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Identification and characterization of bioactive compound berberine in the *Berberis vulgaris* root extract using HR-LC-MS analysis

Abstract

Berberis vulgaris L. is a shrub of family Berberidaceae produces in Asia and Europe. A broad range of medicinally and nutritionally important phytochemical components have been isolated from *B. vulgaris*, which are of medicinal value. Berberine is the main alkaloid isolated from *B. vulgaris*. Chromatography was performed on an Acquity UPLC system (Waters) equipped with a HSS T3 column18 using mixture of water (containing 0.1% formic acid) and acetonitrile (30:70 v/v) as mobile phase. The mass spectrometric detection was performed by selected ion monitoring mode via electrospray ionization in the positive ionisation mode. The results indicate that the approximate berberine content in the *B. vulgaris* root extract, as determined by the proposed method was 0.7266 mg ml-1. The fully validated HR-LC/MS method can be successfully applied for the determination of berberine in *B. vulgaris*, which can be used as a potent therapeutic agent for the treat of hypopigmentary disorders.

Keywords: berberis vulgaris, alkaloid, berberine, hypopigmentation

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Introduction

Ayurvedic medicines and formulations acquired from ancient Indian herbal systems are well-known for their various important applications. Recently, the interest and investigation in medicinal plants have augmented immensely. *Berberis vulgaris L*. is a shrub of family Berberidaceae produces in Asia and Europe; it is named in English as barberry.[1] It is a deciduous shrub growing up to 4m high. The leaves are small oval, 2–5cm long and 1–2cm broad, with a serrated margin; they are borne in clusters of 2-5 together, subtended by a three-branched spine 3–8mm long. The flowers are yellow, 4–6mm across, produced on 3–6cm long panicles in late spring. The fruit is an oblong red berry 7–10mm long and 3–5 mm broad, ripening in late summer or autumn; they are edible but very sour, and rich in Vitamin C.[2] The plant has been used widely for centuries for the treatment of various ailment. A broad range of medicinally and nutritionally important phytochemical components have been isolated from various parts of the plants such as alkaloids, saponins, cardioactive glycosides, anthocyanins, tanins, carbohydrates, protein, lipid, vitamins, fiber contents, β carotein. Phytic acid and phytate phosphorus etc.[3]

Plant is reported to possess twenty two alkaloid from roots, stems, leaves and fruits, which are of medicinal value.[4] The alkaloid content differs from the different areas, different species and different organs.[5] Bereberine is the key alkaloid components with an isoquinolonic nucleus isolated from the roots and bark of *B. vulgaris*.[6] It is an isoquinoline plant alkaloid belongs to the structural class protoberberines. Berberine is the one of the most studied alkaloid among the various naturally occurring protoberberine. It is reported to possess various pharmacological actions including antibacterial, anti-pyretic, anti-hepatotoxic, anti-cancer, anti-lipidemic, anti-hyperglycemic, anti-oxidant agent.[6–8] It has been used in some cases like diarrhoea, haemorrhoids, osteoporosis, leishmaniasis, eye and ear infections, jaundice, kidney and gall bladder stones, wound healing, skin diseases and malaria fever.[2]

Mukherjee et al.[9] have reported that decoction of roots of *B. vulgaris* can be used for skin troubles. Recently we have investigated that the berberine as its helps in pigment dispersal in the skin melanophores of adult *Bufo melanostinctus* via β-2-adrenergic receptor.[10,11] Skin darkening effect of berberine was further supported by ultrastructural analysis of skin melanophores of *B. melanostinctus*.[12] These studies suggest that the berberine could have been used as potential therapeutic agent for the treatment of various hypopigmentary disorders like vitiligo.[11,13]

