REFERENCES


Accepted 23 February 2006
Revised 20 April 2005
Received 24 November 2003

Antiinflammatory Constituents of Teramnus labialis

C. SRIDHAR, A. V. KRISHNARAJU1 AND G. V. SUBBARAJU1*
Department of Pharmaceutical Chemistry, Sri Padmavathi School of Pharmacy, Tirupati-517 507, Laila Impex R & D Centre, Unit-I, Phase-III, Jawahar Autonagar, Vijayawada-520 007, India.

Bioassay-guided fractionation, based on antiinflammatory activity of the methanolic extractives of Teramnus labialis led to the isolation and characterization of vitexin, bergenin, daidzin and 3-O-methyl-D-chiro-inositol as active constituents. Vitexin exhibited a dose-dependent inhibitory activity on 5-lipoxygenase enzyme. The isolated constituents were also screened for their antioxidant activity by nitroblue tetrazolium (NBT) riboflavin photo reduction method. Vitexin exhibited moderate antioxidant activity. This is the first reported occurrence of vitexin, bergenin, daidzin and 3-O-methyl-D-chiro-inositol in T. labialis.

Teramnus labialis Spreng (Family: Fabaceae) is a herb, commonly known as mashaparni (Sanskrit) and mashavan (Hindi), and a well-known medicinal plant in the Ayurvedic system of medicine. It has been reported to be useful in treating rheumatism, tuberculosis, nerve disorders, paralysis and catarrhs1-3. Phytochemical investigation on the seeds of T. labialis yielded fraxidin as the major active constituent4. Bioassay-guided fractionation, based on anti-hyperglycaemic activity of aqueous alcoholic extract of T. labialis, yielded fraxidin as the major active constituent5. In view of the reported use of T. labialis in rheumatism, we have evaluated the antiinflammatory activity of T. labialis, and we report in this paper the isolation and characterization of the active constituents.

*For correspondence
E-mail: subbarajugottumukkala@hotmail.com

The aerial parts of T. labialis were collected from the Tirumala Hills of Chittoor district, Andhra Pradesh, and were authenticated at the Department of Botany, S. V. University, Tirupati. The aerial parts were shade-dried and powdered. The powdered material was extracted, successively, with hexane, ethyl acetate and methanol. The extracts were concentrated under reduced pressure.

The extracts were subjected to antiinflammatory activity by carrageenin-induced rat paw oedema model of winter et al.6. Wistar rats of either sex weighing between 180 and 220 g were procured from NIN, Hyderabad. The rats were divided into five groups, each group consisting of six animals. One group served as negative control (received 1% Tween-80, 10 ml/kg); second group served as positive control (received 25 mg/kg, diclofenac sodium suspended in 1% Tween-80); third, fourth and fifth groups received 250 mg/kg of hexane, ethyl acetate and
methanol extracts suspended in 1% Tween-80 respectively, by oral route.

All experimental protocols have been approved by the Institutional Animal Ethics Committee prior to the conduct of the experiments. Oedema was produced by injecting carrageenin solution 0.1 ml (1% w/v) to subplantar region of the left hind paw of rats of all groups. Drug treatment was given 1 h prior to the carrageenin injection. The paw volume was measured by a plethysmometer at zero and three hours after carrageenin injection. The difference between the initial and the final paw volume gave the oedema volume. The results obtained as mean increase in paw volume and percentage inhibition of oedema are presented in Table 1.

The methanol extract, which showed potent antiinflammatory activity, was further fractionated to isolate the active constituents. The methanol extracts (200 g) were chromatographed over silica gel column and eluted with chloroform and mixture of chloroform and methanol with increasing polarity. The chloroform-methanol (93:7) eluates afforded compound-A (170 mg), mp: 139-140°, [α]D0 -20.0° (c, 0.2, methanol); chloroform-methanol (90:10) eluates yielded compound-B (130 mg), mp: 232-233°; chloroform-methanol (85:15) eluates yielded compound-C (48 mg), mp: 275-276°; and chloroform-methanol (70:30) eluates yielded compound-D (5 g), mp: 180-182°.

