Validation and quality determination of an ethnobotanical lead for osteogenic activity isolated from *Ulmus wallichiana* Planch.: A traditional plant for healing fractured bones

K R Arya* Deepty Sharma† and Brijesh Kumar‡

1Botany Division, 2Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute, Lucknow 226 001, India

Received 29 October 2010; revised 05 April 2011; accepted 11 April 2011

*Ulmus wallichiana* is an important traditional and endangered plant species of Western Himalaya, used for treatment of fractured bones in animal as well as in human beings. This paper presents significant ethnobotanical information of *U. wallichiana* collected from folk tradition of remote hilly localities of Almora and Bageshwar districts of Kumaon Himalaya, Uttarakhand, India. Folk claim is validated through chemical and pharmacological outputs followed by identification and qualitative determination of bioactive compounds.

**Keywords:** Ethnobotanical lead, Fractured bone, Osteogenic, *Ulmus wallichiana*

**Introduction**

Prostratin, an HIV therapeutic that activates latently infected T-cell pool1, is a recent example of potentially beneficial and lucrative compound identified through ethnobotanical work in Samoa2. Western Himalayan sector of Indian Himalaya is one of the richest emporiums for medicinal taxa. Plenty of reports on ethnobotany of Kumaon region have been published3-7. In Indian context, osteoporosis8 is a silent epidemic. Bone forming (osteogenic/anabolic) therapy is limited to only parathyroid hormone (PTH), which being extremely costly, is not widely available in India. *Ulmus wallichiana* Planch. (local name, Chamourmou) is a rare and endemic plant species of Western Himalaya9-12. Bark of this tree is commonly used for treatment of fractured bones in animals as well as in human beings in folk tradition of Uttarakhand Himalaya13,14. This study validates folk traditional knowledge of *U. Wallichiana* through determination of bioactive compounds as well as to explore its possible uses in public domain for treatment of health related disorders with osteoporosis.

**Experimental Section**

Plant species was properly identified according to Flora of District Garhwal, North West Himalaya9. Herbarium specimen was deposited in departmental Herbarium, CDRI, Lucknow, vide voucher specimen No. KRA-24443. During collection, matured trees (15) from different localities (Almora, Bageshwar and Nainital districts of Uttarakhand, India) were selected. Longitudinally (15 cm x 8 cm long) strips of bark from different spots (5-6 strips per tree) were collected for single time to avoid damage and to sustain existing diversity of elite clones in nature.

Fresh bark (1 kg) was collected, crushed minutely and boiled in water (1 l) for ½ h to make fine semi-solid substrate. As bark contains strong fibers and high percentage of mucilage, substrate became laxative and sticky. Fractured part of body was washed and properly sated by experienced bone setter. Cooled semi-solid laxative substrate was thoroughly poured and lapped around fractured part and tightened with a clean cloth followed by giving proper support to fractured part with thick cardboard. Patient was then allowed for minimum 30 days rest.

Initially fresh bark (2 kg) was collected, dried and powdered. Ethanolic extract were evaluated for chemical and pharmacological investigations15-17. Active
compounds, Ulmoside A, B and Naringenin-6-C-β-glucopyranoside (Fig. 1), were identified through Quadrupole Time of Flight High Resolution Mass Spectrometer (Q TOF HRMS). Chemical profiling of *U. wallichiana* was performed using Agilent 1200 series liquid chromatography system (LCS), which was equipped with a quaternary solvent manager and auto sampler coupled to Agilent 6520 accurate mass QTOF mass spectrometer with an Electro Spray Ionization (ESI) interface.

**Results and Discussion**

Folklore information of *U. wallichiana* for bone healing property was recorded during 2006-2008 under ethnomedicinal exploration tour to surrounding remote hilly localities of Taragtal and Chaura villages in Almora and Bageshwar districts of Uttarakhand, India. During interrogations from 10 herbal medicaments and 15 villagers (age 45-80 y), 80% informants revealed that bark of *U. wallichiana* has great potential for healing fractured bones in animals as well as in human beings.

