Journal of Medicinal Plants Studies
Year: **2014**, Volume: **2**, Issue: **2**
First page: (**48**) Last page: (**57**)
ISSN: **2320-3862**
Online Available at **www.plantsjournal.com**

Journal of Medicinal Plants Studies

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 [4, 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 [2, 7, 12, 13, 14, 15, 16, 22] 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% NH_4OH (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 palmatine 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 F_{254} 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, λ max-342.36 nm with the standards Berberine chloride, λmax-341.06 and Palmatine chloride hydrate, λmax-42.24showed 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)

Table1: Rf values of the different alkaloid fractions

Plant parts	Spot No.	Distance of solvent front (in cm)	Spot distance of standard alkaloids		Distance of different fractions (in cm)	Rf value
			Berberine chloride	Palmatine chloride hydrate		
Stem bark	1	8.5			0.55	0.065
	2				1.45	0.171
	3			1.85	1.85	0.218
	4		2.45		2.45	0.289
	5				6.4	0.752

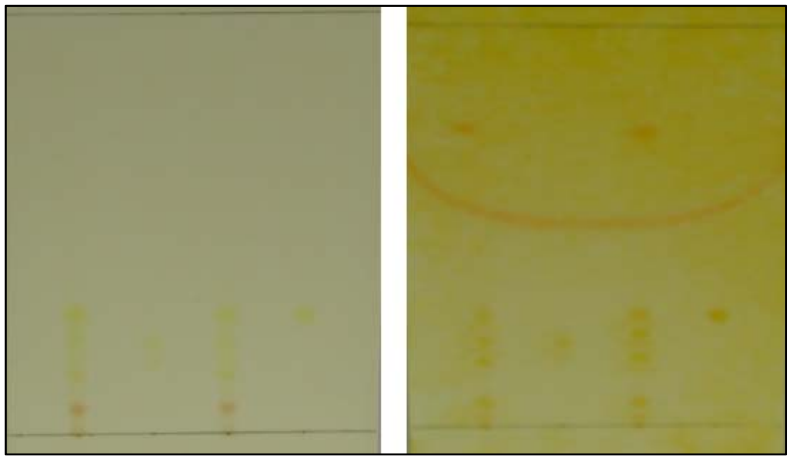


Fig1: TLC fingerprint of the alkaloids extract (a) before spraying the reagent (b) after spraying the reagent

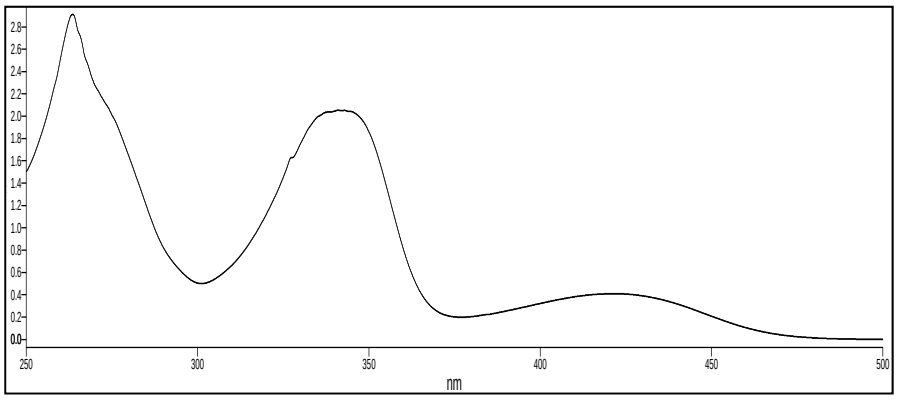


Fig 2a: UV spectrum of Berberine chloride.

