PART-I

Chemical Constituents of

Vitex peduncularis
Section 1: A brief review of phytochemicals reported from different Vitex species

Vitex, the largest genus of family Verbenaceae, comprises about 250 species mostly distributed in warm regions of Europe and temperate regions of Asia. In India about 13 species are found [1]. Most of the species are either trees or aromatic shrubs. Several Vitex species have been used as folk medicine in different countries for the treatment of various diseases and ailments. In India, Vitex agnus-castus, V. negundo, V. peduncularis, V. pubescens and V. trifolia are found throughout the country [2].

Fruits and leaves of Vitex agnus-castus have been used mainly in traditional medicine. Fruits have been used in the treatment of female diseases including menstrual disorders, premenstrual dysphoric disorder, hyperprolactinaemia infertility, acne, menopause, disrupted lactation, breast pain, cyclical mastalgia and inflammatory conditions, diarrhoea and flatulence and leaves are used for increasing milk [3-5]. The leaves and fruits of V. negundo (syn. V. inesia Lam.) have been used in folk medicine for treatment of headache, cold, migraine, eye pain, asthma, chronic bronchitis, gastrointestinal infections, catarrhal fever, dysmenorrhea, and as anthelmintic [6-8]. Infusion of leaves, root bark or young stem bark of V. peduncularis are useful in malarial and black water fever [2].

V. rotundifolia is widely used as folk medicine in Japan for headache, colds, migraine, eye pain, etc [9]. V. trifolia has been used as an anti-inflammatory and sedative for headache, rheumatism and the common cold in Asian countries [10].

Iridoids, flavonoids, diterpenoids, lignans and essential oils are the major classes of phytochemicals of this genus, Vitex. The list of the phytochemicals reported from different Vitex species is provided in Table 1.1.
Table 1.1. List of phytochemicals reported from different *Vitex* species

<table>
<thead>
<tr>
<th>Str. No.</th>
<th>Name and structure</th>
<th>Plant source(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[A] Iridoids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Nishindaside</td>
<td><em>V. negundo</em></td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>V. cannabifolia</em></td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Nishindaside" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Agnuside</td>
<td><em>V. altissima</em></td>
<td>[11,13,14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>V. agnus-castus</em></td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>V. cannabifolia</em></td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Agnuside" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Cis</em>-Eurostoside</td>
<td><em>V. rotundifolia</em></td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Cis-Eurostoside" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Viteoid II</td>
<td><em>V. rotundifolia</em></td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Viteoid II" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10-<em>O</em>-Vanilloylauccubin</td>
<td><em>V. cannabifolia</em></td>
<td>[12]</td>
</tr>
</tbody>
</table>
6. 2'-O-p-Hydroxybenzoyl-6'-O-trans-caffeoyl-8-epi-loganic acid  
   \[ \text{V. altissima} \] [15]

7. 2'-O-p-Hydroxybenzoyl-8-O-epi-loganic acid  
   \[ \text{V. altissima} \] [15]

8. Agnucastoside D  
   \[ \text{V. agnus-castus} \] [18]

9. Agnucastoside C  
   \[ \text{V. agnus-castus} \] [18]

10. Agnucastoside A  
    \[ \text{V. agnus-castus} \] [18]
11 Agnustoside B

\[
\text{V. agnus-castus} \quad [18]
\]

12 6'-O-trans-Feruloylnegundoside

\[
\text{V. altissima} \quad [15]
\]

13 6'-O-trans-Caffeoylnegundoside

\[
\text{V. altissima} \quad [15]
\]

14 Negundoside

\[
\text{V. negundo} \quad [20]
\]

\[
\text{V. altissima} \quad [15]
\]

15 6'-O-p-Hydroxybenzoyl mussaenosidic acid

\[
\text{V. negundo} \quad [21]
\]
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound Name</th>
<th>Species</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>16</td>
<td>Pedunculariside</td>
<td><em>V. peduncularis</em></td>
<td>[19]</td>
</tr>
<tr>
<td>17</td>
<td>Limoniside</td>
<td><em>V. limonifolia</em></td>
<td>[19a]</td>
</tr>
<tr>
<td>18</td>
<td>Geniposide</td>
<td><em>V. cannabifolia</em></td>
<td>[12]</td>
</tr>
<tr>
<td>19</td>
<td>2'-O-p-Hydroxybenzoylgardoside</td>
<td><em>V. altissima</em></td>
<td>[15]</td>
</tr>
<tr>
<td>20</td>
<td>Viteoid 1</td>
<td><em>V. rotundifolia</em></td>
<td>[17]</td>
</tr>
</tbody>
</table>
[B] Diterpenoids

21 (rel $5S$, $6R$, $8R$, $9R$, $10S$) 6-Acetoxy-9-hydroxy-13(14)-labden-16, 15-olide

$V. \ rotundifolia$ [22] $V. \ trifolia$ [25]

22 (rel $5S$, $6S$, $8R$, $9R$, $10S$)-6-Acetoxy-9-hydroxy-13(14)-labden-16, 15-olide

$V. \ rotundifolia$ [22]


$V. \ rotundifolia$ [22]

24 Vitexilactone

$V. \ rotundifolia$ [22] $V. \ trifolia$ [25] $V. \ agnus-castus$ [23]
Review on Different Vitex Species

25 Viteagnuside A  
\[ V. \ agnus-castus \] [23]

26 Viteoside A  
\[ V. \ rotundifolia \] [26]

27 (+) Polyalthic acid  
\[ V. \ rotundifolia \] [27]

28 Viterotulin A  
\[ V. \ rotundifolia \] [28]

29 Viterotulin B  
\[ V. \ rotundifolia \] [28]
Review on Different *Vitex* Species

30. Vitexilactone C  

![Vitexilactone C](image)

*V. negundu var. cannabifolia* [29]

31. 9-Hydroxy 13 (14)-labden-15,16-olide  

![9-Hydroxy 13 (14)-labden-15,16-olide](image)

*V. trifolia* [30]

32. Vitenegusin 1  

![Vitenegusin 1](image)

*V. agnus-castus* [31]

33. Vitexlactam A  

![Vitexlactam A](image)

*V. agnus-castus* [32]

34. *(rel 5S, 6R, 8R, 9R, 10S, 13S, 16S)-6-Acetoxy-9, 13-epoxy-16-methoxy labdan-15, 16-olide*  


*V. rotundifolia* [22]  

*V. agnus-castus* [23]
35. \((\text{rel} 5S, 6R, 8R, 9R, 10S, 13R, 16S)-\)Acetoxy-9, 13-epoxy-16-methoxy labdan-15, 16-oxide

\[ \text{V. rotundifolia} \quad [22] \]

\[ \text{V. agnus-castus} \quad [23] \]


\[ \text{V. rotundifolia} \quad [22] \]

\[ \text{V. agnus-castus} \quad [23] \]


\[ \text{V. rotundifolia} \quad [22] \]

\[ \text{V. agnus-castus} \quad [23, 24] \]

38. \((\text{rel} 5S, 8R, 9R, 10S, 13S, 15S, 16R)-\)9, 13; 15, 16-Diepoxy-15, 16-dimethoxy labdane

\[ \text{V. rotundifolia} \quad [22] \]
39. \((\text{rel } 5S, 8R, 9R, 10S, 13S, 15R, 16S)\)-9, 13; 15, 16-Diepoxy-15, 16-dimethoxy labdane

\[ V. \text{ rotundifolia} \] [22]

40. \((\text{rel } 5S, 8R, 9R, 10S, 13S, 15R, 16R)\)-9, 13; 15, 16-Diepoxy-15, 16-dimethoxy labdane

\[ V. \text{ rotundifolia} \] [22]

41. Previtexilactone

\[ V. \text{ trifolia} \] [25]

42. Viteagnusin E

\[ V. \text{ agnus-castus} \] [24]
43. Viteagnusin I  
\[ V. \text{agnus-castus} \quad [23] \]

44. Viteagnusin J  
\[ V. \text{agnus-castus} \quad [23] \]

45. Negundoal  
\[ V. \text{negundo} \quad [33] \]

46. Negundoin A  
\[ V. \text{negundo} \quad [34] \]

47. Negundoin B  
\[ V. \text{negundo} \quad [34] \]
48. Negundoin C  
V. negundo  [34]

49. Negundoin D  
V. negundo  [34]

50. Negundoin E  
V. negundo  [34]

51. Negundoin G  
V. negundo  [34]

52. Vitetrifolin D  
V. trifolia  [35]  
V. agnus-castus  [23]  
V. rotundifolia  [36]
53. Vitetrifolin E (= Vitexifolin E)  
\[ V. trifolia \] [35]

54. Vitetrifolin F (=Vitexifolin F)  
\[ V. trifolia \] [35]

55. Vitetrifolin G  
\[ V. trifolia \] [35]

56. Vitetrifolin H  
\[ V. rotundifolia \] [28]

57. 13-Hydroxy-5 (10),14-halimadien-6-one  
\[ V. trifolia \] [30]
58. 6α,7α-Diacetoxy 13-hydroxy-8(9),14-labdadiene  
\[ V. trifolia \] [30]

59. 3-Oxo-15,17,18-trihydroxy labda-7,13E-diene  
\[ V. caulisflora \] [37]

60. Viteaginusin A  
\[ V. agnus-castus \] [24]

61. Viteagnusin B  
\[ V. agnus-castus \] [24]

62. Viteagnusin C  
\[ V. agnus-castus \] [24]
63. Viteagnusin D  

\[ V. \text{agnus-castus} \quad [24] \]

64. \textit{8-epi-Sclareol}  

\[ V. \text{agnus-castus} \quad [23,24] \]

65. Vitexifolin A  

\[ V. \text{rotundifolia} \quad [36] \]

66. Vitexifolin B  

\[ V. \text{rotundifolia} \quad [36] \]

67. Norditerpene aldehyde A  

\[ V. \text{trifolia} \quad [25] \]
68. Norditerpene aldehyde B  
*V. trifolia*  
[25]

69. Vitetrifolin B  
*V. trifolia*  
[38]

70. Vitetrifolin C  
*V. trifolia*  
[38]

71. Rotundifuran  
*V. trifolia*  
[38]

72. Dihydrosolidagenone  
*V. trifolia*  
[38]
73. Vitedoin B  
\[ V. \text{negundo} \] [39]

74. Vitexifolin D  
\[ V. \text{rotundifolia} \] [36]

75. Vrinor-\(\gamma\)-lactone  
\[ V. \text{rotundifolia} \] [36]

76. \textit{iso}-Ambreinolide  
\[ V. \text{rotundifolia} \] [36]