Considering the great therapeutic potential of this plant, it was of great importance to qualitatively and quantitatively evaluate the percentage of berberine content in the root extract of *B. vulgaris*. A number of analytical methods have recently been reported for the determination of berberine content in the root extract of *B. vulgaris*. The chromatographic technique provides an excellent precision for the routine determination of the alkaloid content of *B. vurgaris* roots extract. The separation and purification of berberine from *B. vulgaris* by conventional methods such as column chromatography and high-performance liquid chromatography (HPLC) is tedious, time consuming and usually requires multiple chromatography steps.[14] LCMS detection is one of the most powerful analytical tools for organic compound analysis as quantitative and qualitative data can be obtained easily with limited instrument optimisation. In present study aims to isolate berberine from *B. vulgaris* and an attempt has been made to develop and validate HPTLC and LC/MS method for the analysis of berberine content in the root extract of *B. vulgaris*, which would be highly sensitive, having high resolution, shorter retention time and reproducibility.

Materials and method

Analytical grade ethanol and methanol were purchased from Qualigens Fine Chemicls, Mumbai, India. Formic acid (HPLC grade) and Acetonitril (HPLC grade) was procured from Sigma Aldrich (USA). Berberine Chloride Dihydrate was obtained from Alfa Aesar (USA). Daruhaldi or the root of Berberis vulgaris was purchased from local market of Bhopal. The plant sample was identified and authenticated by Dr. Zia ul Hasan, Botanist Department of Botany, Saifia Science College, Bhopal. The voucher specimen (No.:452/Bot/Saifia/14) was deposited at the botanical herbarium at Saifia Science

College, Bhopal (M.P.), India. Preservation of plants material was done according to standard protocol following the literature: technical reports and manuals.

Plant material and preparation of extract

Soxhlet extraction method was performed for the preparation of crude extracts containing high content of berberine from the root of *B. vulgaris*. For preparation of the alcoholic extracts of *B. vulgaris*, 100 g of roots were dried at room temperature in dark and then crushed into coarse powder. The powdered material was then soaked in 100 ml of 80% ethanol overnight and then was exhaustively extracted with 80% ethanol (100 ml X2) in a Soxhlet apparatus at 80°C for 72h. The crude extract was filtered and evaporated to dryness on a water bath set at 100°C. The dried residue of crude extract was cooled in desiccators for 30 min, then filtered via disk of filter paper and accurately weighed for analysis.[15]

Preparation of standard and sample solutions

A stock solution of berberine was prepared by dissolving 4.8mg of standard berberine chloride dihydrate (equivalent to 3.96 mg of berberine) in 10 ml methanol. The standard solution of berberine was prepared by diluting the stock solution to obtain the concentration of 99µg/ml. The sample solution was prepared by weighing dried extracts (10mg), dissolving in each extracting solvent and adjusting to 10ml.

High resolution liquid chromatography mass spectrometry (HR-LC/MS) analysis

The total berberine content in the root extract of *B. vulgaris* was quantitatively determined by HR-LC/MS analysis. Chromatographic separations were performed on an Acquity UPLC system (Waters) equipped with a HSS T3 column18 (100 ×1.0 mm, particle size 1.8µm; Waters); mobile phase consist of water (containing 0.1% formic acid) and acetonitrile (30:70v/v); applying the flow rate of 0.45mL min^{-1}; 0-0.5min. The injection volume was 3.1µL (full loop injection). The thermo stated auto sampler was kept at 4°C.

The mass spectrometer operated using an electrospray ionisation (ESI) source in positive mode and was set for isolation and fragmentation of the berberine molecular ion. Eluted compound was detected from m/z 50 to 3000 using a Micro-TOF-Q hybrid quadrupole time-of-flight mass spectrometer (Bruker micrOTOF-Q-II Daltonics) equipped with an Apollo II electrospray ion source in positive ion modes. MS operating condition were optimized as follows: nebulizer gas: nitrogen; drying gas flow: 7.0 L/min; nebulizer pressure: 1.2 bar; capillary voltage: 4500V; end plate offset: -500V; collision cell RF:130Vpp. drying gas temperature: 200°C. System control and data acquisition were controlled by Bruker Compass Data Analysis 4.0.

