PART-IV

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 anthelminthic [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 4.1.
Table 4.1. 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. negunda</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="image1" 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="image2" alt="Agnuside" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cis -Eurostoside</td>
<td><em>V. rotundifolia</em></td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td><img src="image3" alt="Cis -Eurostoside" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Viteoid II</td>
<td><em>Vitex rotundifolia</em></td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td><img src="image4" alt="Viteoid II" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10-O-Vanilloylaucubin</td>
<td><em>V. cannabifolia</em></td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td><img src="image5" alt="10-O-Vanilloylaucubin" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6 2'-O-p-Hydroxybenzoyl-6'-O-trans-caffeoyl-8-epi-loganic acid  

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

7 2'-O-p-Hydroxybenzoyl-8-O-epi-loganic acid  

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

8 Agnucastoside A  

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

9 Agnucastoside C  

\[ \text{V. agnus-castus} \quad [19] \]
10 Agnucastoside A  
\( V. agnus-castus \) [18]

11 Agnucastoside B  
\( V. agnus-castus \) [18]

12 6’-O-trans-Feruloylnegundoside  
\( V. altissima \) [15]

13 6’-O-trans-Caffeoylnegundoside  
\( V. altissima \) [15]
14 Negundoside  
\[V. \textit{negundo}\] \[\text{[20]}\]  
\[V. \textit{altissima}\] \[\text{[15]}\]  
\[
\begin{align*}
&\text{HO} \\
&\text{p - HO - Benzoyl - O} \\
&\text{OH}
\end{align*}
\]

15 6′-O-\(\text{p}\)-Hydroxybenzoyl mussaenosidic Acid  
\[V. \textit{negundo}\] \[\text{[21]}\]  
\[
\begin{align*}
&\text{HO} \\
&\text{O - Benzoyl - OH(p)} \\
&\text{OH}
\end{align*}
\]

16 Agnucastoside A  
\[V. \textit{agnus-castus}\] \[\text{[19]}\]  
\[
\begin{align*}
&\text{O - Glc - 6 - O - Foliamenthoyl}
\end{align*}
\]

17 Agnucastoside B  
\[V. \textit{agnus-castus}\] \[\text{[19]}\]  
\[
\begin{align*}
&\text{O - Glc - 6 - O - (6, 7 - Dhydrofoliamenthoyl)}
\end{align*}
\]
18 Geniposide \[ V. cannabifolia \] [12]

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

19 \(2\text{'-}O-p\)-Hydroxybenzoylgardoside \[ V. altissima \] [15]

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

20 Viteoid 1 \[ V. rotundifolia \] [17]

[B] Diterpenoids

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Vitex Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>(rel 5S, 6S, 8R, 9R, 10S)-6-Acetoxy-9-hydroxy-13(14)-labden-16, 15-olide</td>
<td><em>V. rotundifolia</em></td>
<td>[22]</td>
</tr>
<tr>
<td>23</td>
<td>(rel 5S, 6R, 8R, 9R, 10S)-6-Acetoxy-9-hydroxy-15-methoxy 13(14)-labden-16, 15-olide</td>
<td><em>V. rotundifolia</em></td>
<td>[22]</td>
</tr>
<tr>
<td>24</td>
<td>Vitexilactone</td>
<td><em>V. rotundifolia</em></td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>V. trifolia</em></td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>V. agnus-castus</em></td>
<td>[23]</td>
</tr>
<tr>
<td>25</td>
<td>Viteagnuside A</td>
<td><em>V. agnus-castus</em></td>
<td>[23]</td>
</tr>
</tbody>
</table>
Review on Different *Vitex* Species

26 Vitexlactam A *V. agnus-castus* [25a]

27 \(\text{rel} 5S, 6R, 8R, 9R, 10S, 13S, 16S\)-6-Acetoxy-9, 13-epoxy-16-methoxy labdan-15, 16-olide (A) *V. rotundifolia* [22] *V. agnus-castus* [23]