77. Vitexifolin E  
\[ V. \text{rotundifolia} \] [36]
78. Vitexifolin C  \[ V. \textit{rotundifolia} \] [36]

79. Abietatriene 3β-ol  \[ V. \textit{trifolia} \] [38]

80. Vitetrifolin A  \[ V. \textit{trifolia} \] [38]

81. Negundoin F  \[ V. \textit{negundo} \] [34]
THESIS, PART-I

Review on Different Vitex Species

[C] Triterpenoids

82. Ursolic acid

\[
\text{\textit{V. negundo} \ [40]}
\]

83. 2α,3α-Dihydroxy-urs-12-en-28-oic acid

\[
\text{\textit{V. agnu-castus} \ [23]}
\]

84. 2α - Hydroxyursolic acid

\[
\text{\textit{V. agnus-castus} \ [23]}
\]

\[
\text{\textit{V. peduncularis} \ [41]}
\]

85. Selvin A

\[
\text{\textit{V. cienkowskii} \ [42]}
\]
86. $2\alpha,3\alpha$-Dihydroxyurs-12, 20 (30)-dien-28-oic acid 

87. $2\alpha,3\alpha,19\alpha$-Trihydroxyurs-12, 20 (30)-dien-28-oic acid 

88. Tormentic acid 

89. Negundonorin A
90. Negundonorin B  
\[ V. \text{negundo} \]  
[33]

91. Cannabifolin A  
\[ V. \text{negundo var. cannabifolia} \]

92. Cannabifolin B  
\[ V. \text{negundo var. cannabifolia} \]

93. Cannabifolin C  
\[ V. \text{negundo var. cannabifolia} \]
94. Cannabifolin D

\[ V.\ negundo\ var.\ cannabifolia \]

95. 2α,3α,24-Trihydroxyurs-12,20 (30)-dien-28-oic acid-28-O-β-D-glucopyranosyl ester

\[ V.\ negundo \]

96. Maslinic acid

\[ V.\ agnus-castus \]

97. 3-epi-Maslinic acid

\[ V.\ agnus-castus \]
98. Acetyloleanolic acid  

![Acetyloleanolic acid](image1)

**V. negundo** [40]

99. 3β-Acetoxy olean-12-en-27-oic acid  

![3β-Acetoxy olean-12-en-27-oic acid](image2)

**V. negundo** [45]

100. 2α,3α - Dihydroxyoleana-5, 12-dien-28-oic acid  

![2α,3α - Dihydroxyoleana-5, 12-dien-28-oic acid](image3)

**V. negundo** [45]

101. 2β, 3α – Diacetoxyoleana-5, 12-dien-28-oic acid  

![2β, 3α – Diacetoxyoleana-5, 12-dien-28-oic acid](image4)

**V. negundo** [45]
102. 2α, 3β - Diacetoxy-18-hydroxyoleana-5, 12-dien-28-oic acid  

V. negundo  [45]

103. 2α,3α,24-Trihydroxy olean-12-en-28-oic acid  

V. negundo var. cannabifolia  [43]

104. Cannabifolin E  

V. negundo var. cannabifolia  [43]

105. Cannabifolin F  

V. negundo var. cannabifolia  [43]
106. Betulinic acid  

\[ V. \text{negundo} \quad [40] \]

[\text{E}]  

Flavonoids

107. Apigenin  

\[ V. \text{pinnata} \quad [46] \]

108. Luteolin  

\[ V. \text{pinnata} \quad [46] \]

109. Luteolin-3'-O-Glc A-Me ester  

\[ V. \text{negundo} \quad [47] \]
110. Vitexin

```
\text{V. peduncularis \ [41]}
\text{V. negundo \ [48]}
```

111. Isovitexin

```
\text{V. negundo \ [48]}
```

112. Isoorientin

```
\text{V. cannabifolia \ [12]}
```

113. Orientin

```
\text{V. cannabifolia \ [12]}
```

114. 2"-O-p - Hydroxybenzoylorientin

```
\text{V. altissima \ [49]}
```
115. Saponaretin  
\[
\text{V. littoralis} \quad (= V. lucens) \quad [50]
\]

116. Schaftoside  
\[
\text{V. polygama} \quad [51]
\]

117. Carlinoside  
\[
\text{V. polygama} \quad [51]
\]

118. 6-Hydroxy quercetin-3,6,7,4'-tetramethyl ether  
\[
\text{V. negundo} \quad [52]
\]

119. Peduncularisin  
\[
\text{V. peduncularis} \quad [41]
\]
120. Pachypodol

\[
\text{MeO} - \text{O} - \text{Me}
\]
\[
\text{OH} - \text{O} - \text{OH}
\]

*V. peduncularis* [41]

121. Penduletin

\[
\text{MeO} - \text{O} - \text{Me}
\]
\[
\text{OH} - \text{O} - \text{Me}
\]

*V. agnus-castus* [53]

122. Casticin (=Vitexicarpin)

\[
\text{MeO} - \text{O} - \text{Me}
\]
\[
\text{OH} - \text{O} - \text{Me}
\]

*V. agnus-castus* [23]
*V. rotundifolia* [54]
*V. negundo* [55]

123. Artemetin

\[
\text{MeO} - \text{O} - \text{Me}
\]
\[
\text{OH} - \text{O} - \text{Me}
\]

*V. rotundifolia* [54]

124. 5, 4'-Dihydroxy-3, 6, 7, 8, 3'-penta methoxyflavone

\[
\text{MeO} - \text{O} - \text{Me}
\]
\[
\text{OH} - \text{O} - \text{Me}
\]

*V. cannabifolia* [12]
125. 5, 3'-Dihydroxy-7, 8, 4'-trimethoxyflavanone  
\[ V. \text{negundo} \quad [56] \]

126. 5, 3'-Dihydroxy-6, 7, 4'-trimethoxyflavanone  
\[ V. \text{negundo} \quad V. \text{rotundifolia} \quad [56, 36] \]

127. (2S) - 5 Hydroxy-7, 4'-dimethoxyflavanone  
\[ V. \text{quinata} \quad [49] \]

128. 2', 4'-Dihydroxy - 4, 6'-dimethoxychalcone  
\[ V. \text{leptobotrys} \quad [57] \]

129. 4'-Hydroxy - 4,2',6'-trimethoxychalcone  
\[ V. \text{leptobotrys} \quad [57] \]
130. 4,2',4', β - Tetrahydroxy - 6 -methoxy- α, β dihydrochalcone  

\[
\begin{array}{c}
\text{HO-}4' \\
\text{2'} \text{O} \\
\text{O} \\
\text{MeO} \quad 6 \\
\text{OH} \\
\end{array}
\]

\[\text{HO-}4' \quad \text{2'} \quad \text{O} \quad \text{MeO} \quad 6 \quad \text{OH} \]

\[\text{V. leptobotrys} \quad [57]\]

131. Vitexcarpan  

\[
\begin{array}{c}
\text{HO} \\
\text{O} \\
\text{O} \\
\text{O} \\
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\end{array}
\]

\[\text{V. agnus-castus} \quad [58]\]

[F] Lignans

132. Negundin A  

\[
\begin{array}{c}
\text{MeO} \\
\text{HO} \\
\text{O} \\
\text{Me} \\
\end{array}
\]

\[
\begin{array}{c}
\text{MeO} \\
\text{HO} \\
\text{O} \\
\end{array}
\]

\[\text{V. negundo} \quad [59]\]

133. Detetrahydroconidendrin  

\[
\begin{array}{c}
\text{MeO} \\
\text{OH} \\
\text{OMe} \\
\end{array}
\]

\[
\begin{array}{c}
\text{MeO} \\
\text{HO} \\
\text{OMe} \\
\end{array}
\]

\[\text{V. negundo} \quad [39]\]

\[\text{V. cannabifolia} \quad [12]\]
134. isomer of Methoxytetrahydro-
    conidendrin  \( V. \text{rotundifolia} \) [60]

135. Methoxytetrahydro-
    conidendrin  \( V. \text{rotundifolia} \) [60]

136. Vitedoamine A  \( V. \text{negundo} \) [39]

137. Vitexdoin I  \( V. \text{negundo} \) [61]
138. Vitexdoin F  
\[ \text{V. negundo} \quad [61] \]

139. Vitexdoin G  
\[ \text{V. negundo} \quad [61] \]

140. Vitexdoin H  
\[ \text{V. negundo} \quad [61] \]

141. Vitrofolal D  
\[ \text{V. rotundifolia} \quad [62] \]
142. Vitrofolal C  
\[ \text{V. rotundifolia} \quad [60] \]

143. Vitrofolal A  
\[ \text{V. rotundifolia} \quad [60] \]

144. Vitrofolal B  
\[ \text{V. rotundifolia} \quad [60] \]

145. Vitrofolal E  
\[ \text{V. negundo} \quad [39,59] \]
\[ \text{V. rotundifolia} \quad [62] \]
\[ \text{V. cannabifolia} \quad [12] \]
146. Vitrofolal F  
\[ V. negundo \ [39,59] \]
\[ V. rotundifolia \ [62] \]
\[ V. cannabifolia \ [12] \]

147. Negundin B  
\[ V. negundo \ [59] \]

148. (+) – Lyoniresinol  
\[ V. negundo \ [59] \]

149. 6-Hydroxy – 4(4-hydroxy-3-methoxy phenyl)-3-hydroxymethyl-7-methoxy-3, 4- dihydro 2- naphthaldehyde  
\[ V. negundo \ [39,63] \]
\[ V. cannabifolia \ [12] \]
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>150.</td>
<td>Vitedoin A</td>
<td>V. negundo [39], V. cannabifolia [12]</td>
<td></td>
</tr>
<tr>
<td>151.</td>
<td>Vitecannaside A</td>
<td>V. cannabifolia [12]</td>
<td></td>
</tr>
<tr>
<td>152.</td>
<td>Vitecannaside B</td>
<td>V. cannabifolia [12]</td>
<td></td>
</tr>
<tr>
<td>153.</td>
<td>Pinoresinol</td>
<td>V. cannabifolia [12]</td>
<td></td>
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<tr>
<td>154.</td>
<td>Vitelignin A</td>
<td>V. negundo [64]</td>
<td></td>
</tr>
</tbody>
</table>
155. Altissinone  
\[ V. cannabifolia \]  
![Altissinone](image)