During chemical investigations, ulmosides A & B (Fig. 1) were isolated from bark\(^{15}\) and showed peak bone achievement and prevention of menopausal bone loss in ovariectomized rats. Whereas, third known compound, naringenin-6-C-β-D glucopyranoside is at 435, also identified from same extract, promotes osteoblast function and inhibited adipogenesis\(^ {16-17}\). Stimulation of osteoblast differentiation is a bone anabolic function that is desirable for osteoporosis therapy\(^ {18}\). A representative Q TOF mass spectrum of bark of *U. wallichiana* (Fig. 2) indicated that peak at m/z 467 and 435 could be due to ulmoside A and peak at 451 could be either due to ulmoside B or eriodictyol-6-C-β-D glucopyranoside. Exact mass values measured using QTOF mass spectrometer is given in Table 1. All samples collected from different altitudinal locations of Uttarakhand provided same spectrums and molecular mass, which confirm presence of active compounds for osteoprotective activity.

Through recorded history, much knowledge can be found\(^ {19}\). An estimate have been made that between 20,000 and 55,000 species of plants have been used medicinally\(^ {21-22}\), of which only small portion has been investigated for drug purposes. About 300 million people in India are suffering with osteoporosis\(^ {22}\). Although incidence of osteoporosis is alarmingly high in India, lack of awareness of disease delays diagnosis and preventive measures. Recent data indicates that Indians have lower bone density than their North American and European counterparts, and that osteoporotic fractures occur 10-
Fig. 2—Q TOF HRMS spectrum and molecular mass of ulmosides A & B and naringenin -6-C-β-glucopyranoside, respectively
ARYA et al: FOLK KNOWLEDGE VALIDATION OF OSTEOGENIC ACTIVITY FROM ULMUS WALLICHIANA

20 years earlier in Indians as compared to Caucasians\(^2\)\(^3\)\(^4\). In India, 50% women (200 million women) have osteoporosis; there are more women than men at any elderly age group\(^2\)\(^2\).

**Conclusions**
This study supports significant advantages for using ethnobotanical knowledge to select candidate species for scientific investigations and bioprospection of lead molecules for pharmaceutical application. Scientific outputs of this study also support and validate knowledge of this patrimony and revealed scope for isolation of novel compounds for modern therapeutic value for osteoprotective drug from *U. wallichiana*. However, plant species can be easily identified and determined through Q TOF HRMS profiling.

**Acknowledgements**
One of the authors (KRA) thanks Director CDRI, Lucknow, for providing facilities. Authors also thank Dr N Chattopadhayay, Coordinator, Reproductive Health Research for financial supports and local herbal medicaments villagers and Forest Department of Uttarakhand for cooperation and support. Another author (DS) thanks CSIR, New Delhi for research grant NWP-0045.

**References**
8. website: www.hinduonnet.com/fline/fl2101/stories/20040116002010400.htm

**Table 1—Comparative evolution and qualitative determination of bark samples of *Ulmus wallichiana* collected from Almora, Bageshwar and Nainital Districts, Uttarakhand, India**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Collection sites of samples (bark)</th>
<th>Peak reported</th>
<th>Molecular formula</th>
<th>Mass</th>
<th>Difference ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Almora</td>
<td>[M+H]+467.1186</td>
<td>C(<em>{21})H(</em>{22})O(_{12})</td>
<td>466.1112</td>
<td>-0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+Na]+489.1002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+H]+451.1227</td>
<td>C(<em>{21})H(</em>{22})O(_{11})</td>
<td>450.1154</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+H]+435.1282</td>
<td>C(<em>{21})H(</em>{22})O(_{10})</td>
<td>434.1208</td>
<td>1.15</td>
</tr>
<tr>
<td>2</td>
<td>Bageshwar</td>
<td>[M+H]+467.1187</td>
<td>C(<em>{21})H(</em>{22})O(_{12})</td>
<td>466.1114</td>
<td>-0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+Na]+489.1001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+H]+451.1238</td>
<td>C(<em>{21})H(</em>{22})H(_{11})</td>
<td>450.1164</td>
<td>-0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+Na]+473.1053</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+H]+435.1289</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Nainital</td>
<td>[M+H]+467.1188</td>
<td>C(<em>{21})H(</em>{22})O(_{10})</td>
<td>434.1216</td>
<td>-0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+H]+451.1237</td>
<td>C(<em>{21})H(</em>{22})H(_{11})</td>
<td>450.1163</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[M+H]+435.1295</td>
<td>C(<em>{21})H(</em>{22})O(_{10})</td>
<td>434.1222</td>
<td>-2.14</td>
</tr>
</tbody>
</table>


20 Penso G, Inventory of medicinal plants and compilation of a list of the most widely used plants, *WHO Document DPM/ WP* (Geneva) 1976, 78.2.


22 Website: www.hindu.onnet.com.