28 \(\text{rel} 5S, 6R, 8R, 9R, 10S, 13R, 16S\)-6-Acetoxy-9, 13-epoxy-16-methoxy labdan-15, 16-olide *V. rotundifolia* [22] *V. agnus-castus* [23]

29 \(\text{rel} 5S, 6R, 8R, 9R, 10S, 13S\)-6-Acetoxy-9, 13-epoxy-15-methoxy labdan-16, 15-olide *V. rotundifolia* [22] *V. agnus-castus* [23]

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

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

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

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

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

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

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

\(V. \text{rotundifolia} \quad [22]\)
34 Previtexilactone  
\[ V. trifolia \] [25]

35 Viteagnusin E  
\[ V. agnus-castus \] [24]

36 Viteagnusin I  
\[ V. agnus-castus \] [23]

37 Viteagnusin J  
\[ V. agnus-castus \] [23]

38 Vitetrifolin D  
\[ V. trifolia \] [26]  
\[ V. agnus-castus \] [23]  
\[ V. rotundifolia \] [27]
Review on Different Vitex Species

39 Vitetrifolin E (= Vitexifolin E) \( V. \text{ trifolia} \) [26]

40 Vitetrifolin F (=Vitexifolin F) \( V. \text{ trifolia} \) [26]

41 Vitetrifolin G \( V. \text{ trifolia} \) [26]

42 Viteaginusin A \( V. \text{ agnus-castus} \) [24]

43 Viteagnusin B \( V. \text{ agnus-castus} \) [24]
44 Viteagnusin C  \( V. agnus-castus \) [24]

45 Viteagnusin D  \( V. agnus-castus \) [24]

46 8-epi. Scareol  \( V. agnus-castus \) [23,24]

47 Vitexifolin A  \( V. rotundifolia \) [27]

48 Vitexifolin B  \( V. rotundifolia \) [27]
49 Norditerpene aldehyde A

50 Norditerpene aldehyde B

51 Vitetrifolin B

52 Vitetrifolin C

53 Rotundifuran

V. trifolia [25, 28]
54 Dihydrsolidagenone  
\[ V. trifolia \]  [28]

55 Vitedoin B  
\[ V. negundo \]  [29]

56 Vitexifolin D  
\[ V. rotundifolia \]  [27]

57 Trisnor-\(\gamma\)-lactone  
\[ V. rotundifolia \]  [27]

58 iso-Ambreinolide  
\[ V. rotundifolia \]  [27]
59  Vitexifolin E  

\[ \text{V. rotundifolia} \] [27]

60  Vitexifolin C  

\[ \text{V. rotundifolia} \] [27]

61  Abietatriene 3β – ol  

\[ \text{V. trifolia} \] [28]

62  Vitetrifolin A  

\[ \text{V. trifolia} \] [28]
[C] Triterpenoids

63 Ursolic acid  \( V.\) negundo  [30]

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

64 2\(\alpha\),3\(\alpha\)-Dihydroxy-urs-12-en-28-oic acid  \( V.\) agnu-castus  [23]

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

65 2\(\alpha\) - Hydroxyursolic acid  \( V.\) agnus-castus  [23]  \( V.\) peduncularis  [31]

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

66 Maslinic acid  \( V.\) agnus-castus  [23]

\[
\begin{align*}
\text{HO} & \quad \text{H} \\
\text{H} & \quad \text{COOH} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{HO}
\end{align*}
\]
67 3-epi-Maslinic acid  
\[ \text{V. agnus-castus} \] [23]

68 Acetyloleanolic acid  
\[ \text{V. negundo} \] [30]

69 3β-Acetoxy olean-12-en-27-oic acid  
\[ \text{V. negundo} \] [32]

70 2α,3α - Dihydroxyoleana-5, 12-dien-28-oic acid  
\[ \text{V. negundo} \] [32]
71. 2β, 3α – Diacetoxyoleana-5, 12-dien-28-oic acid