156. 2α,3β-7-O-Methylcedrusin  
\[ V. negundo \]  
![2α,3β-7-O-Methylcedrusin](image)

157. Viterolignan A  
\[ V. rotundifolia \]  
![Viterolignan A](image)

158. Viterolignan B  
\[ V. rotundifolia \]  
![Viterolignan B](image)

159. 1,6-Dioxo-2 (3), 9 (10)-dehydrofurano-eremophilane  
\[ V. negundo \]  
![1,6-Dioxo-2 (3), 9 (10)-dehydrofurano-eremophilane](image)

[G] Sesquiterpenoids
160. 4,6-Dimethyl-11-dimethoxymethyl-1-oxo-4H, 3,2-dihydropyran 
V. negundo [66]

161. Negunfurol 
V. negundo [33]

162. β – Caryophyllene 
V. negundo [67]

163. Caryophyllene oxide 
V. negundo [67]

164. β – Selinene 
V. negundo [68]

165. α – Seliene 
V. negundo [68]
166. $4\alpha,10\alpha$ – Dihydroxyaromadendrane  

\[ V. \text{agnus-castus} \quad [23] \]

167. Germacrene D  

\[ V. \text{agnus-castus} \quad [23] \]

[H] Monoterpenoid

168. Sabiene  

\[ V. \text{negundo} \quad [67] \]

[I] Ecdysteroids

169. Ajugasterone C  

\[ V. \text{polygama} \quad [69] \\
V. \text{strickeri} \quad [70] \\
V. \text{doniana} \quad [71] \\
V. \text{scabra} \quad [72] \]
170. Tetraacetyljugasterone C  
\[ V. cienkowskii \quad [73] \]

171. Ajugasterone C monoacetonide  
\[ V. polygama \quad V. strickeri \quad [69] \quad [70] \]

172. 20-Hydroxyecdysone  
\[ V. cymosa \quad V. polygama \quad V. strickeri \quad V. scabra \quad [69] \quad [70] \quad [72] \]

173. Turkesterone  
\[ V. polygama \quad V. scabra \quad [69] \quad [72] \]
174. Abutasterone

\[
\text{Abutasterone} \quad \text{V. strickeri} \quad [70]
\]

175. Canescensterone

\[
\text{Canescensterone} \quad \text{V. canescens} \quad [74]
\]

176. 20 - Hydroxyecdysone-20, 22-monoacetonide

\[
\text{20 - Hydroxyecdysone-20, 22-monoacetonide} \quad \text{V. strickeri} \quad [70]
\]

177. Shidasterone

\[
\text{Shidasterone} \quad \text{V. doniana} \quad [71]
\]
178. 21–Hydroxyshidasterone \[V. \textit{doniana}\] [71]

179. Pinnatasterone \[V. \textit{scabra}\] [72]

180. 26–Hydroxypinnatasterone \[V. \textit{cymosa}\] [69]

181. Scabrasterone \[V. \textit{scabra}\] [72]

182. 24-\textit{epi}-Pinnatasterone \[V. \textit{scabra}\] [72]
   C-24 epimer of Pinnatasterone

183. 24-\textit{epi} - Abutasterone \[V. \textit{scabra}\] [72]
   C-24 epimer of Abutasterone
184. (24\(R\)) - 11\(\alpha\), 20, 24 -Trihydroxyecdysone 
\[ \text{V. canescens} \quad [75] \]

185. 11\(\alpha\), 20, 26 – Trihydroxyecdysone and its C-25 epimer 
\[ \text{V. canescens} \quad [75] \]

186. 20,26-Dihydroxyecdysone 
\[ \text{V. scabra} \quad [72] \]

187. 24 – Hydroxyecdysone-2, 3-acetonide 
\[ \text{V. doniana} \quad [71] \]
188. 24-Hydroxyecdysone  
\[V. doniana\] [71]

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{O} & \quad \text{O}
\end{align*}
\]

189. 11β, 24-Dihydroxyecdysone  
\[V. doniana\] [71]

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{O} & \quad \text{O}
\end{align*}
\]

190. 11α-Hydroxyecdysone  
\[V. strickeri\] [70]  
\[V. scabra\] [72]

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{O} & \quad \text{O}
\end{align*}
\]

191. 11β-Hydroxy-20-deoxyshidasterone  
\[V. doniana\] [49]

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{HO}
\end{align*}
\]
Miscellaneous

192. Dimethyl 3,4,3',4'-tetrahydroxy-δ-truxinate  

\[ \text{V. quinata} \quad [49] \]

193. Methyl 10\text{R} - \text{methoxy} - 12 -oxo-9(13), 16\text{E} - \text{phytodienoate}  

\[ \text{V. quinata} \quad [49] \]

194. Methyl 3, 4, 5 - O- tricaffeoylquinate  

\[ \text{V. quinata} \quad [49] \]

195. Methyl 3, 4, - O- dicafeoylquininate  

\[ \text{V. polygama} \quad [76] \]

196. Methyl 3, 5, - O- dicafeoylquininate  

\[ \text{V. polygama} \quad \text{V. cymosa} \quad [76] \]
197. Vicioside

\[
\begin{align*}
\text{Glc} - \text{O} & \quad \text{N} \\
\text{H}_2\text{N} & \quad \text{N} \\
\text{NH}_2 &
\end{align*}
\]

*V. pinnata* [46]

198. \(\beta\)-Sitosterol

\[
\begin{align*}
\text{HO} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H}
\end{align*}
\]

*V. negundo* [40]

199. Rutundial

\[
\begin{align*}
\text{CHO} & \quad \text{O} & \quad \text{H}
\end{align*}
\]

*V. rotundifolia* [77]

200. Tanacetamide

\[
\begin{align*}
\text{OH} & \quad \text{HO} & \quad \text{OH} & \quad \text{OH} \\
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H}
\end{align*}
\]

*V. cienkowskii* [42]

201. \(p\)-Hydroxybenzoic acid

\[
\begin{align*}
\text{COOH} & \quad \text{OH} & \quad \text{OH}
\end{align*}
\]

*V. negundo* [40]
Review on Different *Vitex* Species

- Cinnamoyl
- 6,7-Dihydroxfoliamethoyl
- Foliamenthoyl
- Vanilloyl
- Feruloyl
- Caffeoyl
Pharmacological activities of crude extracts and pure isolated chemicals from different *Vitex* species

The pharmacological activities of crude extracts / pure isolates from several species of *Vitex* such as *V. agnus-castus*, *V. negundo*, *V. rotundifolia*, *V. trifolia*, *V. doniana*, *V. glabrata*, *V. polygama*, *V. megapotamica*, *V. leucoxylon* have been reported. Some of the important pharmacological activities are discussed briefly.

a) *Anti-inflammatory activity*

The EtOH extract of *Vitex glabrata* leaves exhibited significant anti-inflammatory activity in carrageenan-induced paw edema and cotton pellet-induced granuloma formation in rat models. The extract showed significant anti-inflammatory activity in rats at a dose of 400 mg / kg bw, *p.o.* and the activity was comparable to that of standard reference drug, diclofenac sodium (50 mg / kg *p.o.*) [78].

The EtOAc extract of *Vitex altissima* leaves exhibited significant anti-inflammatory activity in rat paw edema model [79].

The CHCl$_3$, EtOAc and *n*-BuOH fractions from methanolic extract of the stem-bark of *Vitex doniana* exhibited significant anti-inflammatory activity on carrageenan-induced paw oedema model in rats at a dose of 100 mg / kg bw, *p.o.* by inhibiting the paw edema volume by 68-72 %, which were comparable to that of reference drug, diclofenac (50 mg / kg *p.o.*) having 81.94 % inhibition. Seven ecdysteroids, 21-hydroxyshidasterone (178), 11β - hydroxy-20-deoxyshidasterone (191), 24-hydroxyecdysone (172), 24-hydroxyecdysone-2,3-acetonide (187), shidasterone (177), ajugasterone C (169) and 11 β, 24-dihydroxyecdysone (189) isolated from these fractions showed significant (*p < 0.05*) inhibitory effect (58-71 % inhibition after 6 h) at 100 mg/kg, *p.o.* on rat paw edema development. The reference drug, diclofenac sodium showed 70% inhibition after 6 h [71].

Iridoid agnuside (2) isolated the BuOH extract of *Vitex peduncularis* stem bark showed significant anti-inflammatory activity by inhibiting the activity of proinflammatory enzymes, COX-2 with IC$_{50}$ values of 0.026 ± 0.015 mg / ml, while it showed mild inhibitory effects on COX-1 [80].
Vitexicarpin (122) isolated from *Vitex rotundifolia* showed anti-inflammatory activity by preventing TNF-α-induced vascular inflammatory process in human umbilical vein endothelial cells (HUVEC) [54].

The CHCl₃ extract of *Vitex negundo* seeds exhibited anti-inflammatory activity in carrageenan-induced rat paw edema model. The extract at a dose of 500 mg / kg bw, p.o. showed 34.8% inhibition of paw edema volume after 3.5 h of injection of carrageenan. The isolated triterpenoid, 2α,3α-dihydroxyoleana-5,12-dien-28-oic acid (100) showed weak inhibition (18.7%) of paw edema volume at a dose of 50 mg / kg p.o. The standard drug, ibuprofen (50mg / kg, p.o.) showed 63.2% inhibition of paw edema volume [45].

The EtOAc extract and its isolate, 6'-O-trans-feruloylnegundoside (12) from *Vitex altissima* leaves exhibited moderate anti-inflammatory activity in the carrageenan induced- rat paw edema model by inhibiting 39% and 20% of the paw edema volume at a dose of 250 mg / kg and 200 mg / kg after 3 h, repectively [81].

Lignan, negundin B (147) isolated from *Vitex negundo* roots exhibited potent anti-inflammatory activity by inhibiting the activity of soybean lipoxygenase and butyryl-cholinesterase (BChE) with IC₅₀ of 6.25 ± 0.5 and 194 ± 4.4 μM, respectively. While another isolated lignan, vitrofolal E (145) isolated from the same plant showed only moderate activity against BChE with IC₅₀ of 35.0 ± 105 μM [59].