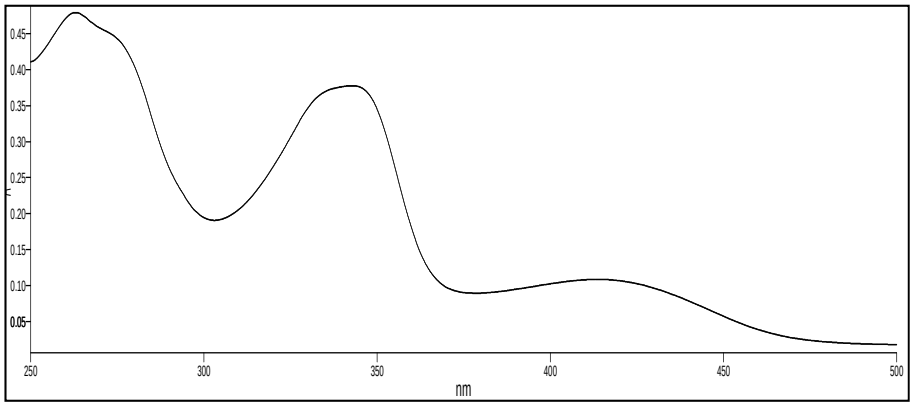


Fig 2b: UV spectrum of fraction-II.

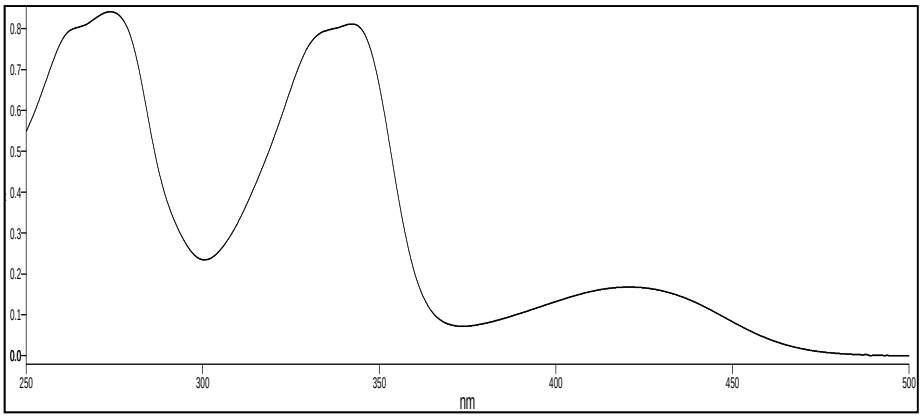


Fig 3a: UV spectrum of Palmatine chloride hydrate.

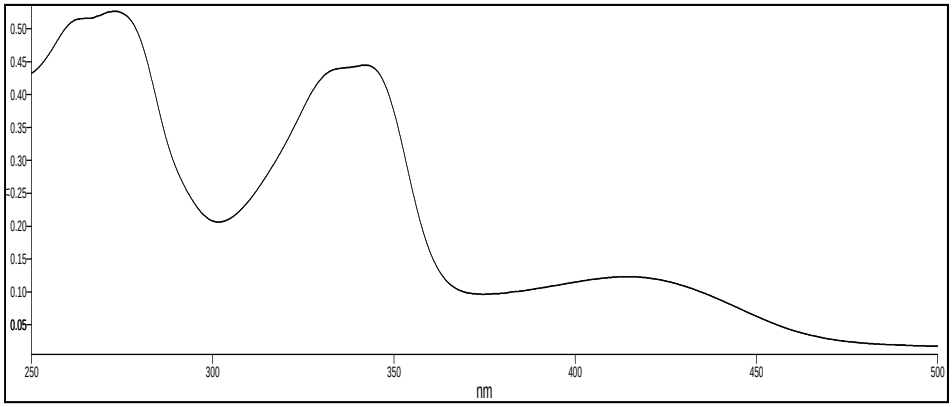


Fig 3b: UV spectrum of fraction-III.