Compound-A, was obtained as colourless crystals from aqueous methanol, mp: 139-140°, analysed for C14 H16 O9 [LC-MS: m/z 327, (M-H)]. The IR spectrum showed bands at 3372 brs (hydroxyl), 1623 (carbonyl), 1182, 1220, 1237, 1570, 1572 and 1610 cm⁻¹ (aromatic). The 1H NMR [500 MHz, d6-DMSO] spectral data contained six aromatic protons constituted by a singlet at δ 8.36 (1H, 8.8 Hz) and 7.22 (1H, d, J=2.1 Hz) and an AA’BB’ spin system [δ 7.40 (2H, d, J=8.6 Hz) and 6.81 (2H, d, J=8.6 Hz)] attributable to a para-disubstituted phenyl unit. A perusal of the above data indicated the presence of an isoflavonoid skeleton. The spectrum also showed a group of signals between δ 3.10 and 3.70, in addition to an anomeric proton signal at δ 5.10 (1H, d, J=7.6 Hz), suggestive of a sugar unit. The 13C NMR [125 MHz, d6-DMSO] spectrum showed six quaternary carbons (δ 116.0, C-10a and 118.1, C-6), one aromatic carbonyl (δ 163.4), three aromatic carbons (δ 151.4, 154.1 and 1445 cm⁻¹), three aromatic protons constituted by a singlet at δ 8.36 (1H, 8.8 Hz) and 7.22 (1H, d, J=2.1 Hz) and an AA’BB’ spin system [δ 7.40 (2H, d, J=8.6 Hz) and 6.81 (2H, d, J=8.6 Hz)] attributable to a para-disubstituted phenyl unit. A perusal of the above data indicated the presence of an isoflavonoid skeleton. The spectrum also showed a group of signals between δ 3.10 and 3.70, in addition to an anomeric proton signal at δ 5.10 (1H, d, J=7.6 Hz), suggestive of a sugar unit. The 13C NMR [125 MHz, d6-DMSO] spectrum showed six quaternary carbons (δ 118.5, 122.0, 123.7, 157.0, 157.2 and 161.0), three aromatic carbons (δ 103.4, 115.6 and 127.0), a β-olefinic carbon (δ 153.3) and a carbonyl carbon resonating at δ 174.8. The signals at δ 60.7, 69.7, 73.2, 76.5, 77.2 and 100.0 are attributable to a sugar moieties.

Compound-B was obtained as a white crystalline solid from aqueous methanol, mp: 232-233°, analysed for C15 H16 O9 [LC-MS: m/z 439, (M+Na)]. The IR spectrum showed bands at 3390 brs (hydroxyl), 1623 (carbonyl), 1514 and 1445 cm⁻¹ (aromatic). The 1H NMR [500 MHz, d6-DMSO] spectral data contained six aromatic protons constituted by a singlet at δ 8.36 (1H, 8.8 Hz) and an ABX spin system, characteristic of a 1,2,4-trisubstituted phenyl unit [δ 8.04 (1H, d, J=8.8 Hz), 7.14 (1H, dd, J=2.2, 8.8 Hz) and 7.22 (1H, d, J=2.1 Hz)] and an AA’BB’ spin system [δ 7.40 (2H, d, J=8.6 Hz) and 6.81 (2H, d, J=8.6 Hz)] attributable to a para-disubstituted phenyl unit. A perusal of the above data indicated the presence of an isoflavonoid skeleton. The spectrum also showed a group of signals between δ 3.10 and 3.70, in addition to an anomeric proton signal at δ 5.10 (1H, d, J=7.6 Hz), suggestive of a sugar unit. The 13C NMR [125 MHz, d6-DMSO] spectrum showed six quaternary carbons (δ 119.3, 122.1, 123.1, 157.1, 157.0 and 161.1), three aromatic carbons (δ 103.4, 115.6 and 127.0), a β-olefinic carbon (δ 153.3) and a carbonyl carbon resonating at δ 174.8. The signals at δ 60.7, 69.7, 73.2, 76.5, 77.2 and 100.0 are attributable to a sugar moiety. The 13C NMR chemical shifts of the sugar moiety matched well with those recorded for bergenin (1).

The table below shows the effect of different extracts of T. labialis on carrageenin-induced rat paw oedema.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Mean oedema volume ±SE (ml)</th>
<th>Percent inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>10 ml/kg</td>
<td>0.63 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac sodium</td>
<td>25</td>
<td>0.15 ± 0.01</td>
<td>76.19</td>
</tr>
<tr>
<td>3</td>
<td>Hexane extract</td>
<td>250</td>
<td>0.61 ± 0.03</td>
<td>3.17</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl acetate extract</td>
<td>250</td>
<td>0.59 ± 0.04</td>
<td>6.34</td>
</tr>
<tr>
<td>5</td>
<td>Methanol extract</td>
<td>250</td>
<td>0.45 ± 0.03</td>
<td>28.57*</td>
</tr>
</tbody>
</table>

Number of animals in each group 6. *P< 0.01, when compared to control

TABLE 1: EFFECT OF DIFFERENT EXTRACTS OF T. LABIALIS ON CARRAGEENIN-INDUCED RAT PAW OEDEMA.
identical with those reported for daidzin\textsuperscript{10,11}(2).