Diterpene, viterotulin A (28), and neolignan viterolignan A (157) isolated from *Vitex rotundifolia* showed anti-inflammatory activity by inhibiting NO production in LPS-induced RAW 264.7 macrophages with IC₅₀ of 16.4 and 21.1 μM, respectively [28].

Labdane diterpenes, negundoins C (48) and E (50) isolated from *V. negundo* seeds showed anti-inflammatory effects by inhibiting NO production by LPS-stimulated RAW 264.7 macrophages, with IC₅₀ values of 0.12 and 0.23 μM, respectively. Further studies revealed that these compounds at 5 μM concentration significantly reduced the levels of iNOS protein to 0.40±0.13% and 41.01±6.02%, respectively, and Cox-2 protein to 2.06±0.53% and 26.40±7.43%, respectively [34].
b) **Antimicrobial activity**

Luteolin-3'-O-glucuronic acid methyl ester (109) and negundoside (14) isolated from *V. negundo* leaves exhibited significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* with MIC value of 6.25 μg/mL. Flucanazole was used as standard drug [47].

Supercritical fluid extract of *Vitex negundo* leaves exhibited strong antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* and mild activity against *Escherichia coli, Pseudomonas aeruginosa* and yeast, *Candida albicans* in disc diffusion assay [82].

Lignans, vinitrolals C (142) and D (141) and detetrahydroconidendrin (133) isolated from *Vitex rotundifolia* aerial parts showed significant antibacterial activity against various methicillin-resistant *Staphylococcus aureus* (MRSA) strains with MIC values in the range of 4-64 μg/mL in broth dilution method [62].

The essential oils obtained by hydrodistillation from the aerial parts of *Vitex rivularis* showed antifungal activity against yeasts and dermatophyte strains with MIC and MLC values ranging from 0.16 – 0.64 μL / mL and 0.32 – 2.5 μL / mL, respectively [83].

Vitelignin A (154) isolated from *V. negundo* seeds showed moderate antifungal activity against *Candida albicans, Cryptococcus neoformans* and *Trichophyton rubrum* with MIC value of 32, 64 and 32 μg/mL, respectively [64].

c) **Anticancer activity**

Flavonoid casticin (122) isolated from the fruits of *Vitex rotundifolia* showed significant cytotoxicity against human lung cancer cells (PC-12) and human colon cancer cells (HCT 116) with GI₅₀ values of 114 and 119 ng / mL, respectively in MTT assay. The standard drug cisplatin showed GI₅₀ of 111 and 794 ng / mL, against PC-12 and HCT 116 cells, respectively [36].

Vitexicarpin (also known as casticin) (122) isolated from the leaves of *Vitex negundo* showed antiproliferative activity against KB, LNCaP and LuI (human lung) cancer cells with ED₅₀ values of 0.5, 0.5 and 0.7 μg / mL, respectively. The activity of the compound was also evaluated *in-vivo* hollow fiber model in mice using the same cancer cells at doses of 10, 20 and 40 mg/kg. With
LNCap cells, the compound inhibited the growth by 0-7.2% at the ip site and 0-2.4% at the sc site. While with \( \kappa B \) cells, it was ineffective at the ip site and inhibited the growth by 0-8.2% at the sc site. The compound was also ineffective in \textit{in-vivo} mouse P-388 leukemia model (135 mg / kg) [55].

Negundonorin A (89) isolated from \textit{V. negundo} seeds showed strong cytotoxicity against breast cancer (ZR-75-30) cells with IC\( _{50} \) value of 0.56±0.19 µg/mL, while negnfulur (161) isolated from the same plant exhibited potent cytotoxicity against HL-60 cells with IC\( _{50} \) of 0.94±0.26 µg/mL [33].

d) \textit{Anti-angiogenic activity}

Vitexicarin (122) isolated from the fruits of \textit{Vitex rotundifolia} showed significant \textit{in-vitro} anti angiogenic activity by inhibiting vascular-endothelial growth factor (VEGF)-induced endothelial cell (EC) proliferation, migration, and capillary-like tube formation on matrigel in a dose-dependent manner (0.1 – 5.0 µM). Further studies using flow cytometric analysis of DNA fragments, and caspase 3 blotting indicated that vitexicarin (0.1-5 µM) inhibited EC proliferation \textit{via} cellcycle arrest and induction of apoptosis. The flavonoid at the concentration of 5 µM also \textit{in-vitro} inhibited sprouting from chorioallantoic membranes (CAMs). In addition, vitexicarin impaired vascularisation in allograft mouse tumor model. These results provide a new light on the traditional use of the plant, for cancer treatment. The plant was commonly used as anti arthritic drug [76].

e) \textit{Antioxidant activity}

6\textquoteright-O-trans-Caffeoylnegundoside (13), 2\textquoteright-O-p-hydroxybenzoylgardoside (19) and 2\textquoteright-O-p-hydroxybenzoyl-6\textquoteright-O-trans-caffeoyl-8-epilogenic acid (6) isolated from \textit{Vitex altissima} leaves exhibited potent antioxidant activity, both in superoxide free-radical scavenging assay (using NBT method) (IC\( _{50} \), 24.3, 32.0 and 31.9 µM, respectively) and in DPPH radical scavenging assay (IC\( _{50} \), 15.2, 10.9 and 11.4 µM, respectively) in comparison to the known antioxidants, BHT and \( \alpha \)-tocopherol, of IC\( _{50} \) values 381 µM, 19 µM, respectively [15].

Lignans isolated from the seeds of \textit{Vitex negundo} showed antioxidant activity both in lipid peroxidation and DPPH methods. The antioxidant activity was higher in DPPH method. Among the tested lignans, vitedoamine A (136), 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-
naphthaldehyde (149), vitrofolal F (146) showed radical scavenging activity similar to \( \alpha \)-tocopherol (standard antioxidant) in DPPH assay [39].

Lignans, vitecannasides A (151) and B (152) and flavonoids, isoorientin (112) and orientin (113) isolated from the fruits of *Vitex cannabifolia* showed stronger antioxidant activity than that of L-cysteine in DPPH assay. The activity of isoorientin (112) and orientin (113) was more than that of \( \alpha \) tocopherol (standard antioxidant) in the same assay [12].

f) **Trypanocidal activity**

Six diterpenoids, two nor aldehyde (67 and 68), vitexifolins E (77) and F (54), vitexilactone (24) and 6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (22) isolated from the fruits of *Vitex trifolia* showed significant *in-vitro* trypanocidal activity against epimastigots of *Trypanosoma cruzi* with MLCs (minimum lethal concentrations) of 11, 36, 34, 34, 66 and 66 \( \mu \)M, respectively [25].

g) **Moulting hormone activity**

24-epi- Pinnatasterone (179) and scabrasterone (181) isolated from the stem bark of *Vitex scabra* exhibited weak *in-vivo* moulting activity with \( EC_{50} \) values of 5.2 \( \times \) 10\(^{-4}\) and 1.0 \( \times \) 10\(^{-3}\) M, respectively based on the activity of 20-hydroxyecdysone (172) (1.6\( \times \)10\(^{-5}\) M) in Musca assay. The low moulting activity of these ecdysteroids was possibly due to lacking of a 22R hydroxyl group in their molecule [72].

h) **Antimutagenic activity**

(+) Polyalthic acid (27) isolated from MeOH extract of *V. rotundifolia* whole plant exhibited antimutagenic activity by suppressing the mutagenicity of Trp P-1 (3-amino-1,4-dimethyl-5H-pyrdo [4,3b] indole) in Umu gene expression assay with ID\(_{50}\) value of 0.29 \( \mu \)M/mL [27].

i) **Anti-osteoporotic activity**

Lignan vitexdoin F (138) isolated from *V. negundo* seeds showed potent anti-osteoporotic effect by inhibiting the proliferation and ALP activity of osteoblastic UMR106 cells at 10\(^{-7}\)M concentration [61].

j) **Vasorelaxant activity**

The CH\(_2\)Cl\(_2\)-MeOH (1:1) extract of *V. cienkowskii* stem bark showed significant endothelium dependent vasorelaxant activity with \( EC_{50} \) value of 12.12 \( \mu \)g/mL in
isolated rat aortic rings precontracted with noradrenaline (1μM). The isolated compounds, mixture of salvin A and maslinic acid, tanacetamide and β-sitosterol glycoside from this bioactive fraction showed vasorelaxant effect in the same model with EC$_{50}$ values of 1.999, 4.256 and 1.178 mg/L, respectively [42].

Tetraacetylajugalactone C (170) isolated from *V. cienkowskii* stem bark produced strong vasorelaxant activity on concentration-dependent manner in rat artery rings pre-contracted with 1μM noradrenaline or 60 mM KCl with IC$_{50}$ values of 8.40 and 36.30 μM, respectively [73].

K) *Antitubercular acivity*

Labdane diterpenes, 9-hydroxy-13 (14)-labden-15,16-olide and isoambreinolide (202) isolated from *V. trifolia* leaves exhibited antitubercular activity against *Mycobacterium tuberculosis* H37Rv in BACTEC-460 assay with MIC value of 100 and 25 μg/mL, respectively [30].
Section 2: A brief review of new flavonols reported during the last five years

Both synthetic and natural products chemists from the beginning of the 21st century are searching for potential drugs from nature. Among various nature-origin phytochemicals, flavonoids have received an increased attention due to their considerable biological benefits. Natural flavonoids play an important role in preventing and managing of modern diseases such as cancers, diabetes, and cardiovascular diseases among others [84].

The flavonoids are a group of a large number of small molecules having a benzene ring (A) linked with γ-pyrone ring (C), which in the position 2 or 3 takes a phenyl ring (B) as a substitute (203, 204). Flavonoids are broadly classified as flavonoids (203) and isoflavonoids (204).

Flavonoids are subclassified into flavones, flavonols, flavanones, dihydroflavonols, chalcones and anthocyanidins (flavan 3-ol) (Fig. 1.1). Similarly isoflavonoids are sub classified into isoflavones, isoflavanones and isoflavans (Fig. 1.1) [85].
Flavonoids exist in plants either as aglycones or as their O-glycosides or C-glycosides. In some cases, O-glycosylation generally reduces the bioactivity of these compounds such as antioxidant, antidiabetic, anti-inflammatory, antibacterial, antifungal, antitumor, antiplasmodial activities [84].

To highlight the distribution and substitution patterns of flavonols, a list of new flavonols reported during the last five years is provided in Table 1.2.