Results and discussion

B. vulgaris is reported to have wide range of pharmacological activities. It is used medicinally in all the traditional medical systems and has a history of usage in Ayurvedic, Iranian as well as Chinese medicines dating back at least 3000 years.[16] Though numbers of RP-HPLC, LC-MS-MS, UPLC-MS/MS, LC MS/MS Q-tof, etc. techniques have been developed for the qualitative and quantitative estimation of berberine in Tinospora cordifolia, various formulation, rat and rabbit plasma.[17–21] But there is no report on the quantitative screening of berberine content in the root extract of *B. vulgaris* using HR-LC/MS analysis. It has been observed that the vacuum evaporated extract of *B. vulgaris* gave yellowish dark brown extract which was subjected to HR-LC/MS analysis for proper qualitative as well as quantitative identification of berberine. In order to identify and quantify the main alkaloid from the root extract of *B. vulgaris* by HR-LC/MS analysis standard berberine chloride dihydrate was used.

The UPLC chromatogram of detected compound i.e. berberine in the root extract of *B. vulgaris* and standard berberine is shown in Figure 1. It is obvious that the composition of mobile phase also affects the separation and ionization of phytocompounds. Therefore, in order to boost the reproducibility, formation and sensitivity of the analysis, several additives in different concentration were incorporated to the mobile phase like formic acid, acetic acid and ammonium acetate. It was observed that the addition of 0.1% formic acid enhance the reproducibility and sensibility of the analysis as single, sharp and symmetrical peaks were obtained The average recovery of berberine at different level was noticed to be 98.75%. The obtained LC-MS total ion chromatograms, UV characteristics and extracted ion chromatogram of *B. vulgaris* root extract along as well as standard berberine are depicted in Figure 1. Many compounds were observed in the UPLC chromatogram of *B. vulgaris*, but the purity of berberine was reported as 45% based on UPLC peak area.

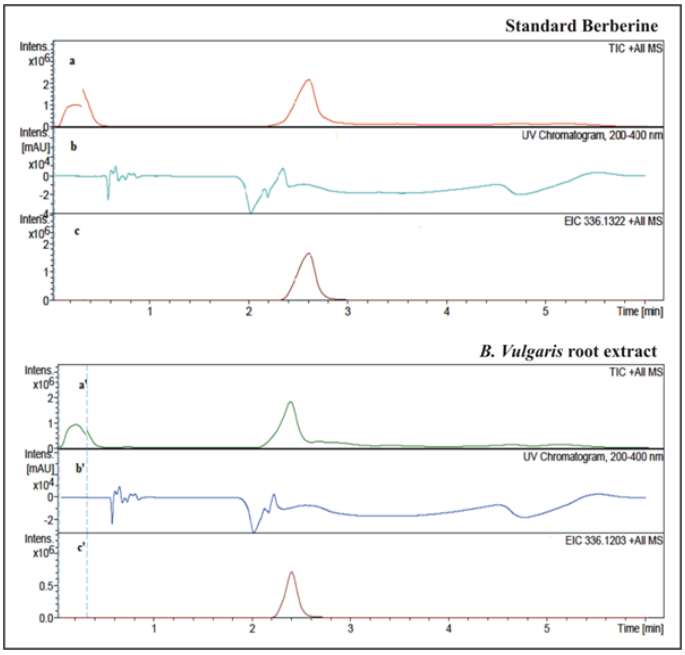

Figure 1 Comparison of standard berberine and root extract of *B. vulgaris* via UPLC-HRLC-MS analysis (a-a') Total ion counts vs. acquisition time (min); (b-b') UV chromatogram; (c-c') Extracted ion counts vs. Acquisition time (min).