72. 2α, 3β - Diacetoxy-18-hydroxyoleana-5, 12-dien-28-oic acid

73. Betulinic acid

[D] Flavonoids

74. Apigenin
75 Luteolin \[V. pinnata\] [33]

76 Luteolin-3'-O-Glc A-Me ester \[V. negundo\] [34]

77 Vitexin \[V. peduncularis\] \[V. negundo\] [31] [35]

78 Isovitexin \[V. negundo\] [35]
79 Isoorientin

V. cannabifolia [12]

80 Orientin

V. cannabifolia [12]

81 2″-O-p – Hydroxybenzoyl orientin

V. altissima [36]

82 5-Hydroxy-3,6,7,3’,4’-Pentamethoxyflavone

V. negundo [37]
Review on Different Vitex Species

83 Peduncularisin

84 Pachypodol

85 Casticin (=Vitexicarpin)

86 Artemetin
87  5, 4’-Dihydroxy-3, 6, 7, 8, 3’-penta methoxy flavones  

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

\[
\begin{align*}
\text{MeO} & \\
\text{OMe} & \\
\text{OH} & \\
\text{OMe} & \\
\text{OMe} & \\
\end{align*}
\]

88  5, 3’-Dihydroxy-7, 8, 4’-trimethoxy-flavanone  

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

\[
\begin{align*}
\text{MeO} & \\
\text{OMe} & \\
\text{OH} & \\
\text{OMe} & \\
\text{OMe} & \\
\end{align*}
\]

89  5, 3’-Dihydroxy-6, 7, 4’-trimethoxy flavanone  

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

\[ \text{V. rotundifolia} \quad [27] \]

\[
\begin{align*}
\text{MeO} & \\
\text{OMe} & \\
\text{OH} & \\
\text{OMe} & \\
\text{OMe} & \\
\end{align*}
\]

90  2S - 5 Hydroxy-7, 4’-dimethoxy flavanone  

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

\[
\begin{align*}
\text{MeO} & \\
\text{OMe} & \\
\text{OH} & \\
\end{align*}
\]
91 2', 4'-Dihydroxy - 4, 6'-dimethoxy-chalcone  
\[ \text{V. leptobotrys} \] [41]

92 4'-Hydroxy - 4, 2', 6' - trimethoxy-chalcone  
\[ \text{V. leptobotrys} \] [41]

93 4,2',4', β - Tetraydroxy - 6 -methoxy-α, β dihydrochalcone  
\[ \text{V. leptobotrys} \] [41]

[E] Lignans

94 Negundin A  
\[ \text{V. negundo} \] [42]
95 Detetrahydroconidendrin

\[ \text{V. negundo} \quad [29] \\
\text{V. cannabifolia} \quad [12] \]

96 Isomer of Methoxydetetrahydroconidendrin

\[ \text{V. rotundifolia} \quad [43] \]

97 Methoxydetetrahydroconidendrin

\[ \text{V. rotundifolia} \quad [43] \]

98 Vitedoamine A

\[ \text{V. negundo} \quad [29] \]
99 Vitrofolal D  
\[ V. \text{rotundifolia} \quad [44] \]

100 Vitrofolal C  
\[ V. \text{rotundifolia} \quad [43] \]

101 Vitrofolal A  
\[ V. \text{rotundifolia} \quad [43] \]

102 Vitrofolal B  
\[ V. \text{rotundifolia} \quad [43] \]
103 Negundin B

\[
\begin{align*}
&\text{CH}_2\text{OH} \\
&\text{H} \\
&\text{CH}_2\text{OH} \\
&\text{MeO} \\
&\text{OH} \\
&\text{OH} \\
&\text{OMe}
\end{align*}
\]

\[V. \text{negundo}\] [42]

104 (+) – Lyoniresinol

\[
\begin{align*}
&\text{MeO} \\
&\text{HO} \\
&\text{OMe} \\
&\text{OMe} \\
&\text{MeO} \\
&\text{OH} \\
&\text{OH} \\
&\text{OMe}
\end{align*}
\]

\[V. \text{negundo}\] [42]

