**QUANTITATIVE ESTIMATION OF BERBERINE CONTENT OF BERBERIS ASIATICA FROM DIFFERENT ALTITUDE OF GARHWAL HIMALAYA**

ALOK MAITHANI¹, VERSHA PARCHA¹, AND DEEPAK KUMAR²

¹Dept of Pharmaceutical Chemistry, Sardar Bhagwan Singh PG Institute of Biomedical Sciences and Research, Balawala, Dehradun.²Dept of Pharmaceutical Chemistry, Dolphin PG College of Natural Sciences, Manduwala, Dehradun. Email: alok_maithani1@rediffmail.com

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**ABSTRACT**

Objective: *Berberis asiatica* is a popular medicinal plant of Garhwal Himalaya and its root is traditionally used for curing various ailments like jaundice, conjunctivitis, and diabetes mellitus. In the present study berberine content in *B. asiatica* growing at seven different altitudes of Garhwal Himalayas was quantitatively determined by HPTLC analysis.

Methods: Ethanolic extracts of various plant samples were analyzed with standard berberine (purified) in different concentrations 1-5μl. The peak areas of each standard and test samples were obtained from the system and a calibration graph was plotted between concentrations vs. Peak area. Results: The plant samples belonging to lowest altitude region was found to possess maximum concentration of berberine which was 2.94% however berberine content was non-linearly distributed among the other samples of higher altitudes.

Keywords: *Berberis asiatica*, Berberine, quantitative determination, isoquinoline alkaloid, and HPTLC.

**Introduction:**

*Berberis asiatica*, belongs to family Berberidaceae, is a well-known shrub of Garhwal Himalaya and regionally known as "Kingoro". In Ayurvedic medicinal system it is named as "Daruharidra" or 'Wood Turmeric' due to similar properties as of turmeric[1]. *Berberis asiatica* is reported to possess many biological activities like antimicrobial, wound healing, hepatoprotective, anti-acne, & cytotoxicity etc[2-6].

The plant yields fairly large quantity of alkaloids in which isoquinoline type alkaloids like berberine, palmatine, jettorhizine, and columbamine are the most studied phytoconstituents[7] (Figure 1). Thus the major pharmaceutical properties of *B. asiatica* are attributed due to the presence of alkaloidal content of plant. Berberine is 8- substituted derivative of isoquinoline. It exhibits significant role in management of various enteric infections, specially bacterial dysentery [8]. It is also experimentally claimed to be hepatoprotective, antiabetic [9], anti-inflammatory [10] and antimicrobial [11] agent. The medicinal importance of berberine and other phytochemicals present in *Berberis asiatica* increases its demand in global market India consumes 500 tones of roots of this plant alone and also Indian government exports 60 to 70 tones of roots of *Berberis asiatica* per year[12]. Roots of the plant are collected in fairly large quantities in Tehri Garhwal of Uttarakhand during augst - September and are sold in drug market of Dehradun and Hardwar.

Looking into the tremendous potential of curing disease and excellent revenue realized through the disposal of this plant, it was thought worth effective to quantitatively estimate percentage of berberine content in *Berberis asiatica* growing in different altitudes of Garhwal Himalaya range.

**Material & Methods**

**Chemicals & Instruments**

All the solvents like petroleum ether, ethanol, and butanol were of analytical grade and purchased from Rankem Ltd. Standard berberine was an isolated and purified compound and received as a gift from Department of Chemistry, HNB Garhwal (A Central) Universiti, Srinagar Garhwal. HPTLC analysis was performed from Centre for Aromatic Plants (CAP), Selaqui, Dehradun.

**Procurement of Plant Material**

Roots of *Berberis asiatica* were collected from seven places of different altitudes, of Garhwal Himalayas, ranging from 1700-5500 feet (table 1) and were identified by Dr. S.A.S. Vishwas (with a specimen no. 10127), in F.R.I Dehradun. The samples of the plants were submitted to the herbarium of SBSPGI, Dehradun.