The compound-C was obtained as a yellow solid, mp: 275-276°, analysed for C\textsubscript{21}H\textsubscript{20}O\textsubscript{10} [LC-MS: m/z 431, (M-H)]. The IR spectrum of the compound showed bands at 3381 (hydroxyl), 1652 (carbonyl), 1568, 1501 cm\textsuperscript{-1} (aromatic). The \textsuperscript{1}H NMR [400 MHz, d\textsubscript{6}-DMSO] spectrum showed a chelated hydroxyl proton (δ 13.18, 1H, s), an aromatic singlet (δ 6.28, 1H, s), a downfield signal (δ 6.79, 1H, s), suggestive of a flavonoid\textsuperscript{12} and an AA’BB’ spin system [δ 8.03, (2H, d, J=8.5 Hz) and 6.09 (2H, d, J=8.5 Hz)], attributable to a para-disubstituted phenyl unit. In addition, the \textsuperscript{1}H NMR spectrum showed a series of signals attributable to a para-disubstituted phenyl unit. In addition, the \textsuperscript{1}H NMR spectrum showed bands at 3403 (hydroxyl) and 1072 cm\textsuperscript{-1} (ether). The IR spectrum of the compound showed bands at 3381 (hydroxyl), 1652 (carbonyl), 1568, 1501 cm\textsuperscript{-1} (aromatic).

The IR spectrum of vitexin, bergenia, daidzin and 3-O-methyl-D-chiro-inositol were screened for their antioxidant activity by the nitroblue tetrazolium (NBT) riboflavin photo-reduction method\textsuperscript{17}. The reaction mixture comprises of EDTA (6 \textmu M) containing 3 \mu g NaCN, riboflavin (2 \mu M), NBT (50 \mu M), various concentrations of the test substances and phosphate buffer (58 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured at 560 nm before and after illumination. The percentage inhibition of superoxide generation was calculated by comparing the absorbance values of the control and compound treated tubes. The IC\textsubscript{50} values are obtained from the plot drawn, concentration vs percent inhibition. The results of antioxidant studies have been presented in Table 2.

The present investigation reveals that the methanol extract of \textit{T. labialis} possess strong antiinflammatory activity. The isolation and characterization of vitexin, bergenin and daidzin, the known antiinflammatory compounds, from the methanolic extractives of \textit{T. labialis}, substantiates the traditional use of \textit{T. labialis} in treating rheumatism. It showed further that the vitexin may be exhibiting antiinflammatory activity by inhibiting 5-lipoxygenase pathway. This is the first report on identification of vitexin, bergenin, daidzin and 3-O-methyl-D-chiro-inositol in the extracts of \textit{T. labialis}.

### TABLE 2: ANTIOXIDANT ACTIVITY OF CHEMICAL CONSTITUENTS OF \textit{T. LABIALIS}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Superoxide-radical scavenging IC\textsubscript{50} (\mu g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitexin</td>
<td>62</td>
</tr>
<tr>
<td>Daidzin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bergenin</td>
<td>100</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>90</td>
</tr>
</tbody>
</table>

The compounds, vitexin, bergenin, daidzin and 3-O-methyl-D-chiro-inositol were screened for 5-lipoxygenase inhibitory activity using colorimetric method of Gay \textit{et al.}\textsuperscript{16}. The assay mixture contained 50 mM phosphate buffer (pH 6.3), 5-lipoxygenase, various concentrations of test substances and linoleic acid (80 mM) in a total volume of 0.5 ml. After 5 min incubation of above reaction mixture, 0.5 ml ferric-xyleneol orange reagent (in perchloric acid) was added and OD was measured after 2 min at 585 nm using a spectrophotometer. Controls were run along with test in a similar manner except using vehicle instead of test substance solution. Percent inhibition was calculated by comparing absorbance of test with that of control. Vitexin exhibited a dose-dependent inhibitory activity on 5-lipoxygenase enzyme (percent inhibition:dose in \mu M, 12.16:500; 22.2:1000; 36.49:2000; 64.48:3000). Daidzin, bergenin and 3-O-methyl-D-chiro-inositol did not exhibit any activity even at 1000 \mu M dose.
Figure 1: Names of the chemical structures

ACKNOWLEDGEMENTS

The authors thank Sri G. Ganga Raju, Chairman, Laila Impex; and Smt. P. Sulochana, Correspondent, Sri Padmavathi School of Pharmacy, for encouragement; Dr. B. Lakshmana Raju for NMR spectral data; and Dr. K. Madhava Chetty, Department of Botany, S. V. University, Tirupati, for authentication of plant species.

REFERENCES