**Table 1.2  List of new flavonols reported during the last five years (since 2010)**

<table>
<thead>
<tr>
<th>Str. No.</th>
<th>Name and Structure</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>8-C-(1,1-Dimethyl-2-propen-1-yl)-galangin</td>
<td><em>Platanus acerifolia</em> (Platanaceae)</td>
<td>[86]</td>
</tr>
</tbody>
</table>

**Fig. 1.1  Skeletons and ring designations of flavonoids.**

![Skeletons and ring designations of flavonoids.](image-url)
206. 7-O-(3-Methyl-2-butenyl)-galangin  
Platanus acerifolia  
(Platanaceae) [86]

207. 7,8-(2″,3″,3″-Trimethyl-2″,3″-dihydrofuran) galangin  
Platanus acerifolia  
(Platanaceae) [86]

208. 3″,4″-Dihydro-3″,4″-dihydroxypongaflavone (= Derrisin B)  
Derris indica  
(Fabaceae) [87]

209. 6-p-Hydroxybenzyl kaempferol  
Cudrania cochinicensis [88]
| 210. | 8-C-(1,1-Dimethyl-2-propen-1-yl)-kaempferide | *Platanus acerifolia* [86] (Platanaceae) |
| 211. | 7-O-(3-Methyl-2-butenyl)-kaempferide | *Platanus acerifolia* [86] (Platanaceae) |
| 212. | (-) (2''S)-7,8-(2'',3'',3'''-Trimethyl-2'',3'''-dihydrofuran) kaempferide | *Platanus acerifolia* [86] (Platanaceae) |
| 213. | 6-p-Hydroxybenzyl quercetin | *Cudrania cochinchinensis* [88] |
214. 3-Hydroxy-3',4'-dimethoxy-7-(γ,γ'-dimethylallyloxy) flavone  Sophora interrupta [89]

215. 3,7,8,3'-tetrahydroxy-5-methoxy-4'-benzoyloxy flavone  Anisotes trisulens [90]  (Acanthaceae)

216. Officinin A  Alpinia officinarum [91]  (Zingiberaceae)

217. 2',3',5',5,7-Pentahydroxy-3,4'-dimethoxy flavone  Eriosema robustum [92]  (Fabaceae)
Pharmacological activities
Free flavonols and their glycosides possess some pharmacological properties. Some of these activities are briefly discussed.

a) Antioxidant activity
Flavonols, 6-p-hydroxybenzyl kaempferol (209), 6-p-hydroxybenzyl quercetin (213), kaempferol (220) and quercetin (221) isolated from Cudrania cochinchinensis exhibited significant radical scavenging activities in DPPH and ABTS assays with IC$_{50}$ value of 25.67, 18.52, 23.29 and 10.71 µM in DPPH assay and TEAC values of 0.731, 3.63, 1.50 and 4.18 mM in ABTS assay, respectively [88].

$2',3',5',7$-Pentahydroxy-$3',4'$-dimethoxyflavone (217) and $2',3',5',5,7$-pentahydroxy-$4'$-methoxyflavone (218) isolated from Eriosema robustum twigs exhibited significant antioxidant activity in DPPH assay with IC$_{50}$ values of 1.13 and 1.19 mg/mL, comparable to that of positive control L-ascorbic acid (IC$_{50}$, 1.00 mg/mL) [92].
b) **Antidiabetic activity**

Derrisin B (208) isolated from *Derris indica* stem bark showed antidiabetic effect by inhibiting the formation of advanced glycation end products with IC$_{50}$ value of 18.0 μM and this inhibitory activity was stronger than that of positive control aminoguanidine [87].

c) **Memory cognition effect**

Quercetin (221) at a dose of 10, 20, 40 mg/kg, *p.o* impaired cognitive function in mice by suppressing pAKt and pCAMKII and hence decreasing pCREB expression in hippocampus [94].

d) **Antiviral activity**

Quercetin (221) showed antiviral activity by suppressing hepatitis C virus through inhibition of nonstructural protein 3 (NS3) activity [95].

Quercetin (221) also exhibited mild antiviral activity against Japanese encephalitis virus (JEV) with IC$_{50}$ value of 212.1 μg/mL [96].

e) **Anti-inflammatory activity**

Quercetin pentamethyl ether (222) exhibited strong anti-inflammatory activity in carrageenan induced paw edema model in rats [97].

f) **Hepatoprotective activity**

Quercetin (221) showed significant hepatoprotective effect in alcohol-induced hepatotoxic rats [98].
PART-I

Section 3: A brief history and taxonomical description and classification of Vitex peduncularis

Plate 1.1. Photographs of Vitex peduncularis
Vitex peduncularis Wall. var. roxburghiana C. B. Clarke (local name: God Harina) of family Verbenaceae is distributed in different parts of India and other Asian countries [1]. The plant has been used for centuries in traditional medicine for treatment of malarial, black water and black fevers [2,99]. Phytochemical investigations on the plant reported flavonoids, iridoids and triterpenoids [41,80,100].

V. peduncularis is a large tree with opposite leaves, flowers in axillary panicles, patioles faintly winged, Calyx campanulate, limb 3-5 toothed; corolla 2 lipped and stamens 4, didynamous. Fruits are obovoid drupes, enclosed by enlarged calyx and seeds are exalbuminous (Plate 1.1) [1].

Taxonomically, the plant, V. peduncularis was classified as per Bentham & Hooker System of classification [101].

Class : Dicotyledons
Sub class : Gamopetalae
Series : Bicarpellatae
Co hort : Lamiales
Order : Verbenales
Family : Verbenaceae
Genus : Vitex
Species : Vitex peduncularis Wall.
Section 4: Isolation, Structure elucidation and antileishmanial activity of vitecetin.

Isolation:
Vitecetin (223) was isolated from the CHCl₃ soluble phase of methanolic extract of *Vitex peduncularis* leaves by repeated column chromatography over silica gel in yellow cubes, mp 202°C. It was homogeneous in TLC in different solvent systems [silica gel G, Rf 0.47 in CHCl₃-MeOH = 8:1].

Structure elucidation:

a) The molecular formula
The molecular formula of the compound was determined as C₁₈H₁₆O₈ from the quasi-molecular mass ion at m/z 361.0920 [M+H]⁺ (Calcd for C₁₈H₁₇O₈: 361.0923) and analysis of its ¹³C-DEPT NMR spectral data.

b) The UV spectrum
The UV spectrum of the compound in MeOH (Fig. 1.2) showed absorption maxima at λₘₐₓ 252 (band II), 263 sh, 360 (band I) nm, characteristic of flavonoids [102]. The bathochromic shifts of both the characteristic bands I and II in presence of AlCl₃ at λₘₐₓ 272 (band II), 310 and 428 (band I) nm, indicated the presence of ortho-dihydroxy grouping in ring B and 5-hydroxyl group in ring A of the flavone molecule. In presence of NaOMe in MeOH, it decomposed after 2 min and showed absorption maxima at λₘₐₓ 268 nm. It suggested the presence of C-3 substitution [102].

c) The IR spectrum
The IR spectrum of the compound in KBr (Fig. 1.3) showed absorption bands for hydroxyl (3472 cm⁻¹), alkoxy (2872 cm⁻¹) and α,β-unsaturated carbonyl (1643 cm⁻¹) functions.

d) The ¹H-NMR spectrum
The 600 MHz ¹H-NMR spectrum of the compound in DMSO-d₆ (Fig. 1.4) (Table 1.3) displayed the signals for two pair of meta-coupled aromatic protons [δH 6.37 and 6.75 (each 1H, d, J= 2.4 Hz)] and [δH 7.32 and 7.26 (each 1H, d, J= 2.4 Hz)], assigned to H-6 and H-8; H-2' and H-6', respectively [103-105]. Furthermore, the ¹H-NMR spectrum showed signals for three aromatic methoxyls.
[δ<sub>H</sub> 3.81, 3.85 and 3.87 (each 3H, s)], suggesting its flavonol trimethylether like structure.

e) The 13C-NMR spectrum
The 150 MHz 13C-NMR spectrum of the compound in DMSO-<em>d</em><sub>6</sub> (Fig. 1.5) (Table 1.3) showed 18 carbon signals, which on DEPT experiments revealed for 3 methyl, 5 methine and 10 quaternary carbons. The carbon resonance values of the compound were very similar to that of myricetin, 3,5,7,3′,4′,5′-hexahydroxyflavone (224), except for methoxyl carbons [106]. The methoxyl carbon resonance at δ<sub>C</sub> 59.8 coupled with its proton resonance at δ<sub>H</sub> 3.81 suggested its location between two ortho-substituted groups and hence its position was assigned to C-3 [105,106]. The downfield shift of C-3 resonance by 2.0 ppm compared to that of C-3 having a free hydroxyl group in myricetin also supported the methoxyl substitution of C-3 [104].

f) The NOESY spectrum
In the NOESY spectrum, the correlation of methoxyl protons (δ<sub>H</sub> 3.87) with H-6 (δ<sub>H</sub> 6.37) and H-8 (δ<sub>H</sub> 6.75), and of methoxyl protons (δ<sub>H</sub> 3.85) with H-2′ (δ<sub>H</sub> 7.32) suggested the location of other two methoxyl groups at C-7 and C-3′ positions, respectively (Fig. 1.6).

g) The HMBC spectrum
In the 2D HMBC spectrum, the correlation of H-2′ (δ<sub>H</sub> 7.32) with C-3′ (δ<sub>C</sub> 56.1) also supported the location of one methoxyl at C-3′ position (Figs. 1.7 and 1.7a).

h) The HSQC spectrum
The 1H-13C-HSQC spectrum of the compound in DMSO-<em>d</em><sub>6</sub> (Fig. 1.8) supported the assignment of proton and carbon resonances of methine and methoxyl groups.

i) The FAB-MS
The FAB-MS of the compound (Fig. 1.9) showed mass ions at <em>m/z</em> 361 [M+H]<sup>+</sup>, 360 [M]<sup>+</sup>, 359 [M-H]<sup>+</sup>, 345, 317, 299 and 167, characteristic of myricetin trimethyl ether skeletal structure (Scheme 1.1) [107].

j) Conclusion
On the basis of these evidences, the structure of the compound was determined as myricetin-3,7,3′-tri-O-methyl ether (223) (Fig. 1.6). It is a new compound.
### Table 1.3