**Preparation of Extracts**

Finely dried roots were defatted with petroleum ether (60-80°C) and then extracted with 80% ethanol (80 parts of ethanol and 20 parts of water). Extraction was carried out for 40 hrs, solvent was distilled off and extract was concentrated using thin film evaporator until a viscous and sticky mass obtained. The extracts of different plant materials are designate S1 – S7 in the increasing order of altitudes [13] (Table 1).

**HPTLC Analysis**

The HPTLC method provides a simple, low cost and a good statistical analysis that proves an efficient method in order to quantitatively determine berberine in ethanolic extracts of roots of *Berberis asiatica* of the concern altitudes. The instrument used for the estimation was Camag semi automatic sample applicator, Camag TLC scanner, CATS software for interpretation of the data.
Pure berberine was used as standard material in HPTLC analysis. The mobile phase used was n-propanol: formic acid : water (90 : 1 : 9) and ethanol was used as solvent to dissolve the standard and test sample. TLC plates were prewashed with ethanol and activated by keeping at 105°C for about 30 minutes. Spotting of sample and standard was applied at the lower end of the TLC plate. 1-5μl standard stock solution and 5μl of each test sample was spotted (fig3). The plate was developed in mobile phase chamber and scanned at 366 nm, slit dimension 6.00 X 0.45 nm, scanning speed 100 nm/sec, data resolution 1nm/step and the source of radiation was a deuterium lamp (D2 & W). The peak areas of each standard and test samples were obtained from the system and a calibration graph was plotted between concentrations vs. Peak area (fig2)

The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter day and intra-day assay precision repeatability of measurement & repeatability of sample application. The mobile phase constitutes n-propanol: formic acid: water (90 : 1 : 9) gave Rf values of 0.31±0.05 for berberine. The percentage quantities of berberine in different sample /were calculated by graph between Rf value and relative height of the peak. Followed by application of linear regression equation Y = 26.016+3.715 (Figure 2).

RESULTS AND DISCUSSION

Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants. Berberis asiatica is one such wonder plant which grows freely in the hilly region of Indian subcontinent. It is recommended strongly in Ayurvedic literature for treatment of various human ailments which include internal and external use of each part of the plant. It is chief source of berberine, an isoquinoline type alkaloid, and most of the pha
dontaneous or mineralization or

Acknowledgement

The author is immensely thankful to HRDI, Selaqui, Dehradun for their kind help in providing HPTLC facility and to Dr. S.A.S. Viswas, H.O.D, Department of Botany, F.R.I, Dehradun for their co-operation in identification of plant. The study was also extended to evaluate the antidiabetic effect of plant extract and isolated berberine.

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Table 1: Distribution of berberine in different Berberis asiatica samples procured from different altitudes of Garhwal Himalayas

<table>
<thead>
<tr>
<th>S.No</th>
<th>Symbol</th>
<th>Region</th>
<th>Heights(ft.)</th>
<th>HPTLC Results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S₁</td>
<td>D.I.T</td>
<td>1,700</td>
<td>2.94</td>
</tr>
<tr>
<td>2</td>
<td>S₂</td>
<td>Yamkeshwar block, Pauri Garhwal</td>
<td>2,000ft.</td>
<td>2.25</td>
</tr>
<tr>
<td>3</td>
<td>S₃</td>
<td>Srinagar,Pauri Garhwal</td>
<td>2,200ft.</td>
<td>2.79</td>
</tr>
<tr>
<td>4</td>
<td>S₄</td>
<td>Shiv Mandir</td>
<td>2,500</td>
<td>2.46</td>
</tr>
<tr>
<td>5</td>
<td>S₅</td>
<td>Kolukhet</td>
<td>3,500</td>
<td>2.67</td>
</tr>
<tr>
<td>6</td>
<td>S₆</td>
<td>Chamba, Tehri Garhwal</td>
<td>5,000ft.</td>
<td>2.48</td>
</tr>
<tr>
<td>7</td>
<td>S₇</td>
<td>Mussorie</td>
<td>5,500</td>
<td>2.20</td>
</tr>
</tbody>
</table>

REFERENCES


