.50 (1H, d, J=11.2, H-12), 5.39 (1H, d, J=11.2, H-11), 3.55 (1H, d, J=5.8, H-15), 3.54 (1H, m, H-2), 3.52 (1H, dd, J = 10.5 4.0, H-21), 1.89 (2H, d, J= 4.4, H-19), 1.86 (1H, d, J = 4.4, H-18), 1.62 (1H, m, H-8) 1.58 (2H, m, H-7), 1.28 (2H, m, H-16), 1.23 (2H, m, H-1), 1.20 (2H, m, H-3), 1.19 (2H, dd, J=12.0 4.0, H-22), 1.17 (2H, m, H-10), 0.97 (3H, s, H-23), 0.92 (3H, s, H-26), 0.89 (1H, s, H-5), 0.89 (3H, s, H-29), 0.88 (3H, s, H-28), 0.87 (2H, m, H-6), 0.86 (3H, s, H-30), 0.85 (1H, s, H-25), 0.83 (1H, s, H-27), 0.72 (3H, s, H-24).

$^{13}$C NMR (125 MHz, CDCl$_3$, $\delta$, ppm): 31.1 (C-1), 72.5 (C-2), 29.2 (C-3), 37.0 (C-4), 55.2 (C-5), 18.2 (C-6), 31.9 (C-7), 41.0 (C-8), 44.2 (C-9), 38.2 (C-10), 123.2 (C-11), 142.3 (C-12), 42.2 (C-13), 30.2 (C-14), 71.1 (C-15), 31.1 (C-16), 36.4 (C-17), 45.8 (C-18), 48.4 (C-19), 33.3 (C-20), 70.7 (C-21), 20.2 (C-22), 27.1 (C-23), 19.1 (C-24), 18.1 (C-25), 20.1 (C-26), 19.8 (C-27), 20.3 (C-28), 28.1 (C-29), 25.2 (C-30).

Recolumn chromatography of fraction Fr-6 with CHCl$_3$:EtOAc (9:1, v/v) afforded a white amorphous powder of 2β,19α-glutin-7,21-diene-2,19-diol (6).
Melting point: 165-166°C.

\[ \alpha \]_D^22 (c 0.25, CHCl_3): -91.4°

R_f: 0.49 (CHCl_3:EtOAc, 8:2 v/v).

**IR (KBr, v, cm\(^{-1}\)):** 3430.2, 2941.0, 1587.6, 1457.3, 1343.6, 1229.7, 1119.5, 1073.5, 945.6, 869.6.

**\(^1\)H NMR (500 MHz, CDCl_3, \(\delta\), ppm, J/Hz):** 5.41 (1H, dd, J =11.5, 5.5, H-7), 5.19 (1H, d, J = 11.2, H-22), 5.16 (1H, d, J= 11.2, H-21), 3.53 (1H, m, H-2), 3.51 (1H, d, J= 3.7, H-19), 2.34 (2H, m, H-6), 1.81 (2H, dd, J=10.5, 3.6, H-16), 1.78 (2H, dd, J=10.5, 3.6, H-15), 1.63 (1H, d, J = 3.7, H-18), 1.53 (2H, dd, J=10.6 5.2, H-12), 1.50 (1H, m, H-10), 1.22 (2H, dd, J=10.6 5.2, H-11), 1.21 (2H, m, H-3), 1.12 (2H, m, H-1), 0.96 (3H, s, H-23), 0.92 (3H, s, H-28), 0.91 (3H, s, H-26), 0.91 (3H, s, H-27), 0.89 (3H, m, H-5), 0.89 (3H, s, H-29), 0.87 (3H, s, H-30), 0.85 (3H, s, H-25), 0.75 (3H, s, H-24).

**\(^1\)3C NMR (125 MHz, CDCl_3, \(\delta\), ppm):** 31.2 (C-1), 71.2 (C-2), 29.3 (C-3), 38.2 (C-4), 50.1 (C-5), 38.2 (C-6), 125.0 (C-7), 140.4 (C-8), 43.3 (C-9), 37.6 (C-10), 30.3 (C-11), 34.3 (C-12), 41.1 (C-13), 41.1 (C-14), 29.3 (C-15), 25.3 (C-16), 35.6 (C-17), 45.5 (C-18), 74.1 (C-19), 33.2 (C-20), 127.1 (C-21), 126.3 (C-22), 26.3 (C-23), 20.2 (C-24), 19.2 (C-25), 21.2 (C-26), 19.2 (C-27), 20.2 (C-28), 27.6 (C-29), 24.9 (C-30).