105 Vitrofolal E

\[
\begin{align*}
&\text{MeO} \\
&\text{HO} \\
&\text{CHO} \\
&\text{OH} \\
&\text{OMe}
\end{align*}
\]

\[V. \text{negundo}\] [42,29]

\[V. \text{rotundifolia}\] [44]

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

106 Vitrofolal F

\[
\begin{align*}
&\text{MeO} \\
&\text{HO} \\
&\text{CHO} \\
&\text{OH} \\
&\text{OH} \\
&\text{OMe}
\end{align*}
\]

\[V. \text{negundo}\] [42,29]

\[V. \text{rotundifolia}\] [44]

\[V. \text{cannabifolia}\] [12]
107 6-Hydroxy – 4(4-hydroxy-3-methoxy phenyl-3-hydroxymethyl-7-methoxy-3, 4-dihydro 2-naphthaldehyde

V. negundo [45,29]
V. cannabifolia [12]

108 Vitedoin A

V. negundo [29]
V. cannabifolia [12]

109 Vitecannaside A

V. cannabifolia [12]

110 Vitecannaside B

V. cannabifolia [12]
111 Pinoresinol

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

112 Altissinone

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

113 2α,3β-7-O-Methylcedrusin

\[ \text{V. negundo} \quad [29] \]

[F] Sesquiterpenoids

114 β – Caryophyllene

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

115 Caryophyllene oxide

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

116 β – Selinene

\[ \text{V. negundo} \quad [48] \]
Review on Different *Vitex* Species

117  \( \alpha \)-Seliene  

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

118 4\(\alpha\),10\(\alpha\) - Dihydroxyaromadendrane  

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

119 Germacrene D  

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

120 Sabiene  

\[ \text{\textit{V. negundo}} \]  [47]

121 Ajugasterone C  

\[ \text{\textit{V. polygama}} \]  [49]  
\[ \text{\textit{V. strickeri}} \]  [50]  
\[ \text{\textit{V. doniana}} \]  [51]  
\[ \text{\textit{V. scabra}} \]  [52]
122 Ajugasterone C monoacetonide
V. polygama [49]
V. strickeri [50]

123 20 – Hydroxyecdysone
V. cymosa [49]
V. polygama [49]
V. strickeri [50]
V. scabra [52]

124 Turkesterone
V. polygama [49]
V. scabra [52]

125 Abutasterone
V. strickeri [50]
126 20-Hydroxyecdysone-20,22-monoacetonide

\[ \text{V. strickeri} \quad [50] \]

127 Shidasterone

\[ \text{V. doniana} \quad [51] \]

128 21-Hydroxyshidasterone

\[ \text{V. doniana} \quad [51] \]

129 24-\textit{epi}-Pinnatasterone

\[ \text{V. scabra} \quad [52] \]
[H] Ecdysteroids

130 26 – Hydroxypinnatasterone  
\[ V. cymosa \] \[49\]

131 Scabrasterone  
\[ V. scabra \] \[52\]

132 Pinnatasterone  
C-24 epimer of 24-\textit{epi}-Pinnatasterone  
\[ V. scabra \] \[52\]

133 24-\textit{epi} - Abutasterone  
C-24 epimer of Abutasterone  
\[ V. scabra \] \[52\]

134 \( (24\,^R) \)-11\(\alpha\), 20, 24-Trihydroxy-ecdysone  
\[ V. canescens \] \[53\]
135 11α, 20, 26 – Trihydroxy-ecdysone and C-25 epimer  

\[ \text{V. canescens} \quad [53] \]

136 20,26-Dihydroxyecdysone  

\[ \text{V. scabra} \quad [52] \]

137 24 – Hydroxyecdysone-2, 3-acetonide  

\[ \text{V. doniana} \quad [51] \]

138 24-Hydroxyecdysone  

\[ \text{V. doniana} \quad [51] \]
**139** 11β, 24-Dihydroxyecdysone  \( V.\ doniana \) [51]