$^1$H (600 MHz) and $^{13}$C (150 MHz) NMR spectral data of 223 in DMSO-$d_6$ (δ values).\(^a\)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta^b_C$</th>
<th>$\delta^c_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>155.9 (C)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>138.2 (CH)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>178.1 (C)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>161.0 (C)</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>97.9 (CH)</td>
<td>6.37 (d, 2.4 Hz)</td>
</tr>
<tr>
<td>7</td>
<td>165.2 (C)</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>92.4 (CH)</td>
<td>6.75 (d, 2.4 Hz)</td>
</tr>
<tr>
<td>9</td>
<td>156.3 (C)</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>104.1 (C)</td>
<td>-</td>
</tr>
<tr>
<td>1'</td>
<td>119.6 (C)</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>105.2 (CH)</td>
<td>7.32 (d, 2.4 Hz)</td>
</tr>
<tr>
<td>3'</td>
<td>148.2 (C)</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>137.9 (C)</td>
<td>-</td>
</tr>
<tr>
<td>5'</td>
<td>145.6 (C)</td>
<td>-</td>
</tr>
<tr>
<td>6'</td>
<td>109.8 (CH)</td>
<td>7.26 (d, 2.4 Hz)</td>
</tr>
<tr>
<td>3-OCH(_3)</td>
<td>59.8 (CH(_3))</td>
<td>3.81 (s)</td>
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<td>7-OCH(_3)</td>
<td>56.2 (CH(_3))</td>
<td>3.87 (s)</td>
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<tr>
<td>3'-OCH(_3)</td>
<td>56.1 (CH(_3))</td>
<td>3.85 (s)</td>
</tr>
<tr>
<td>5-OH</td>
<td>-</td>
<td>12.68 (s)</td>
</tr>
<tr>
<td>4'-OH</td>
<td>-</td>
<td>9.33 (s)</td>
</tr>
<tr>
<td>5'-OH</td>
<td>-</td>
<td>8.30 (s)</td>
</tr>
</tbody>
</table>

\(^a\)All assignments are based on HMBC, HSQC and COSY experiments; \(^b\)Multiplicities were determined by DEPT experiment; \(^c\)J in Hz.
Fig. 1.6. Structures of compounds 223, 224 and selected HMBC and NOESY correlations in 223.

Scheme 1.1. FAB-mass fragmentation of compound 223.
Fig. 1.2. UV spectrum of vitexin (223) in MeOH
Fig. 1.3. IR spectrum of vitecetin (223) in KBr
Fig. 14: H-NMR spectrum of vitexin (223) in DMSO-d$_6$. 

**II C**
Fig. 1a. 1H-NMR spectrum (expanded) of viscinin (22) in DMSO-d6.
Fig. 1.5. $^{13}$C-NMR spectrum of vtececin (223) in DMSO-$d_6$. 
Fig. 1.5a. $^{13}$C-NMR spectrum (expanded) of viocecin (223) in DMSO-d$_6$. 

VPL-5  13C-NMR in DMSO.

$IICB$
Fig. 1.5K. "C-NMR spectrum (expanded) of vincaxin (223) in DMSO-d_6.

VEL-5  "C-NMR in DMSO
Fig. 1.5: 1^H-NMR spectrum of vitexin (223) in DMSO-d_6.
Fig. 1.7. HMBC NMR spectrum of vitecitin (223) in DMSO-d$_6$. 
Fig. 1.7a. HMBC NMR spectrum (expanded) of vitecetin (223) in DMSO-d$_6$
Fig. 1.7b. HMBC NMR spectrum (expanded) of vinecetin (223) in DMSO-d$_6$
Fig. 1.7c. HMBC NMR spectrum (expanded) of vioceatin (223) in DMSO-d$_6$
Fig. 1.8. HSQC-NMR spectrum of vitexin (223) in DMSO-d$_6$. 
Fig. 1.8a. HSQC-NMR spectrum (expanded) of vitecetin (223) in DMSO-d$_6$. 
Fig. 1.9. FAB-MS spectra of vitexin (223).

Note: INDIAN INSTITUTE OF CHEMICAL BIOLOGY

Sample: P. vitexinifera

Spectrum Type: Normal

Ion Mode: HCT/MS

Inlet: 0.42 mm

Bp: m/z 154.1058

Int.: 59,000

Cut Level: 0.20%

Output m/z range: 80-800

154.1

100
Antileishmanial activity of vitaecin

The leaves of *Vitex peduncularis* has been used in traditional medicine for treatment of black fever [108]. To find out the bioactive principles, we carried out the antileishmanial assay of vitaecin and other isolated chemicals from *V. peduncularis* against both *Leishmania donovani* promastigote and amastigote forms.

Among the isolated compounds, only vitaecin exhibited potent antileishmanial activity, better than that of standard antileishmanial drug, sodium antimonygluconate (SAG) having IC\(_{50}\) values for promastigotes, 2.4 and 58.5 µM, and for amastigotes 0.93 and 36.2 µM, respectively (Tables 1.4 and 1.5) (Figs. 1.10 and 1.11) [109,110].

Table 1.4. *In vitro* antileishmanial activity of vitaecin (223) against *L. donovani* promastigotes

<table>
<thead>
<tr>
<th>Compound</th>
<th>In vitro activity Anti-promastigote activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC(_{50}) ± SD(^{b})</td>
</tr>
<tr>
<td></td>
<td>(µM)</td>
</tr>
<tr>
<td>vitaecin</td>
<td>2.4 ± 0.57</td>
</tr>
<tr>
<td>SAG(^{e})</td>
<td>58.5 ± 7.42</td>
</tr>
</tbody>
</table>

\(^{a}\)IC\(_{50}\): Concentration that causes 50 % inhibition of extracellular promastigotes.

\(^{b}\)SD: standard deviation

\(^{c}\)IC\(_{90}\): Concentration that causes 90 % inhibition of extracellular promastigotes.

\(^{e}\)SAG- Sodium antimony gluconate (Reference drug)

IC\(_{50}\) and IC\(_{90}\) values are the average of three independent assays expressed as average ± standard deviation (SD).
Table 1.5. Antileishmanial activity of vitecetin (223) against intramacrophage amastigote of *L. donovani* and cytotoxicity against THP-1 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity against intracellular amastigote.</th>
<th>Cytotoxicity</th>
<th>Selectivity index (SI)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; ± SD&lt;sup&gt;b&lt;/sup&gt; (µM)</td>
<td>CC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; ± SD (µM)</td>
<td></td>
</tr>
<tr>
<td>vitecetin</td>
<td>0.93 ± 0.21</td>
<td>123.7 ± 9.41</td>
<td>133.01</td>
</tr>
<tr>
<td>SAG&lt;sup&gt;e&lt;/sup&gt;</td>
<td>36.2 ± 5.83</td>
<td>364.3 ± 23.09</td>
<td>10.06</td>
</tr>
</tbody>
</table>

<sup>a</sup>IC<sub>50</sub>: Concentration that causes 50 % inhibition of intracellular amastigotes. 
<sup>b</sup>SD: standard deviation 
<sup>c</sup>CC<sub>50</sub>: Concentration that causes 50 % mortality of THP-1 cells. 
<sup>d</sup>SI: Selectivity Index. CC<sub>50</sub> for macrophages/IC<sub>50</sub> for intracellular amastigotes. 
<sup>e</sup>SAG- Sodium antimony gluconate (Reference drug) 

IC<sub>50</sub> and CC<sub>50</sub> values are the average of three independent assays expressed as average ± SD.

Figure 1.10. Effect of vitecetin (223) on *L. donovani* promastigote viability by MTT assay. Stationary phase *L. donovani* AG83 promastigote cells (1 X 10<sup>6</sup>/100µl) were incubated with vitecetin ( ) and standard drug SAG ( ) at 10 concentrations in two fold serial dilution starting from 200 µM for 92 h.
The promastigote viability assay was performed by the spectrometric reading of the MTT-formazan formed at 570 nm, and the data were expressed as % of viable cells. The experiments were repeated 3 times, yielding similar results. Data expressed as mean ± SD.

**Figure 1.11.** Effect of vitecetin (223) on *L. donovani* intracellular amastigotes and cytotoxicity of THP-1 cells. (a) Differentiated THP-1 Macrophages were cultured in complete RPMI media overnight, infected with *L. donovani* promastigotes for 4 h at macrophage: parasite ratio of 1:10. Non-ingested promastigotes were washed and the cells were incubated with vitecetin (▲) and SAG (●) at ten concentrations in two fold serial dilutions starting from 200 µM. The cells were incubated for another 48 h in coverslip culture, stained with Giemsa stain and numbers of intracellular amastigotes per 100 macrophages were counted. Infection rates were normalized to 100%. The curves showed the number of parasites per 100 peritoneal macrophages. The experiment was repeated three times, yielding similar results and data were expressed as mean ± SD. **indicates P<0.005 and *indicates P<0.001 in comparison to untreated infected macrophages. (b) THP-1 cells were cultured in complete RPMI 1640 medium and incubated with both vitecetin and SAG at ten concentrations in two fold serial dilutions starting from 200 µM for 48 h. Cell viability assay was performed by MTT method. Spectrometric reading of the MTT-formazan formed was taken at 650 nm and the data were expressed as % of viable cells. The experiment was repeated 3 times, yielding similar results and data were expressed as mean ± SD.
To characterize the effector mechanism of vitecetin against *Leishmania* parasite infected THP-1 macrophage cells, RT-PCR (real-time polymerase chain reaction) analysis of inducible nitric oxide synthase-2 (iNOS2) was done followed by measurement of nitric oxide generation by Griess reaction [111,112]. The results indicated that compound vitecetin (223) induced a potent host-protective response by enhancing NO generation and iNOS2 expression in infected macrophages to prevent the progression of *Leishmania* parasite. Both NO generation and iNOS2 expression in *L. donovani* infected macrophages was greater than that of SAG (Fig. 1.12).

