

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

ALOK MAITHANI*¹, 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_maithanii@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 1-5 μ l. 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 "Kingor". 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, jetrorrhizine, 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 iso-quinoline. It exhibits significant role in management if various enteric infections, specially bacterial dysentery [8]. It is also experimentally claimed to be hepatoprotective, antidiabetic [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 consume 500 tones 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 august - September and are sold in drug market of Dehradun and Haridwar.

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) University, Srinagar Garhwal. HPTLC analysis was performed from Centre for Aromatic Plants (CAP), Selaqui, Dehradun.

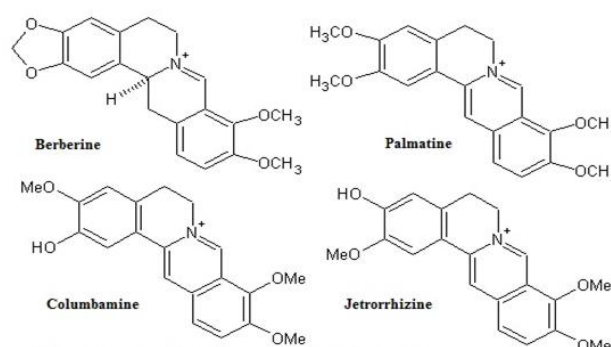


Figure (1): Isoquinoline type alkaloids in *B asiatica*

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 $^{\circ}$ 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 (fig.3). 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 (fig.2)

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 R_f values of 0.31±0.05 for berberine. The percentage quantities of berberine in different sample /were calculated by graph between R_f value and relative height of the peaks followed by application of linear regression equation $Y = 26.016 + 3.715 X$ (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 plants¹⁴. *Berberis asiatica* is one such wonder plant which grows freely in the hilly region of Indian subcontinent. It is recommended strongly in Auyurvedic 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 pharmaceutical properties of *B asiatica* are attributed due to this phyto-constituent. Therefore, it become important to find out variation in berberine content of *Berberis asiatica* which grows abundantly to the hills of Garhwal Himalayas. In present study concentration of berberine in different samples (different altitudes) was determined, by HPTLC analysis.

From the results (table 1) it has been observed that the berberine content was highest (2.94%) in the plant from lowest altitude (sample S₁) whereas the concentration was lowest (2.20%) in the plant belonging to highest altitude region (sample S₇). However the percentage of berberine was random among the plants of intermediate altitudes. The similar kinds of results have also been reported by Andolah HC et al., in 2010 in their study^[15]. These reports suggested a possible effective role of warmer soil condition and low UV exposure to the higher berberine content in plants of lower altitude region. Moreover, a positive relation between soil potassium level and berberine concentration observed in their experiments which can also be a marker parameter. However, the higher alkaloidal content is also importantly governed by the soil nitrogen and N-mineralization. Under wild condition the consumption of inorganic nitrogen by the roots of plants depends on the soil type, quality and quantity of organic matters (C/N ratio), latitude longitude and microbial activity [16, 17]. Dependency of secondary metabolite production is largely vary with the habitate which has been confirmed in a number of reports. Since highest percentage of berberine in our study was found to be in sample S₁ (lowest altitude) thus it can be suggested that N-mineralization or C/N ratio may be highest in that region. A thorough study is required before arriving for final conclusion which can lead cultivate new genom by applying biotechnological approaches. This will undoubtedly improve the commercial exploitation of the plant as well as will produce revenue to the rural people of the local areas.

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Table1: Distribution of berberine in different *Berberis asiatica* samples procured from different altitudes of Garhwal Himalayas

S.No.	Symbol	Region	Heights(ft.)	HPTLC Results (%)
1.	S ₁	D.I.T	1,700	2.94
2.	S ₂	Yamkeshwar block, Pauri Garhwal	2,000ft.	2.25
3.	S ₃	Srinagar,Pauri Garhwal	2,200ft.	2.79
4.	S ₄	Shiv Mandir	2,500	2.46
5.	S ₅	Kolukhet	3,500	2.67
6.	S ₆	Chamba, Tehri Garhwal	5,000ft.	2.48
7.	S ₇	Mussorie	5,500	2.20

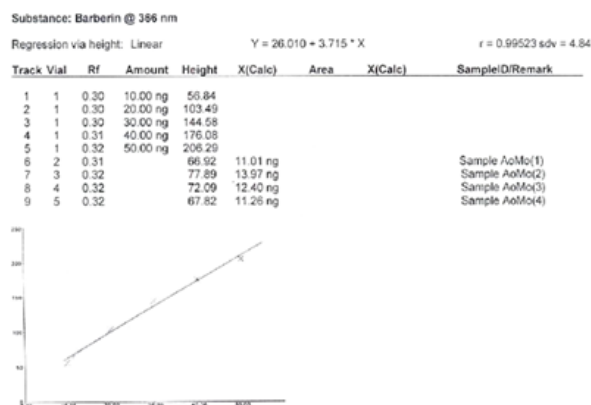


Figure 2: Development of Calibration Curve

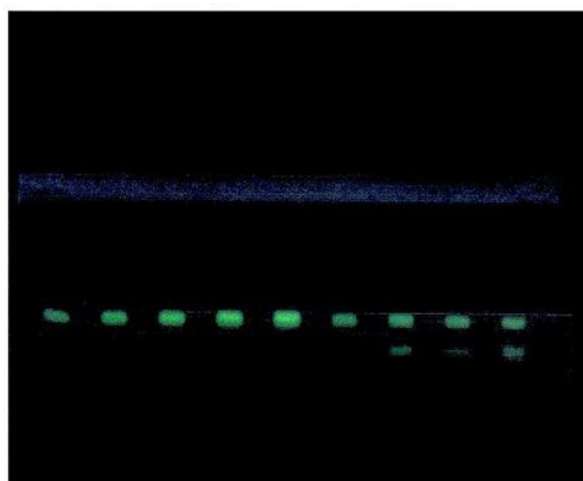


Fig. 3 Development of HPTLC Chromatogram

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