The shade dried aerial part of *Lespedeza juncea* (1.8 Kg) was chopped and soaked in hexane (5L x 4) at room temperature for 48 hrs. The defatted plant material was extracted with methanol (7.5L x 4) at room temperature for 72 hrs and the resulting extract was concentrated to a gum (92 g). The methanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form slurry. This slurry was
loaded onto a silica gel column (60-120 mesh, 500 g). The column was eluted with CHCl₃:MeOH (90:10; v/v) to get 20 fractions each 150 ml. These fractions were combined to get a single fraction Fr-1. Polarity of chloroform was increased to CHCl₃:MeOH (75:25; v/v) to get 15 fractions each 150 ml, all these fractions were combined as Fr-2. The polarity of eluted solvent was then increased to CHCl₃:MeOH (50:50; v/v) to get 15 fractions each 150 ml. These fractions were combined as Fr-3. The polarity was then increased to CHCl₃:MeOH (25:75; v/v) to get 20 fractions each 100 ml. All these fractions were combined to get Fr-4. The polarity was then increased to CHCl₃:MeOH (15:85; v/v) to get 15 fractions each 100 ml. All these fractions were combined to get Fr-5. The repeated column chromatography of Fr-3 with solvents CHCl₃ and MeOH afforded a yellow amorphous powder of Lesjunceol (7), which was again recrystallized from pure methanol. The repeated column chromatography of Fr-5 with solvents CHCl₃, EtOAc and MeOH of increasing polarity afforded a white amorphous powder of Lesjuncerol (8), which was again recrystallized from pure methanol.

6.3.7 Lesjunceol (7)

IUPAC Name: 2, 5, 7, 9-tetrahydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone

Physical State: Light yellow powder (63 mg), crystallized as needles in methanol.

Melting point: 240.2 °C.

[α]D²⁵ (c 0.30, MeOH): +102°

Rf : 0.50 (CHCl₃:MeOH, 8:2 v/v).


IR (KBr, ν, cm⁻¹): 3450.5, 2954.3, 1310.2, 702.3, 668.0.

ESI-MS: m/z 371 [M+Na], (calculated for C₁₅H₁₆O₈).
Table-20: Flow chart showing extraction and isolation from aerial parts of *Lespedeza juncea* Pers.

**Lespedeza juncea** Pers.

Stem bark (1.8 Kg)

Extraction with methanol (7.5L x 4 wash)

Methanol extract (92 g)

Column chromatography (CC)

Fr1
CH2Cl2:MeOH (90:10)

Fr2
CH2Cl2:MeOH (75:25)

Fr3
CH2Cl2:MeOH (50:50)

Fr4
CH2Cl2:MeOH (25:75)

RCC

Fr5
CH2Cl2:MeOH (15:85)

RCC

*CC: Column Chromatography; RCC: Re-column chromatography

\(^1\text{H-NMR (500 MHz, DMSO, } \delta, \text{ ppm, J/Hz):} 4.14 (1H, d, J = 7.7, H-2), 3.34 (1H, dd, J = 7.7, 4.3, H-3), 6.10 (1H, s, H-8), 5.11 (1H, J = 7.8, H-9), 7.41 (1H, J = 8.5, H-2'), 7.34 (1H, J = 8.5, H-3'), 7.32 (1H, d, J = 8.6, H-5'), 7.38 (1H, d, J = 8.6, H-6'), 3.82 (3H, s, -OCH\textsubscript{3}).
13C-NMR (125 MHz, DMSO, δ, ppm): 67.1 (C-2), 51.2 (C-3), 198.2 (C-4), 102.2 (C-4a), 154.2 (C-5), 128.1 (C-6), 158.3 (C-7), 94.5 (C-8), 157.5 (C-8a), 72.2 (C-9), 139.2 (C-1'), 129.5 (C-2'), 128.4 (C-3'), 155.2 (C-4'), 127.5 (C-5'), 135.5 (C-6'), 60.1 (OCH3-6).

6.3.8 Lesjuncerol (8)

IUPAC Name: 2, 5, 7, 9-tetrahydroxy-6-methoxy-8-methyl-3-(4'-hydroxybenzyl)-4-chromanone

Physical State: White amorphous powder (52 mg), crystallized as needles in methanol.

