Isolation of Protoberberine Alkaloids from Stem Bark of *Mahonia manipurensis* Takeda Using RP-HPLC

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*Mahonia manipurensis* Takeda belongs to angiospermic family Berberidaceae. The plant is endemic to the North Eastern region of India in the states of Manipur, Nagaland and part of Indo-Burma region. Earlier works on alkaloid phytochemistry of the genus leads to the isolation and characterization of some quaternary protoberberine alkaloids such as berberine, jatrorrhizine, palmatine, umbellatine, coloumbamine, etc. These alkaloids are found in the root, rhizome, bark and leaves of the plant. In the present investigation, chromatographic techniques such as Thin Layer Chromatography (TLC) and reverse phase High Performance Liquid Chromatography (RP-HPLC) were used to separate and isolate two different compounds of alkaloid from the crude extract of *Mahonia manipurensis* stem bark.

Comparison of both the chromatographic fingerprints as well as with the spectroscopic data of UV and MS spectra of the two compounds with the standards Berberine chloride and Palmatine chloride hydrate and also with literature data showed that the values of these two compounds are comparable with the standards indicating that the two compounds isolated in this study are identified as these compounds.

**Keyword:** *Mahonia manipurensis*, isolation, protoberberine alkaloid, chromatography and spectroscopy.

1. **Introduction**

The genus *Mahonia* belongs to the family Berberidaceae. There are 109 different species of *Mahonia* in the world [10]. About 13 different species are recorded from India [1] of which 11 species occurred from Northeast India. 4 species viz. *Mahonia feddei* Ahrendt, *Mahonia magnifica* Ahrendt, *Mahonia manipurensis* Takeda and *Mahonia roxburghii* (DC.) Takeda are found in Manipur [20]. Many species of this genus are well known medicinal plants widely used in folk medicine [18, 21]. The main biologically active compounds in *Mahonia* plant is alkaloid. These alkaloids are found in the root, rhizome, bark and leaves of the plant. The alkaloids berberine, jatrorrhizine, palmatine and oxyacanthine have been isolated from the root of *Mahonia manipurensis* Takeda [14, 5]. Some of the principle alkaloids which are widely distributed in this genus include berberine, coloumbamine, jatrorrhizine, palmatine and umbellatine. Pharmacological studies carried out by various workers showed that the plants belonging to the genus *Mahonia* exhibits antibacterial, antifungal, anticancer, antioxidant, antiproliferative and anti-inflammatory effects [12, 7, 12, 13, 14, 15, 16, 21] thus support the claimed of the uses of these plants in traditional or folkloric medicine.

2. **Materials and Methods**

2.1 **Plant collection and identification**

The stem bark of *Mahonia manipurensis* and herbarium specimen were collected in the foot...
hill of Mt. Tenipu, Senapati district, Manipur in
the month of April-2009, identified from Flora of
India, 1993; Flora of Manipur, 2000 and further verified from Kew Herbarium,
Edingburg. A voucher specimen (Coll. No. 188-M) was prepared from the collected plant and
deposited in the herbarium of the Department of
Botany, NEHU, Shillong.

2.2 Alkaloid extraction
The plant stem bark was removed, dried in oven
and pulverized into fine powder using grinder.
About 100 g of the fine powder plant sample was
extracted with 1000 ml of 80% methanol in 2.5
liters beaker with stirring at interval in room
temperature. The extract was filtered and then
concentrated to 1/5th of the original volume in a
Buchi rota vapor under reduced pressure. The
concentrated extract was then used for extraction
of alkaloid following Harborne method [9].

2.3 Chromatography analysis
2.3.1 Thin layer chromatography (TLC)
The presence of alkaloids in the crude extract was
initially analyzed by TLC using hexane, ethyl
acetate and methanol (56:20:5) as the mobile
phase. The purified fraction that showed positive
reagent test (Dragendroff’s reagent) was collected
and subjected to further analysis using
Chloroform, ethylacetate, diethylamine, methanol
and 20% NH4OH (6:24:1.5:6:0.3) as the mobile
phase. After the plate developed, it is put to dry at
room temperature and then spray with
Dragendorff’s reagent to detect and visualized the
fractions which are active with the reagent.

2.3.2 HPLC analysis
2.3.2.1 Apparatus
The HPLC system (Waters Alliance, Milford,
MA, USA) consisted of a Waters 515 HPLC
Pump, an automatic thermostatic column
compartment, a degasser and Waters 2489
UV/VIS Detector.

2.3.2.2 Reagents and materials
HPLC-grade methanol and water for analysis of
protoberberine alkaloids were purchased from
Sisco Research Laboratory (SRL), Mumbai
(India).Analytical grade formic acid (98-100%) was purchased from Sd fine-CHEM Ltd.
(Mumbia). The standard compounds of Berberine
chloride and Palmatine chloride hydrate were purchased from Sigma Aldrich.

2.3.2.3 Chromatographic conditions
HPLC chromatography was performed at room
temperature on a Reverse Phase (RP) column
WATER SYMMETRY C18 (5 μm, 250 mm x
4.6 mm ID). The mobile phase for the alkaloids
of different fractions was methanol and formic
acid buffer (0.1% v/v). The flow rate was
maintained at 1 ml/min and the mobile phase
gradient for the column was 20-40% methanol for
35 mins.