The identities, retention time and observed molecular and protonated ions for individual components are depicted in Figure 2. It was observed that the highest peak was obtained at retention time 2.3-2.5min belonging to the phytocompound berberine in *B. vulgaris*. With the standard reference graph, the berberine compound is elucidated using the molecular weight. Quantification of berberine was based on the sum of ions with m/z=36.1226 and 453.3392 from the MS spectrum of the parent ion Figure 3. MS/MS chromatogram showed similar fragmentation patter for both the standard berberine and compound from the root extract of *B. vulgaris*. Berberine concentration in the *B. vulgaris* root extract determined by HR-LC/MS was 0.7266mgml^{-1} of extract. The most important phytocompound identified in the root

extract of *B. vulgaris* is berberine, which is known to exhibit various outstanding pharmacological activity.[1,22]

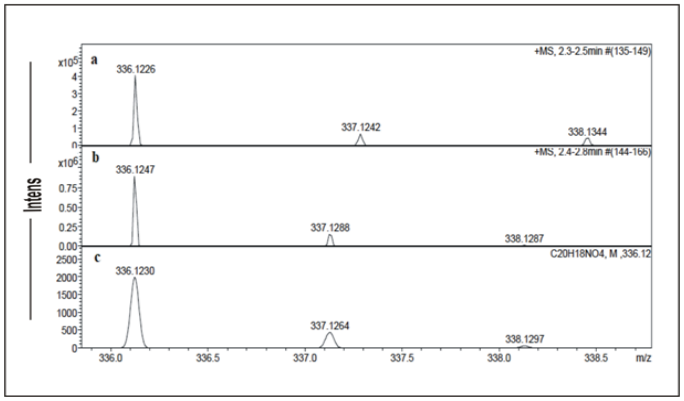

Figure 2 Comparative analysis of LC-MS base peak intensity chromatograms derived from positive ionization mode (m/z = 50-3000). Count vs. Mass-to-charge (m/z): (a) *B. vulgaris* root extract (b) standard Berberine (c) theoretical composition.

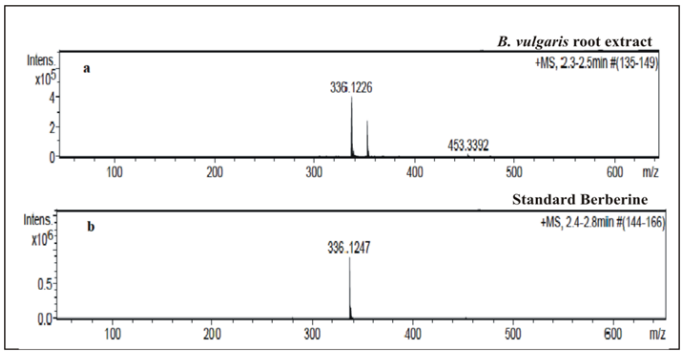

Figure 3 Full scan MS of (a) *B. vulgaris* root extract (b) standard Berberine in mobile phase.

The result showed that the exact mass of berberine content of *B. vulgaris* ($[M-H]^+$ m/z 336.1226) was identical to that of the standard berberine ($[M-H]^+$ m/z 336.1247), matching the theoretical composition of $C_{20}H_{18}NO_4$ ($[M-H]^+$ m/z 336.1230) (Figure 2). The UV absorbance spectra of sample (root extract of *B. vulgaris*) and berberine during LC-UV-MS were recorded and absorbance maxima were noticed at 267nm (Figure 1). The inclusive results of present investigation has proved that HR-LC/MS is the highly precise analytical technique which can be extensively used for isolation and quantification of berberine content in the root extract of *B. vulgaris*.

Conclusion

The present investigation described and justified a simple, sensitive HR-LC/MS technique for the characterization and quantitative estimation of the main alkaloid i.e. berberine content in the root extract of *B. vulgaris*. The HR-LC/MS analysis was implicated for the detailed studies of berberine in the extract. After incorporating required modification, the present analytical method is proposed to determine the alkaloid content in the various samples.

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Conflicts of interest

The authors have no conflict of interest to declare.

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