Melting point: 190.4°C.

[α]D 25° (c 0.30, MeOH): +114°

Rf: 0.49 (CHCl3:MeOH, 7.2:2.5 v/v).

UV (MeOH, λmax, nm): 243.3, 280.4, 308.5 (log ε 4.02, 3.99, 4.17).

IR (KBr, ν, cm⁻¹): 3410.4, 2956.2, 1692.3, 1303.3, 735.5, 670.2.

ESI-MS: m/z 385 [M+Na], (calculated for C18H18O8)

1H-NMR (500 MHz, DMSO, δ, ppm, J/Hz): 4.10 (1H, d, J = 7.6, H-2), 3.25 (1H, dd, J = 7.6, 4.2, H-3), 5.25 (1H, J = 7.8, H-9), 7.31 (1H, J = 8.5, H-2'), 7.21 (1H, J = 8.5, H-3'), 7.25 (1H, J = 8.6, H-5'), 7.32 (1H, J = 8.6, H-6'). 3.78 (3H, s, -OCH3), 2.45 (3H, s, -CH3).

13C-NMR (125 MHz, DMSO, δ, ppm): 68.2 (C-2), 52.2 (C-3), 191.8 (C-4), 103.2 (C-4a), 154.5 (C-5), 128.2 (C-6), 157.3 (C-7), 98.5 (C-8), 150.2 (C-8a), 75.1 (C-9), 139.1 (C-1'), 130.1 (C-2'), 127.7 (C-3'), 154.5 (C-4'), 129.1 (C-5'), 134.2 (C-6'), 59.4 (OCH3-6), 15.2 (CH3-8).

The shade dried root of Lespedeza juncea (500 g) was chopped and soaked in methanol (8L x 4) at room temperature for 72 hrs and the resulting extract was concentrated to a gum (42 g). The methanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form slurry. This slurry was loaded onto silica
gel column (60-120 mesh, 500 g). The column was eluted with CHCl₃:MeOH (75:25; v/v) to get 25 fractions each 150 ml. These fractions were combined to get a single fraction Fr-1. Polarity of chloroform was increased to CHCl₃:MeOH (50:50; v/v) to get 25 fractions each 150 ml, all these fractions were combined as Fr-2. The polarity of eluent was then increased to CHCl₃:MeOH (25:75; v/v) to get 25 fractions each 150 ml. These fractions were combined as Fr-3. The repeated column chromatography of Fr-2 with solvents CHCl₃ and MeOH afforded a colorless amorphous powder of Meoside A (9), which was again recrystallized from pure methanol. The repeated column chromatography of Fr-3 with solvents CHCl₃, EtOAc and MeOH of increasing polarity afforded a white amorphous powder of Meoside B (10), which was again recrystallized from pure methanol.

**6. 3. 9  Meoside A (9)**

**Physical State:** Colorless amorphous powder (35 mg), crystallized as needles in methanol.

**Melting point:** 183.3 °C.

**Rₚ:** 0.47 (CHCl₃:MeOH, 1:1 v/v).

**[α]₀̂̂₅ (c 0.25, EtOH):** - 148°

**UV (MeOH, λmax, nm):** 281.5.

**IR (KBr, ν, cm⁻¹):** 3450.3, 2929.8, 1599.1, 1580.3, 1523.8, 1453.8, 983.3.

**ESI-MS:** m/z 505 [M+H], calculated for C₂₂H₃₂O₁₃.
Table-21: Flow chart showing extraction and isolation from root of *Lespedeza juncea* Pers.

\[\text{Lespedeza juncea Pers.} \]
\[\text{Root (500 g)}\]
\[\text{Extraction with methanol (8L x 4 wash)}\]
\[\text{Methanol extract (42 g)}\]
\[\text{Column chromatography (CC)}\]

\[\begin{align*}
\text{Fr-1} & \quad \text{CHCl}_3;\text{MeOH} \\
& \quad (75:25) \\
\text{Fr-2} & \quad \text{CHCl}_3;\text{MeOH} \\
& \quad (50:50) \\
\text{Fr-3} & \quad \text{CHCl}_3;\text{MeOH} \\
& \quad (25:75) \\
\text{RCC} & \quad (9) \\
\text{RCC} & \quad (10)
\end{align*}\]