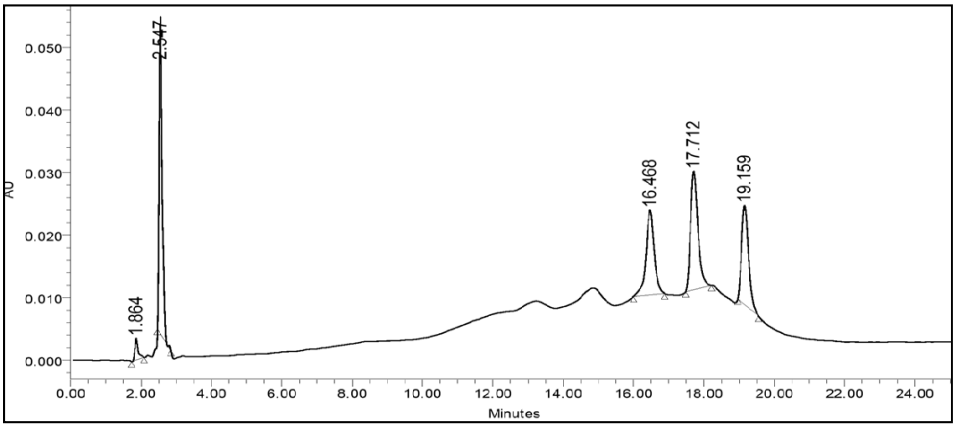


Fig 4: HPLC chromatogram of purified alkaloid fraction.

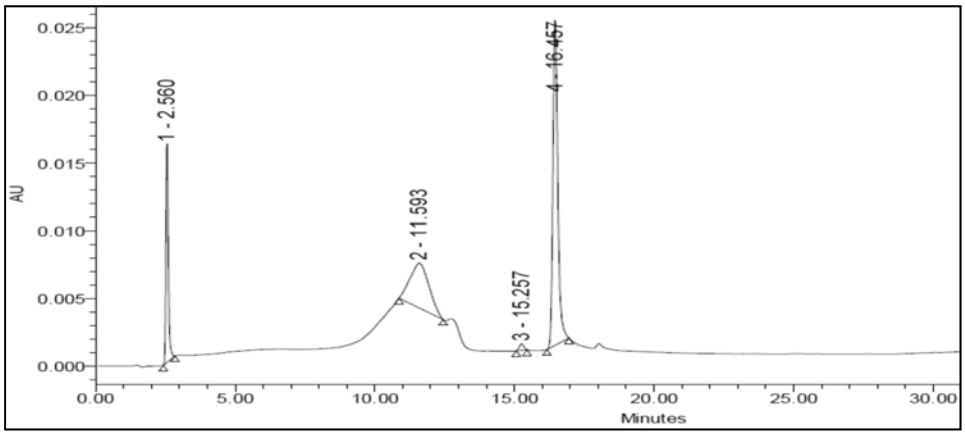


Fig 5a: HPLC chromatogram of Berberine chloride.

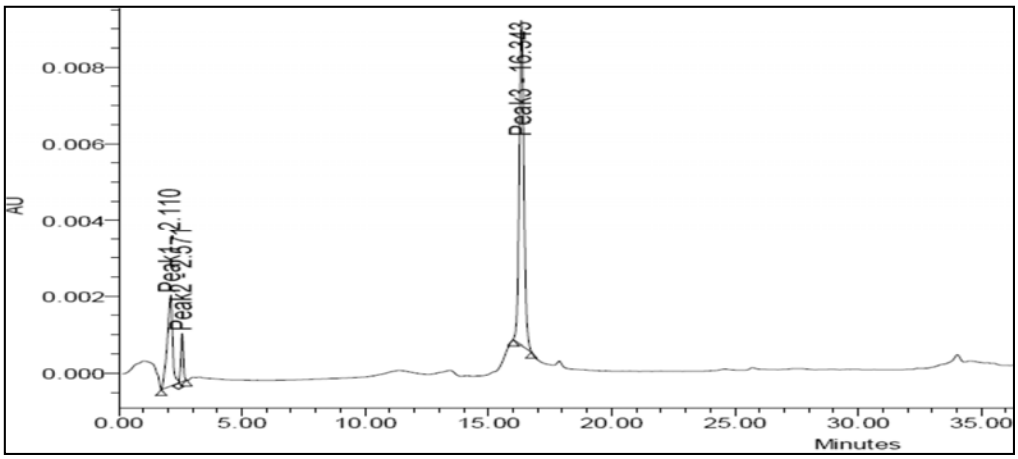


Fig 5b: HPLC chromatogram of FR-II from *M. manipurensis*.