**Figure 1.12.** Vitecetin mediated antileishmanial activity via NO generation and iNOS2 expression in *L. donovani* infected macrophages. (a) Differentiated THP-1 macrophages (10^6 cells/mL) were incubated with *L. donovani* promastigote (macrophage: parasite ratio, 1:10) for 4 h. Non-ingested promastigotes were removed and macrophages were cultured for another 20 h. Uninfected macrophages were either kept untreated or treated with vitecetin (IC_50, 0.93 µM) and SAG (IC_50, 32.3 µM). The cells were kept for 48 h for maximum nitrite generation and cell-free supernatants collected were subjected to nitrite generation assay by Griess reaction as mentioned in the Experimental Part. Data for nitrite generation were expressed as mean ± SD from triplicate experiments, yielding similar results (micromoles of nitrite). **indicates P<0.001 level of significance with respect to infection. (b) Effect of vitecetin treatment on iNOS2 mRNA expression in *L. donovani* infected THP-1 macrophages, as measured by real-time PCR. THP-1 macrophages infected with *L. donovani* promastigotes (1 macrophage: 10 parasites) and treated with vitecetin (IC_50, 0.93 µM) and SAG (IC_50, 36.2 µM) for 5 h, following which RNA was isolated for RT-PCR analysis.
Data are presented as fold changes compared with uninfected untreated control macrophages. The data represent the means ± SD of data from three independent experiments that yielded similar results. *indicates P<0.05 level of significance with respect to infected macrophages.

The toxicity of vitecetin towards the host THP-1 cells was evaluated. It was found that the compound 223 was less toxic than standard drug SAG having CC$_{50}$ values of 123.7 µM and 364.3 µM, respectively (Table 1.5) [113].

All these findings indicated that vitecetin could be a potent candidate for treatment of visceral leishmaniasis. Moreover, further study of vitecetin on its pharmacokinetic and toxicological properties will help to develop potential antileishmanial drug of herbal or semi-synthetic herbal origin.
Section 5: Isolation and structure elucidation of 4′-acetoxy-5-hydroxy-6,7-dimethoxyflavone.

Isolation:
4′-Acetoxy-5-hydroxy-6,7-dimethoxyflavone (225) was isolated from the CHCl₃ soluble fraction of methanolic extract of *Vitex peduncularis* leaves by repeated column chromatography over silica gel in light yellow amorphous solid. It was homogeneous in TLC in different solvent systems [silica gel G, R\(_f\): 0.41 in CHCl₃-MeOH = 8:1].

Structure elucidation:

a) The molecular formula
The molecular formula of the compound was determined as C\(_{19}\)H\(_{16}\)O\(_7\) from the quasi-molecular mass ion at \(m/z\) 357.0973 [M+H]⁺ (Calcd for C\(_{19}\)H\(_{17}\)O\(_7\): 357.0974) and analysis of its \(^{13}\)C-DEPT NMR spectral data.

b) The UV-Vis spectrum
The UV-Vis spectrum of the compound in MeOH (Fig. 1.13) showed absorption maxima at \(\lambda_{max}\) 272 and 310 nm, characteristic of flavonoids [102].

c) The IR spectrum
The IR spectrum of the compound in KBr (Fig. 1.14) showed the absorption bands for hydroxyl (3467 cm\(^{-1}\)), ester (1755 and 1254 cm\(^{-1}\)) and \(\alpha,\beta\)-unsaturated carbonyl (1643 cm\(^{-1}\)) functions.

d) The \(^1\)H-NMR spectrum
The 600 MHz \(^1\)H-NMR spectrum of the compound in DMSO-\(d_6\) (Fig. 1.15) (Table 1.6) displayed the signals for two aromatic protons [\(\delta_H\) 6.36 and 6.74 (each 1H, s)], characteristic of a 5,6,7-trioxygenated flavones [102]. The \(^1\)H-NMR spectrum also showed two signals of A\(_2\)X\(_2\) spin system [\(\delta_H\) 7.95 and 7.26 (each 2H, d, \(J= 8.5\) Hz)], characteristic of a C-4′-oxygengated B-ring of flavones [102]. Furthermore, the signals for two methoxyl [\(\delta_H\) 3.79 and 3.86 (each 3H, s)], one acetoxyethyl [\(\delta_H\) 2.38 (3H, s)] and a phenolic hydroxyl [\(\delta_H\) 12.68 (1H, s)], suggested the presence of two methoxyl, one acetoxyethyl and one hydroxyl group in the compound. The downfield shift of phenolic hydroxyl suggested its location at C-5 [102].
e) The $^{13}$C-NMR spectrum

The 150 MHz $^{13}$C-NMR spectrum of the compound in DMSO-$d_6$ (Fig. 1.16) (Table 1.6) showed 17 carbon signals, which on DEPT experiments revealed for 3 methyl, 4 methine and 10 quaternary carbons. The downfield methoxyl carbon resonance at $\delta_C$ 59.2 indicated its location between two oxygenated carbons and hence its position was assigned at C-6. The carbon resonance values of the compound were very similar to that of salvigenin (226) except for one methoxyl signal at C-4$'$ [106].

<table>
<thead>
<tr>
<th>No.</th>
<th>H/C</th>
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<th>$\delta_C^c$</th>
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<tr>
<td>2</td>
<td>–</td>
<td>165.1 (C)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.36 s</td>
<td>103.8 (CH)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>181.3 (C)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>153.3 (C)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>131.0 (C)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>161.2 (C)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.74 s</td>
<td>92.1 (CH)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>151.2 (C)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>106.2 (C)</td>
<td></td>
</tr>
<tr>
<td>1$'$</td>
<td>–</td>
<td>130.1 (C)</td>
<td></td>
</tr>
<tr>
<td>2$'$, 6$'$</td>
<td>7.95 d (8.5)</td>
<td>128.0 (CH)</td>
<td></td>
</tr>
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<td>3$'$, 5$'$</td>
<td>7.26 d (8.5)</td>
<td>119.3 (CH)</td>
<td></td>
</tr>
<tr>
<td>4$'$</td>
<td>–</td>
<td>156.1 (C)</td>
<td></td>
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<tr>
<td>MeO-6</td>
<td>3.86 s</td>
<td>59.2 (CH$_3$)</td>
<td></td>
</tr>
<tr>
<td>MeO-7</td>
<td>3.79 s</td>
<td>56.1 (CH$_3$)</td>
<td></td>
</tr>
<tr>
<td>AcO-4$'$</td>
<td>2.38 s</td>
<td>21.1 (CH$_3$), 170.2</td>
<td></td>
</tr>
<tr>
<td>HO-5</td>
<td>12.68 s</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

$^a$600 MHz for $^1$H and 150 MHz for $^{13}$C-NMR data; $^b$J values in Hz in parenthesis
$^c$Assignment based on HSQC and HMBC data
f) **The NOESY spectrum**
In the NOESY spectrum, the correlation between H-8 ($\delta_H$ 6.74) and methoxyl protons ($\delta_H$ 3.79) indicated the location of one methoxyl group at C-7 (Fig. 1.17). Therefore, the position of the acetoxymethyl group was assigned at C-4'.

g) **The FAB-MS**
The FAB-MS of the compound (Fig. 1.18) showed mass ions at $m/z$ 357 [M+H]$^+$, 329 [M-28]$^+$, 315 [M-42]$^+$, 197, 161 and 118, which could be rationalized by its flavones methyl ether like skeletal structure (Scheme 1.2) [107].

h) **Alkaline hydrolysis**
The alkaline hydrolysis of the compound with 0.5 N methanolic NaOH afforded 4’,5-dihydroxy-6,7-dimethoxyflavone, cirsimaritin (227).

i) **Conclusion**
On the basis of all these findings, the structure of the compound was established as 4’-acetoxy-5-hydroxy-6,7-dimethoxyflavone (225) (Fig. 1.17). It is a new natural product.

![Diagram](image-url)

**Fig. 1.17.** Structures of compounds 225-227 and selected NOESY correlation in 225.
Scheme 1.2. FAB mass fragmentation of 225.
Fig. 1.13. UV spectrum of compound 225 in MeOH.
Fig. 1.14. IR spectrum of compound 225 in KBr.
Fig. 1.15. $^1$H-NMR spectrum of compound 225 in DMSO-$d_6$. 
Fig. 1.15a. $^1$H-NMR spectrum of compound 225 in DMSO-d$_6$
Fig. 1.1(b): 1H NMR spectrum (expanded) of compound 218 in DMSO-d6.
THESIS, PART-I

Isolation and Structure Elucidation

Natural Products Chemistry
Fig. 1.16e. $^{13}$C-NMR spectrum (expanded) of compound 225 in DMSO-d$_6$. 
Fig. 1.10c. 13C-NMR spectrum of compound 225 in DMSO-46.
Fig. 1.16c. HSQC NMR spectrum of compound 225 in DMSO-d6
Fig. 1.18. FAB-MS of compound 225
Section 6: Isolation and Structure elucidation of vitexin.

Isolation:
Vitexin (228) was isolated from the EtOAc soluble fraction of the methanolic extract of *Vitex peduncularis* leaves by column chromatography (CC) over Diaion HP-20 followed by silica gel CC of the residue obtained from 75% MeOH in H₂O eluate of Diaion HP-20 column chromatography in yellow powder, mp 263-265°C. It was homogeneous in TLC in different solvent systems [silica gel G, Rf 0.4 in CHCl₃-MeOH-H₂O = 18:6:1].

Structure elucidation:

a) **The molecular formula**
The molecular formula of the compound was assigned as C₁₂H₂₀O₁₀ from the quasi-molecular mass ion at m/z 433.1131 [M+H]+ (Caled for C₁₂H₂₁O₁₀: 433.1135) in HR-FAB-MS and analysis of its ¹³C-DEPT NMR spectral data.

b) **The UV spectrum**
The UV spectrum of the compound in MeOH (Fig. 1.19) showed absorption maxima at λₘₐₓ 270 (logε, 3.90) and 335 (3.85) nm, characteristic of flavonoids [102].

c) **The IR spectrum**
The IR spectrum of the compound in KBr (Fig. 1.20) showed the absorption bands for hydroxyl (3380 cm⁻¹) and α,β-unsaturated carbonyl (1652 cm⁻¹) functions.

d) **The ¹H-NMR spectrum**
The 600 MHz ¹H-NMR spectrum of the compound in DMSO-ᴅ (Fig. 1.21) (Table 1.7) showed the signals of an A₂X₂ coupling system [δH 8.02 and 6.94 (each 2H, d, J= 9.0 Hz)], characteristic of C-4’ oxygenated B-ring of a flavone. Furthermore, the signals for two one-proton singlets [δH 6.78 and 6.27 (each 1H, s)], an anomic sugar proton [δH 4.68 (1H, d, J= 10.2 Hz)] and one methylene protons [δH 3.83 (1H, dd, J= 9.6 and 1.8 Hz) and 3.76 (1H, dd, J= 10.2 and 6.0 Hz)] suggested the presence of trisubstituted A-ring of a flavone having a sugar moiety [114].
e) **The $^{13}$C-NMR spectrum**

The 150 MHz $^{13}$C-NMR spectrum of the compound in DMSO-$d_6$ (Fig. 1.22) (Table 1.7) recorded 19 carbon signals, which on DEPT experiments showed for 1 methylene, 9 methine and 9 quaternary carbons. The HMQC spectrum showed a correlation of anomeric proton at $\delta_H$ 4.68 with $\delta_C$ 73.4 suggesting the attachment of sugar to flavone moiety through C-linkage. The carbon chemical resonances of the compound were very similar to those reported for vitexin [106,114,115].

f) **FAB-MS**

The FAB-MS of the compound (Fig. 1.23) recorded mass ions at $m/z$ 433 [M+H]$^+$, 315, 269 and 118. The genesis of these ions could be rationalized by its flavone C-glycoside structure (Scheme 1.3) [107].

g) **Conclusion**

On the basis of the foregoing facts, the structure of the compound was established as apigenin-8-C-β-D-glycopyranoside or vitexin (228) (Fig. 1.24). It is a known natural product.