*CC: Column Chromatography; RCC: Re-column chromatography

\[^{1}H \text{ NMR (500 MHz, CD}_3\text{OD, } \delta, \text{ ppm, J/Hz):} \]
\[7.12 \ (1\text{H, s, H-3}), \ 6.99 \ (1\text{H, s, H-6}), \ 6.32 \ (1\text{H, dd, J = 2.1, 14.1, H-7}), \ 6.28 \ (1\text{H, m, H-8}), \ 1.90 \ (3\text{H, dd, J = 1.5, 7.8, H-9}), \ 3.82 \ (3\text{H, s, H-10}), \ 4.91 \ (1\text{H, d, J = 7.4, H-1'}), \ 3.47 \ (1\text{H, m, H-2'}), \ 3.46 \ (1\text{H, m, H-3'}), \ 3.42 \ (1\text{H, m, H-4'}), \ 3.65 \ (1\text{H, m, H-5'}), \ 4.10 \ (1\text{H, dd, J = 2.1, 12.3, H-6'α}), \ 3.78 \ (1\text{H, m, H-6'β}), \ 4.73 \ (1\text{H, d, J =} \]

146
7.1, H-1"), 3.52 (1H, m, H-2") 3.45 (1H, m, H-3"), 3.76 (1H, m, H-4"), 3.59 (1H, m, H-5"), 3.81 (1H, dd, J=3.2,12.1 H-6"a), 3.44 (1H, m, H-6"b).

$^{13}$C NMR (125 MHz, CD$_3$OD, δ, ppm): 146.1 (C-1), 130.0 (C-2), 119.2 (C-3), 149.4 (C-4), 148.2 (C-5), 100.1 (C-6), 132.1 (C-7), 125.2 (C-8), 19.2 (C-9), 55.6 (C-10), 101.6 (C-1'), 74.3 (C-2'), 77.7 (C-3'), 70.1 (C-4'), 78.4 (C-5'), 65.2 (C-6'), 102.8 (C-1'"), 71.2 (C-2'"), 73.2 (C-3'"), 69.6 (C-4'"), 74.3 (C-5'"), 65.4 (C-6'").

6.3.10 Meoside B (10)

Physical State: White amorphous powder (25 mg), crystallized as needles in methanol.

Melting point: 192.4°C.

Rf: 0.49 (CHCl$_3$:MeOH, 4:6 v/v).

UV (MeOH, $\lambda_{max}$, nm): 285.1.

IR (KBr, v, cm$^{-1}$): 3429.4, 2952.5, 1567.7, 1506.7, 1453.3, 922.4.

ESI-MS: m/z 521 [M+H], calculated for C$_{22}$H$_{32}$O$_{14}$

$[\alpha]_{D}^{25}$ (c 0.25, MeOH): + 124°

$^1$H NMR (500 MHz, CD$_3$OD, δ, ppm, J/Hz): 3.00 (2H, t, H-2), 1.68 (2H, m, H-3), 1.01 (3H, t, H-4), 6.88 (1H, s, H-7). 6.98 (1H, s, H-10), 4.89 (1H, d, J = 7.3, H-1'), 3.41 (1H, m, H-2'), 3.39 (1H, m, H-3'), 3.42 (1H, m, H-4'), 3.32 (1H, m, H-5'), 4.11 (1H, dd, J = 3.0 11.3, H-6'α), 3.98 (1H, m, H-6'β). 4.96 (1H, d, J=7.2, H-1'"), 3.32 (1H, m, H-2'"), 3.29 (1H, m, H-3'"), 3.33 (1H, m, H-4'"), 3.18 (1H, m, H-5'"), 4.60 (1H, dd, J=3.2 11.1 H-6"α), 3.98 (1H, m, H-6"β).

$^{13}$C NMR (125 MHz, CD$_3$OD, δ, ppm): 205.6 (C-1), 47.2 (C-2), 18.2 (C-3), 15.4 (C-4), 115.7 (C-5). 155.8 (C-6). 98.4 (C-7), 154.2 (C-8), 156.6 (C-9), 125.6 (C-10), 102.6 (C-1'), 73.3 (C-2'), 77.9 (C-3'). 70.5 (C-4'). 78.7 (C-5'), 62.2 (C-6'), 100.8 (C-1'"), 72.2 (C-2'"), 75.2 (C-3'"), 70.6 (C-4'"), 76.3 (C-5'"), 61.8 (C-6'").