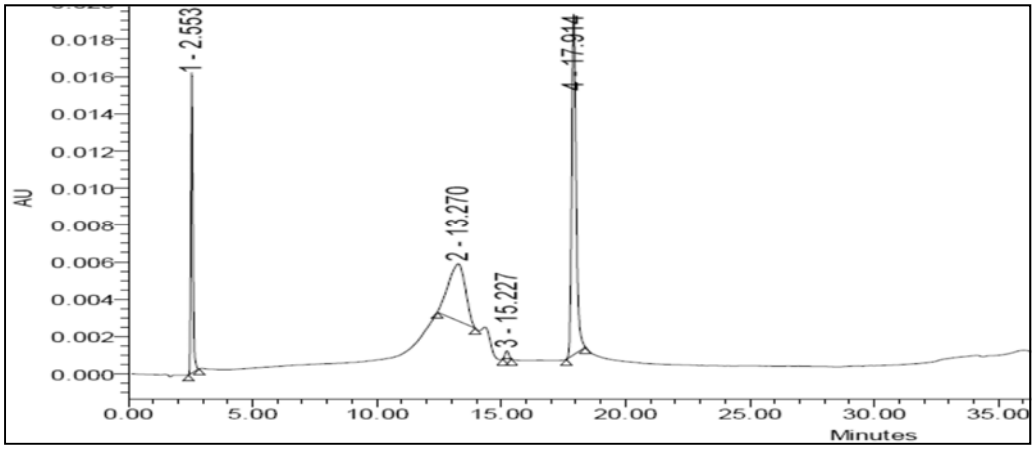


Fig 6a: HPLC chromatogram of Palmatine chloride hydrate.