![Fig. 1.24. Structure of compound 228](image-url)
Table 1.7. $^1$H-(600 MHz) and $^{13}$C-(150 MHz)-NMR spectral data for compound 228 (in DMSO-$d_6$, $\delta$, ppm)

<table>
<thead>
<tr>
<th>H/C-Position</th>
<th>$\delta_H^a$</th>
<th>$\delta_C^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-</td>
<td>164.0 (C)</td>
</tr>
<tr>
<td>3</td>
<td>6.78 s</td>
<td>102.3 (CH)</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>182.1 (C)</td>
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<tr>
<td>5</td>
<td>-</td>
<td>161.2 (C)</td>
</tr>
<tr>
<td>6</td>
<td>6.27 s</td>
<td>98.2 (CH)</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>162.6 (C)</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>104.6 (C)</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>156.0 (C)</td>
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<tr>
<td>10</td>
<td>-</td>
<td>104.1 (C)</td>
</tr>
<tr>
<td>1'</td>
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<td>121.6 (C)</td>
</tr>
<tr>
<td>2',6'</td>
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<td>129.0 (CH)</td>
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<tr>
<td>3',5'</td>
<td>6.94 d (9.0)</td>
<td>115.84 (CH)</td>
</tr>
<tr>
<td>4'</td>
<td>-</td>
<td>160.4 (C)</td>
</tr>
<tr>
<td>1''</td>
<td>4.68 d (10.2)</td>
<td>73.4 (CH)</td>
</tr>
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<td>2''</td>
<td>4.99 dd (10.2, 9.3)</td>
<td>70.9 (CH)</td>
</tr>
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<td>3''</td>
<td>5.01 dd (9.3, 9.0)</td>
<td>78.7 (CH)</td>
</tr>
<tr>
<td>4''</td>
<td>4.61 dd (9.0, 9.1)</td>
<td>70.6 (CH)</td>
</tr>
<tr>
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<td>4.71 m</td>
<td>81.9 (CH)</td>
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<tr>
<td>6''</td>
<td>3.83 dd (9.6, 1.8)</td>
<td>61.3 (CH$_2$)</td>
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<tr>
<td></td>
<td>3.76 dd (10.2, 6.0)</td>
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<tr>
<td>5-OH</td>
<td>13.17 s</td>
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<tr>
<td>7-OH</td>
<td>10.84 br s</td>
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<tr>
<td>4'-OH</td>
<td>10.35 br s</td>
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$^a$J values in Hz in parenthesis  
$^b$Assignment based on HSQC and HMBC data.
Scheme 1.3. FAB mass fragmentation of vitexin (228).
Fig. 1.19. UV spectrum of vitexin (228) in MeOH.
Isolation and Structure Elucidation

Fig. 1.20. IR spectrum of vitsin (228) in KBr.
Fig. 1.21. $^1$H-NMR spectrum of vitexin (228) in DMSO-$d_6$
Fig. 1.21a. $^1$H-NMR spectrum (expanded) of vitexin (228) in DMSO-d$_6$. 

ICB
Fig. 1.22. $^{13}$C-NMR spectrum of vitexin (228) in DMSO-$d_6$. 

VPL-7  $^{13}$C-NMR in DMSO
Fig. 1.22a. $^{13}$C-NMR spectrum (expanded) of vitexin (228) in DMSO-$d_6$. 

\[ \text{VPL-7 13C-NMR in DMSO} \]

\begin{tabular}{cccccccccc}
129.01 & 121.64 & 115.84 & 104.63 & 104.07 & 102.48 & 98.16 & 81.88 & 78.88 & 73.40 & 70.65 & 70.55 & 61.71 \\
\end{tabular}
Fig. 1.23. FAB-MS of viexin (228)
Section 7: Isolation and Structure elucidation of 2α-hydroxyursolic acid.

Isolation:

2α-Hydroxyursolic acid (229) was isolated from the CHCl₃ soluble fraction of methanolic extract of *Vitex peduncularis* leaves by column chromatography in white powder. It was homogeneous in TLC in different solvent systems [silica gel G, Rₑ 0.4 in CHCl₃-MeOH = 8:1].

Structure elucidation:

a) The molecular formula

The molecular formula of the compound was assigned as C₃₀H₄₈O₄ from its quasi-molecular mass ion at m/z 495.3460 [M+Na]+ (Calcd for C₃₀H₄₈O₄Na: 495.3450) in HR-ESI-MS and analysis of its ¹³C-DEPT NMR spectral data.

b) The IR spectrum

The IR spectrum of the compound in KBr (Fig. 1.25) showed the absorption bands for hydroxyl (3423 cm⁻¹) and carboxyl (1693 cm⁻¹) functions.

c) The ¹H-NMR spectrum

The 500 MHz ¹H-NMR spectrum of the compound in C₅D₅N (Fig. 1.26) (Table 1.8) displayed the signals for five tertiary methyls [δH 0.99, 1.06, 1.09, 1.22 and 1.29 (each 3H, s)], two secondary methyls [δH 1.00 and 0.96 (each 3H, d, J= 6.0 Hz)], one olefinic proton [δH 5.48 (1H, m)] and one methine proton [δH 2.64 (1H, d, J= 13.5 Hz)], suggesting its ursane-12-ene like structure [116]. Furthermore, the signals for two oxymethine protons [δH 4.12 (1H, m) and 3.42 (1H, d, J= 11.5 Hz)] suggested the presence of 2,3-dihydroxy grouping in ring A. The large coupling constant of one oxymethine proton indicated their trans-diaxial relation.

d) The ¹³C-NMR spectrum

The 125 MHz ¹³C-NMR spectrum of the compound in C₅D₅N (Fig. 1.27) (Table 1.8) showed 30 carbon signals, which on DEPT experiments revealed for 7 methyl, 8 methylene, 8 methine and 7 quaternary carbons. Two olefinic carbon resonances at δC 125.6 (CH) and 139.2 (C), coupled with two methine carbon resonances at δC 68.6 and 83.8 and a quaternary carbon resonance at δC 179.7, corroborated its 2α-hydroxyursolic acid like structure [117].
**Table 1.8.** $^1$H-(500 MHz) and $^{13}$C-(125 MHz)-NMR Spectral data for compound 229 (in C$_5$D$_5$N, $\delta$, ppm)

<table>
<thead>
<tr>
<th>H/C-Position</th>
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<th>$\delta_C$</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td></td>
<td>48.0 (CH$_2$)</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>3.42 d (11.5)</td>
<td>83.8 (CH)</td>
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<tr>
<td>4</td>
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<td>39.9 (C)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>55.9 (CH)</td>
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<tr>
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<td>19.0 (CH$_2$)</td>
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<tr>
<td>11</td>
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<tr>
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<td>5.48 m</td>
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<tr>
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<td>139.2 (C)</td>
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<tr>
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<td>-</td>
<td>179.7 (C)</td>
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<td>21.5 (CH$_3$)</td>
</tr>
<tr>
<td>30</td>
<td>0.96 d (6.0)</td>
<td>17.5 (CH$_3$)</td>
</tr>
</tbody>
</table>

$^a$J values in Hz in parenthesis; $^b$Assignment based on HSQC and HMBC data
e) The FAB-MS

The FAB-MS of the compound (Fig. 1.28) recorded mass ions at $m/z$ 495 [M+Na]$, 249, 224, 202 and 146, which could be rationalized by its 2-hydroxyursolic acid structure (Scheme 1.4) [118].

f) Conclusion

The spectral data of the compound were very similar to those reported for 2α-hydroxyursolic acid (= corosolic acid) [116,119]. On the basis of all these evidences, the structure of the compound was assigned as 2α-hydroxyursolic acid or 2α,3β-dihydroxyurs-12-en-28-oic acid (229) (Fig. 1.29). It is a known natural product.

![Fig. 1.29. Structure of 2α-hydroxyursolic acid (229).](image)

![Scheme 1.4. FAB mass fragmentation of compound 229](image)
**Fig. 1.25.** IR spectrum of 2\(\alpha\)-hydroxyursolic acid (229) in KBr.
Fig. 1.26. $^\text{1}$H-NMR spectrum of 2$\alpha$-hydroxyursolic acid (229) in $\text{C}_2\text{D}_2\text{N}$. 

$\text{C}_2\text{D}_2\text{N}$
Fig. 1.26a. $^1$H-NMR spectrum (expanded) of 2α-hydroxyursolic acid (229) in C$_2$D$_2$N
Fig. 1.26. 1H-NMR spectrum (expanded) of 2α-hydroxyursolic acid (229) in CD$_3$N.
Fig. 1.26c. $^1$H-NMR spectrum (expanded) of 2α-hydroxyursolic acid (229) in C$_2$D$_3$N
Fig. 1.27. $^1$H NMR spectrum of 24-hydroxydocosahexaenoic acid (229) in CDCl$_3$. 

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Fig. 1.72a. 1H-NMR spectrum of (expanded) 20-g-hydroxy-solnic acid (229) in CD3N.
Fig. 1.27b. $^{13}$C-DEPT NMR spectrum of 20α-hydroxyursolic acid (229) in C$_5$D$_5$N.
Fig. 128. ES-MS of 2C-hydrouricosolic acid (229).
References


References


References


References


References