**140** 11α-Hydroxyecdysone  \( V.\ strickeri \) [50]  \( V.\ scabra \) [52]

**141** 11β-Hydroxy-20-deoxyshidasterone  \( V.\ doniana \) [36]

[I] **Miscellaneous**

**142** Dimethyl 3,4,3',4'-tetrahydroxy-δ-truxinate  \( V.\ quinata \) [36]
Review on Different Vitex Species

143 Methyl 10 R - methoxy - 12 - oxo-9(13), 16E – phytodienoate

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

144 Methyl 3, 4, 5 – O- tricaffeoylquinate

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

145 Methyl 3, 4, - O- dicafeoylquininate

\[ \text{V. polygama} \quad [54] \]

146 Methyl 3, 5, - O- dicafeoylquininate

\[ \text{V. polygama} \quad [54] \]
\[ \text{V. cymosa} \quad [49] \]

147 Vicioside

\[ \text{V. pinnata} \quad [33] \]

148 β - Sitosterol

\[ \text{V. negundo} \quad [30] \]
\[ p\text{-Hydroxybenzoic acid} \]

\[ V. \text{negundo} \quad [30] \]

Cinnamoyl

6,7-Dihydroxfoliamenthoyl

Foliamenthoyl

Vanilloyl

Feruloyl

Caffeoyl
PHARMACOLOGICAL ACTIVITIES OF CRUDE EXTRACTS AND PURE ISOLATED CHEMICALS FROM DIFFERENT VITEX SPECIES

The available literature on Vitex species revealed that pharmacological activities of crude extracts / pure isolates from several species of Vitex such as Vitex agnus-castus, V. negundo, V. rotundifolia, V. trifolia, V. doniana, V. glabrata, V. polygama, V. megapotamica, V. leucoxylon have been evaluated. 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.) [55].

The EtOAc extract of Vitex altissima leaves exhibited significant anti-inflammatory activity in rat paw edema model [56].

The CHCl₃, EtOAc and n-BuOH fractions from methanolic extract of the stem-bark of Vitex doniana exhibited significant anti-inflammatory activity on carrageenin-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 (128), 11β - hydroxy-20-deoxyshidasterone (141), 24-hydroxyecdysone (138), 24-hydroxyecdysone-2,3-acetonide (137), shidasterone (127), ajugasterone C (121) and 11β, 24-dihydroxyecdysone (139) 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 due to to carrageenan-induced
inflammation in rats. The reference drug, diclofenac sodium showed 70% inhibition after 6 h [51].

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 showed mild inhibitory effects on COX-1 [57].

*Vitexicarpin* (85) at the dose of 5-100 nM isolated from *Vitex rotundifolia* showed anti-inflammatory activity by preventing TNF-α-induced vascular inflammatory process in human umbilical vein endothelial cells (HUVEC) [38].

The CHCl$_3$ 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 (70) 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 [32].

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, respectively.

Lignan, negundin B (103) isolated from *Vitex negundo* roots exhibited potent anti-inflammatory activity by inhibiting the activity of soybean lipooxygenase and butyryl-cholinesterase (BChE) with IC$_{50}$ of 6.25 ± 0.5 and 194 ± 4.4 μM, respectively. While another isolated lignan, vitrofolal E (105) from the same plant showed only moderate activity against BChE with IC$_{50}$ of 35.0 ± 105 μM [42].
b. Anti-microbial activity

Luteolin-3’-O-glucuronic acid methyl ester (76) and negundoside (14) isolated from *V. negundo* leaves exhibited significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* at MIC of 6.25 µg/ml using fluconazole as standard drug [34].

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 [58].

Lignans, vitrofolals C (100) and D (99) and detetrahydroconidendrin (95) isolated from *Vitex rotundifolia* subterranean part 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 [44].

The essential oils obtained by hydro distillation 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 [59].

c. Anticancer activity

Flavanoid casticin (85) 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 [27].