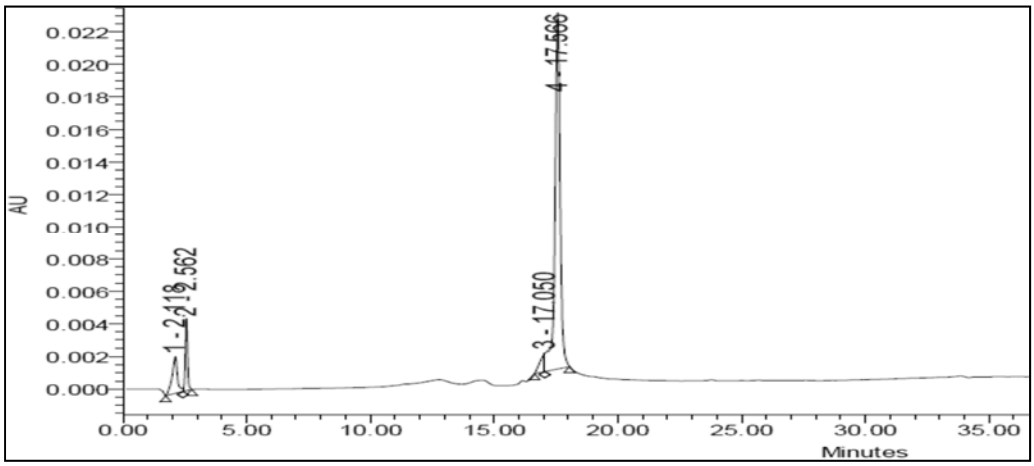
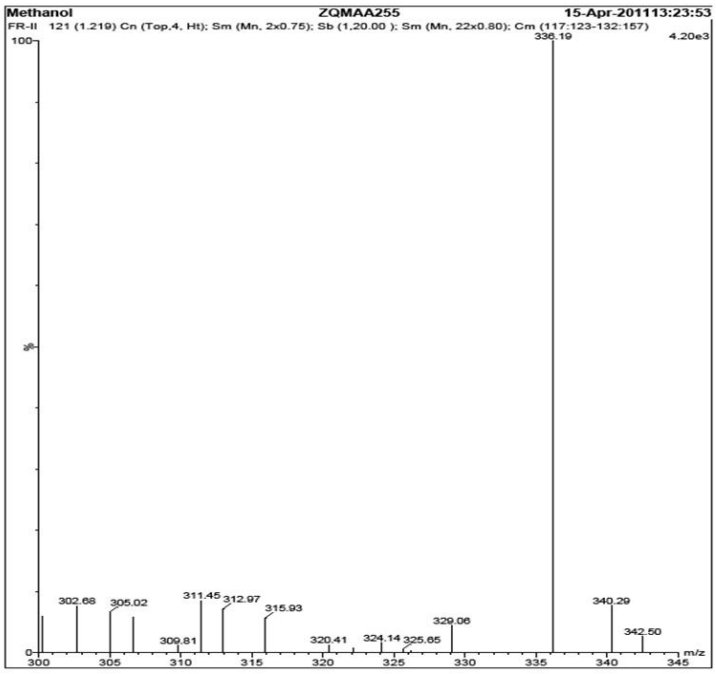
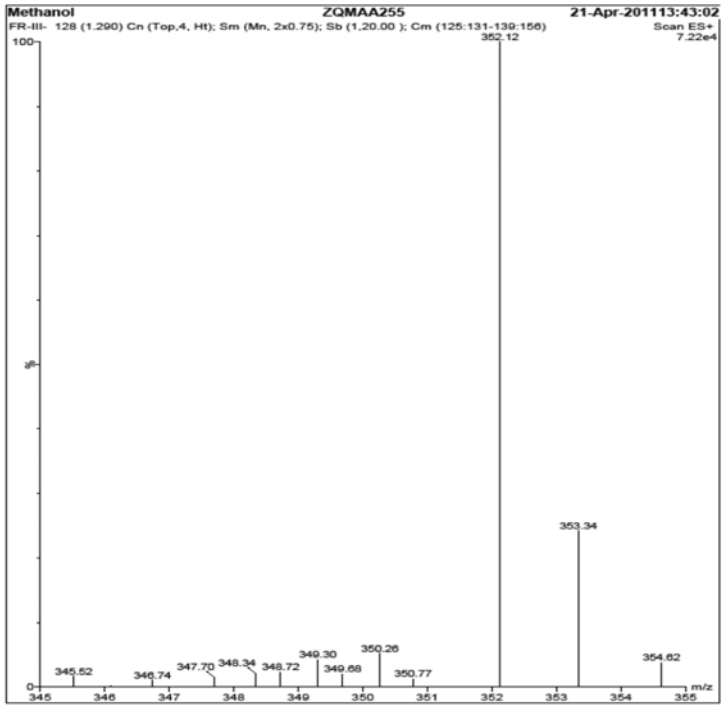


Fig 6b: HPLC chromatogram of FR-III from *M. manipurensis*.



(a)



(b)

Fig 7: ESI-MS spectrum of (a) FR –II and (b) FR-III of protoberberine alkaloid.