2.3.3 Spectroscopy analysis
2.3.3.1 UV-VIS analysis
Each fraction II and III were scrapped from the
preparative TLC glass plates along with Silica gel
and collected in 2.5 ml eppendorf tube. The
mixture is dissolved in 1.5 ml of HPLC grade
water and shakes vigorously for about 1 minute.
It is then centrifuge at 6000 rpm for 5 minutes
using mini SPINWIN centrifuge (TARSON). The
process is repeated 3 times and the supernatant
where the compound gets dissolved is collected
by pipetting in another 2.5 ml eppendorf tube.
Further, the supernatant is filtered using
membrane filter nylon-66 of 0.22 μm pore size
(AXIVA). About 1.2 ml of the supernatant is
transfer into 1.4 ml capacity quartz cuvette and
the absorbance is scan from 250 nm to 500 nm
using Perken Elmer UV-VIS lambda-25
spectrophotometer (figs. 2b & 3b) and also
compared with the UV spectra of the standards
berberine chloride and palmitate chloride hydrate
(figs. 2a & 3a).

2.3.3.2 ESI-MS Spectroscopic analysis
Each fraction II and III were scrapped from the
pre-coated TLC Silica gel G F254 aluminum back
plate of size 10 cm x 5 cm x 0.2 mm and
collected in 1.5 ml eppendorf tube. The mixture is dissolved in 0.5 ml of HPLC grade methanol and shakes vigorously for 1 minute. It is then centrifuge at 6000 rpm for 5 minutes using mini SPINWIN centrifuge (TARSON). The supernatant is collected and filtered using membrane filter nylon-66 of 0.22μm pore size (AXIVA) and the same is taken for Mass Spectra using LC-MS spectrometer Waters ZQ-4000 model. The mass spectra thus generated are shown (figs. 7a, b).

3. Results
The Rf values of the different alkaloid fractions separated from the crude extract using TLC (Table-1) in the present investigation were compared with the standards (fig.1a & b) and observed that the values of the two fractions (Fr-II & III) match with the two standards. Also comparison of UV spectra (fig. 2a, b-3a, b) of the two fractions Fr-II, λmax-342.86 and Fr-III, λmax-342.36 nm with the standards Berberine chloride, λmax-341.06 and Palmatine chloride hydrate, λmax-42.24 showed that the values are comparable with the two standards. Further, retention time of HPLC chromatograms of the two fractions (Fr-II and Fr-III) showed that the values are comparable with the standards (figs.5a & b-6a & b). In addition, ESI-MS spectra of the two fractions are also shown in figs.7a, b with base peak molecular weight of 336.19 and 352.12 corresponding to the standard molecular weight of the alkaloids berberine and palmatine respectively. The different steps involved from extraction of the crude alkaloids to separation and isolation of the compounds are presented schematically in flow chart (fig. 8)

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Spot No.</th>
<th>Distance of solvent front (in cm)</th>
<th>Spot distance of standard alkaloids</th>
<th>Distances of different fractions (in cm)</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem bark</td>
<td>1</td>
<td>8.5</td>
<td>Berberine chloride: 1.45</td>
<td>0.55</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>Palmatine chloride hydrate: 1.85</td>
<td>1.45</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td>1.85</td>
<td>0.218</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td>2.45</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td>6.4</td>
<td>0.752</td>
</tr>
</tbody>
</table>

**Fig1:** TLC fingerprint of the alkaloids extract (a) before spraying the reagent (b) after spraying the reagent
Fig 2a: UV spectrum of Berberine chloride.

Fig 2b: UV spectrum of fraction-II.

Fig 3a: UV spectrum of Palmatine chloride hydrate.
Fig 3b: UV spectrum of fraction-III.

Fig 4: HPLC chromatogram of purified alkaloid fraction.

Fig 5a: HPLC chromatogram of Berberine chloride.
Fig 5b: HPLC chromatogram of FR-II from *M. manipurensis*.

Fig 6a: HPLC chromatogram of Palmatine chloride hydrate.

Fig 6b: HPLC chromatogram of FR-III from *M. manipurensis*. 
**Fig 7:** ESI-MS spectrum of (a) FR –II and (b) FR-III of protoberberine alkaloid.
4. Discussion and conclusion

At present, a number of analytical tools (chromatographic and spectroscopic) have been used to analyze alkaloids in plant samples or crude drugs. Thin Layer Chromatography (TLC) is one of the most popular and widely used separation techniques because of its ease of use, cost effectiveness, high sensitivity, speed of separation as well as its capacity to analysis multiple samples simultaneously. The technique can be utilized for separation, isolation, identification and quantification of components in a mixture. It can also be utilized on a preparative scale to isolate a particular component. However, the technique lacks quantitative precision, complete resolution and separation power. Therefore at present, Reverse Phase (RP) - High Performance Liquid Chromatography (RP-HPLC) is the most commonly used chromatography technique for qualitative and quantitative analysis of protoberberine and other plant alkaloids. Several HPLC or HPLC coupled with Mass Spectroscopy or diode array detector (DAD) methods have been reported for the determination of protoberberine alkaloids. In the present investigation, phytochemical analysis of protoberberine alkaloids from *Mahonia manipurensis* Takeda stem bark extract.
resulted in the separation and isolation of two compounds marked as FR-II and FR-III. A comparison of both chromatographic fingerprints of TLC and HPLC as well as with the spectroscopic data of UV and MS spectra of the two fractions with the standards Berberine chloride and Palmatine chloride hydrate and also with literature data showed that the values of these two fractions are comparable with the two standards indicating that the two fractions isolated in this study are identified as these compounds.

5. Acknowledgement
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6. References
11. Luo X, Chen B, Yao S. Simultaneous analysis of protoberberine, indolequinoline and quinolone alkaloids.

(a) Berberine

(b) Palmatine