Vitexicarpin (also known as casticin) (85) 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 kB 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 in-vivo mouse P-388 leukemia model (135 mg / kg) [39].

d. Anti-angiogenic activity

Vitexicapin (85) isolated from the fruits of Vitex rotundifolia showed significant 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 fragment, and caspase 3 blotting indicated that vitexicarpin (0.1-5 μM) inhibited EC proliferation via cellcycle arrest and induction of apoptosis. The flavonoid at the concentration of 5 μM also in-vitro inhibited sprouting from chorioallantoic membranes (CAMs). In addition, vitexicarpin 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 [54].

e. Antioxidant activity

6’-O-trans-Caffeoylnegundoside (13), 2’-O-p-hydroxybenzoylgardoside (19) and 2’-O-p-hydroxybenzoyl-6’-O-trans-caffeoyl-8-epiloganic acid (6) isolated from Vitex altissima leaves exhibited potent anti-oxidant activity, both in superoxide free-radical scavenging assay (using NBT method) (IC₅₀, 24.3, 32.0 and 31.9 μM, respectively) and in DPPH radical scavenging assay (IC₅₀, 15.2, 10.9 and 11.4 μM, respectively) in comparison to the known antioxidants, BHT and α-tocopherol, 381 μM, 19 μM, respectively [15].

Lignans isolated from the seeds of 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 (98),
6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy 3,4-dihydro-2-naphthaldehyde (107), vitrofolal F (106) showed activity similar to α-tocopherol (standard antioxidant) in DPPH assay [29].

Lignans, vitecansides A (109) and B (110) and flavonoids, isoorientin (79) and orientin (80) isolated from the fruits of *Vitex cannabifolia* showed stronger antioxidant activity than that of L-cysteine in DPPH assay. The activity of isoorientin (79) and orientin (80) was more than that of α tocopherol (standard antioxidant) in the same assay [12].

f. *Trypanocidal activity*

Six diterpenoids, two nor aldehyde (49 and 50) vitexifolins E (59) and F (40), 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 µM, respectively[25].

g. *Moulting hormone activity*

24-epi- Pinnatasterone (129) and scabrasterone (131) isolated from the stem bark of *Vitex scabra* exhibited weak *in-vivo* moulting activity with EC₅₀ values of $5.2 \times 10^{-4}$ and $1.0 \times 10^{-3}$ M, respectively based on the activity of 20-hydroxyecdysone (123) ($1.6\times10^{-5}$ M) in Musca assay. The low moulting activity of these ecdysteroids was possibly due to lacking of a 22R hydroxyl group in their molecule [52].
Section 2: Brief history, taxonomical description and classification of

*Vitex peduncularis*

Plate-4. Photographs of *Vitex peduncularis* (family: Verbenaceae)
Vitex peduncularis Wall ex Schauer syn. Vitex peduncularis f. roxburghiana (C. B. clarke) Moldenke, Vitex peduncularis var. roxburghiana Clarke (local name: God Harina) is distributed in India, Eastern Himalayas, Burma, Vietnam and Malaysia. In India, it is found in North-Eastern States, Bihar and Bengal [1, 2] (Plate 4). It is a large tree with pubescent young shoots, greyish bark, trifoliate leaves in lanceolate leaflets, pale yellow flowers in April-May and obovoid shaped drupes in June-July [1].

The taxonomic classification of Vitex peduncularis as per Bentham & Hooker System of classification [61].

Class: Dicotyledons
Sub-class: Gamopetalae
Series: Bicarpellatae
Cohort: Lamiales
Order: Verbenales
Family: Verbenaceae
Genus: Vitex
Species: Vitex peduncularis Wall.
Section 3: Isolation and structure elucidation of vitecin

Isolation:

Vitecin (150) was isolated as brown needles, mp 232\(^0\)C, from EtOAc fraction of MeOH extract of *Vitex peduncularis* leaves by silica gel column chromatography. It was homogeneous on TLC in different solvent systems [Silica gel G, \(R_f = 0.53\) in CH\(_2\)Cl\(_2\)-EtOAc, 5 : 1].