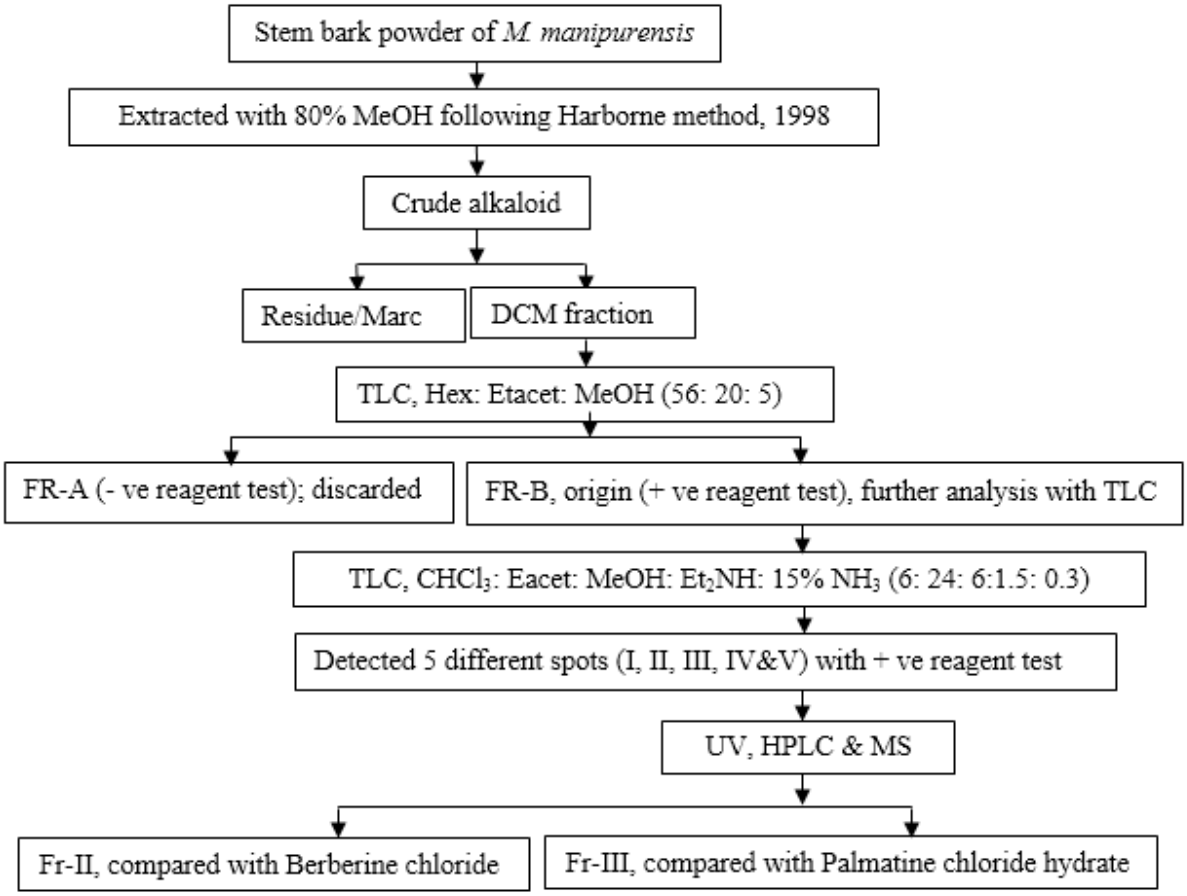
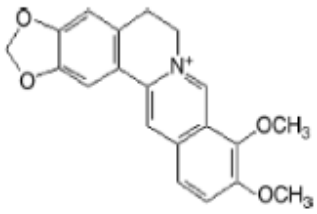


Fig 8: Schematic flow chart for isolation of protoberberine alkaloids from stem bark of *M. manipurensis*

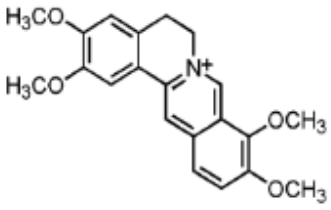
4. Discussion and conclusion

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 [8]. 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 [3, 6, 11, 17, 19]. 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



(a) Berberine



(b) Palmatine

5. Acknowledgement
The authors are grateful to University Grant Commission (UGC), New Delhi for giving financial assistant in the form of monthly stipend through Rajiv's Gandhi National Scholarship for SC/ST candidates.

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