Structure elucidation:

a) **The Molecular formula:**

The molecular formula of the compound was determined as C\(_{18}\)H\(_{16}\)O\(_8\) from its quasi-molecular mass ion at \(m/z\) 361.0918 [M + H]\(^+\) (Calcd for C\(_{18}\)H\(_{17}\)O\(_8\): 361.0923) in HR-FAB-MS and analysis of its \(^{13}\)C-/DEPT NMR data.

b) **The UV spectrum:**

The UV-Vis spectrum of the compound in MeOH (Fig. 4.1) showed absorption maxima at \(\lambda_{max}\) 252 (band II), 263 sh, 360 (band I) nm, characteristic of flavanoids [62]. In presence of AlCl\(_3\), it showed bathochromic shifts of both the bands at \(\lambda_{max}\) 268, 310 and 432 nm (Fig. 4.1a). This significant bathochromic shift of both the bands indicated the presence of ortho-dihydroxyl grouping in ring B and 5-hydroxyl group in ring A of the flavone nucleus [62]. In presence of NaOMe, its solution in MeOH was decomposed after 2 min and showed \(\lambda_{max}\) at 268 nm. It suggested its possible substitution at C-3 position [62].

c) **The IR spectrum:**

The IR spectrum of the compound in KBr (Fig. 4.2) showed absorption bands for hydroxyl (3472 cm\(^{-1}\)), alkoxy (2872 cm\(^{-1}\)) and \(\alpha, \beta\) unsaturated carbonyl (1643 cm\(^{-1}\)) functions.
d) The $^1$H-NMR spectrum:

The $^1$H-NMR spectrum of the compound in DMSO-$d_6$ (Fig. 4.3) (Table-4.3) showed proton signals for a 5,7-deoxygenated A-ring of a flavone moiety [$\delta_H$ 6.37 and 6.75 (each 1H, d, $J = 2.4$ Hz)], a 3',4',5'-trioxoygreated B ring of flavone moiety [$\delta_H$ 7.16 (2H, s, $J = 2.4$ Hz) and three methoxyls [$\delta_H$ 3.81, 3.85 and 3.86 (each 3H, s)]. The $^1$H-NMR spectrum also recorded three phenolic hydroxyl proton signals [$\delta_H$ 12.58 (1H, s) and 9.33 (2H, s)]. The downfield chemical shift of one hydroxyl proton at $\delta_H$ 12.58 indicated the presence of a free hydroxyl group at C-5 position [62]. The $^1$H-NMR spectral data could be assigned by considering its myricetin-tri-O-methyl ether structure [63-65].

e) The $^{13}$C-NMR spectrum:

The $^{13}$C-NMR spectrum of the compound in DMSO-$d_6$ (Fig. 4.4) (Table-4.3) recorded 16 carbon signals, which on DEPT experiments revealed for 3 methyl, 3 methine and 10 quaternary carbons. Two methoxyl carbon resonances at $\delta_C$ 59.8 and 60.0 suggested their location between two ortho-substituted groups and hence their positions would be C-3 and C-4'.

The downfield chemical shift of C-3 resonance by 2.0 ppm compared to that of C-3 in myricetin also supported the location of one methoxyl group at C-3 [66].

f) The HSQC spectrum:

The HSQC spectrum of the compound (Fig. 4.5, 4.5b, 4.6) supported the assignments of methine and methoxyl protons.

g) The HMBC spectrum:

The HMBC spectrum of the compound (Fig. 4.6a and 4.7) showed correlation between $\delta_H$ 3.81 and $\delta_C$ 59.8, and between $\delta_H$ 3.81 and $\delta_C$ 138.2 suggesting the location of one methoxyl at C-3 position. Similarly, the
correlation between $\delta_H$ 3.85 and $\delta_C$ 138.0 in the HMBC spectrum confirmed the position of another methoxyl group at C-4'.

**h) The NOESY spectrum:**

The NOESY spectrum of the compound (Fig. 4.7) showed correlation between methoxyl protons ($\delta_H$ 3.86) and H-6 ($\delta_H$ 6.37), H-8 ($\delta_H$ 6.75) confirming the location of one methoxyl group at C-7 position.

**i) The FAB-MS:**

The FAB-MS of the compound (Fig. 4.8) recorded mass ions at $m/z$ 361 [M + H]$^+$, 360 [M]$^+$, 359 [M - H]$^+$, 345, 317, 299 and 167. The formation of these ions could be rationalized by considering its myricetin-3,7,4'-tri-O-methyl ether structure (Scheme 4.1) [67].

Scheme 4.1. Plausible FAB-MS Fragmentation of 150
j) Conclusion:

On the basis of the forgoing evidence, the structure of the compound, vitecin was elucidated as myricetin-3,7,4'-tri-O-methylether (150) (Fig. 4.9). It is a new natural product.

![Fig. 4.9. Structure of vitecin (150)](image)

![Fig. 4.7. Key HMBC and NOESY correlations in 150](image)
Table 4.2. $^1$H- (600 MHz) and $^{13}$C-(150 MHz) NMR spectral data of compound 150 in DMSO-d$_6$ (δ, ppm)$^a$

<table>
<thead>
<tr>
<th>C/H No</th>
<th>$\delta_C$ (δ, ppm)</th>
<th>$\delta_H$ (δ, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>155.9 (C)</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>138.2 (C)</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>178.1 (C)</td>
<td>—</td>
</tr>
<tr>
<td>4a</td>
<td>104.1 (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)</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)</td>
</tr>
<tr>
<td>8a</td>
<td>156.3 (C)</td>
<td>—</td>
</tr>
<tr>
<td>1'</td>
<td>119.6 (C)</td>
<td>—</td>
</tr>
<tr>
<td>2',6'</td>
<td>109.2 (CH)</td>
<td>7.16 s</td>
</tr>
<tr>
<td>3',5'</td>
<td>145.6 (C)</td>
<td>—</td>
</tr>
<tr>
<td>4'</td>
<td>138.0 (C)</td>
<td>—</td>
</tr>
<tr>
<td>3 - OMe</td>
<td>59.8 (CH$_3$)</td>
<td>3.81 s</td>
</tr>
<tr>
<td>7 - OMe</td>
<td>56.2 (CH$_3$)</td>
<td>3.86 s</td>
</tr>
<tr>
<td>4' - OMe</td>
<td>60.0 (CH$_3$)</td>
<td>3.85 s</td>
</tr>
<tr>
<td>5 - OH</td>
<td>—</td>
<td>12.58 brs</td>
</tr>
<tr>
<td>3', 5'-OH</td>
<td>—</td>
<td>9.33 s</td>
</tr>
</tbody>
</table>

$^a$All assignments are based on HSQC and HMBC experiments.
Fig. 4.1. UV spectrum of vitexin in MeOH

Absorbance vs. Wavelength
THESIS, PART-IV

Isolation and Structure Elucidation

Fig. 4.2. IR spectrum of Vitisin (150) in KBr.

Sample 054 by Chemistry on Wednesday, August 21, 2013

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Fig. 4.3 1H-NMR spectrum of Vitexin (150) in DMSO-d$_6$
Isolation and Structure Elucidation

Fig. 4.3a. 1H-NMR spectrum (expanded) of Vitisin (150) in DMSO-"d_6"
Fig. 4.4: $^1$H-NMR spectrum of Vitexin (150) in DMSO-$d_6$
Fig. 4.4a. $^{13}$C-NMR spectrum (expanded) of Vitecin (150) in DMSO-$d_6$. 
Fig. 4.5. HSQC NMR spectrum of Vitexin (150) in DMSO-d$_6$. 

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Fig 4b: HSQC NMR spectrum (expanded) of Vincin (150) in DMSO-$d_6$.
Fig. 4.6. HSQC NMR spectrum of Vitecin (150) in DMSO-d$_6$.
Fig. 1.8 FAB-MS of viletin (150)
Fig. 4.6: 13C-NMR spectrum (Expanded) of Vitecin (150) in DMSO-d_6.
Fig. 4.4c. $^{13}$C-NMR (DEPT) spectra of Vitecin (150) in DMSO-$d_6$
References


