Biocatalysis and Agricultural Biotechnology 26 (2020) 101622

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

Isolation, characterization of Berberine from *Berberis aristata* DC for eradication of resistant *Helicobacter pylori*

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ARTICLE INFO

Keywords:
H. pylori
Isoquinolone alkaloid
Berberine
Berberis aristata DC
Disc diffusion

ABSTRACT

Objective: *H. pylori* have gradually acquired resistance to the commonly used antibiotics because of their use in many parasitic and anaerobic infections, which leads to treatment failure of various gastric and duodenal diseases associated with *H. pylori* infection. The present research work aimed to isolate and characterize the bioactive compound from the methanol extract of stem of *Berberis aristata* DC. This is traditionally used for the treatment of dyspepsia, dysentery and diarrhea against antibiotic-resistant gastric pathogen *H. pylori*.

Methods: The *in vitro* antimicrobial activity of Berberine an active isolated compound from methanol extract of stem of *Berberis aristata* DC against drug-resistant *H. pylori* strain isolated from North Indian GERD patients. The *H. pylori* strain was only collected from those, who were devoid of any kind of anti-*H. Pylori* therapy. The methodology was in determining the Minimum inhibition concentration (MIC) using the microdilution method and disk diffusion method.

Results: *H. pylori* isolate was included in this study. Berberine from methanol extract of stem of *Berberis aristata* DC showed its potency on *H. pylori*-infected isolated from GERD patients with a maximum inhibition at 0.000075 μg/ml.

Conclusion: Prevalence of metronidazole resistance ranges between 50 and 90% in developing countries including India. The emergence of dual drug resistance was reported in various studies. This study suggests that Berberine an isolated compound from methanol extract of stem of *Berberis aristata* DC used commonly known as Daru Haldi potentially active for the treatment of drug-resistant *H. pylori* infection. Berberine from methanol extract of stem of *Berberis aristata* DC with a concentration of 0.000075 μg/ml shows a positive effect safely and effectively.

1. Introduction

Helicobacter pylori (*H. pylori*) having spiral (shape), gram negative (micro aerophilic) bacterium, usually found in humans 'gastric mucosa, which can live for decades (Safavi, 2016). *H. pylori* is the gastric pathogen affecting more than 50% of the world population (Lahat et al., 2017). Most of them remain asymptomatic in whole life and survive without any major clinical outcomes (Lahat et al., 2017). *H. pylori* commonly induce the upper gastrointestional (GI) disease such as induction of peptic ulcer (duodenal and gastric), gastric cancer, gastric mucosal linked lymphoid tissue lymphoma and chronic gastritis (Wyle, 1991). *H. Pylori* is a typically infection related with the asymptomatic gastritis, But more serious health effects, such as duodenal and stomach ulcers and finally induces the stomach cancers; occur in up to 10% or 1% of contaminated patients, respectively. Every year approximately 780, 000 cases of *H. pylori* induced gastric cancer found worldwide, which are the 6.2% cases among the all cases of cancer (Debruekeleer and Renaut, 2018). However, 90% of cases develop duodenal ulcers and 70% of cases develop gastric ulcers (Preda et al., 2009) and a small proportion are diagnosed with MALT lymphoma or gastric malignancies including

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https://doi.org/10.1016/j.bcab.2020.101622
Received 31 January 2020; Received in revised form 12 April 2020; Accepted 22 April 2020
Available online 30 April 2020
1878-8181/

gastric cancer (El Khadir et al., 2017). It is well documented that humans are the primary host for *H. pylori* and in the recent studies that 100% population was infected from the *H. pylori* (Courtois et al., 2018; Yang et al., 2014). Last few decades, antibiotics mostly used for the treatment of the infection of *H. pylori*. As per the global result, Northern, Southern, Northern and European America populations are still infected, while in the eastern and southern Europe, Asia and South America, the incidence of *H. pylori* higher than 50% (Debraekeleer and Remaut, 2018). Therefore, the eradication of *H. pylori* is the main aim that would significantly affect the world/global health. During the past decade, many effective therapies have been developed to counter *H. pylori* infection. Current therapy is based on a combination of proton pump inhibitor (PPI) with either amoxicillin or metronidazole and clarithromycin (Talebi Bezmin Abadi, 2017). Unfortunately, during the last few decades, the prevalence of resistance to some of the commonly used antibiotics has increased and hence the complete eradication of *H. pylori* is still not be achieved. Resistance to amoxicillin, clarithromycin and metronidazole is widespread, and maybe the reason behind this is the constant use of these drugs against *H. pylori* infections (Talebi Bezmin Abadi, 2017). In our recent study showed the maximum resistance towards Levofloxacin (73.2%) and metronidazole (48.5%) which are the major drugs in the treatment of *H. pylori* (Savoldi et al., 2018; Seck et al., 2013). If this trend continues the therapeutic guidelines recommended may become ineffective.

Because of the incomplete cure and drug resistance reported in the triple therapy described above and its possible side effects, alternative medicines are gaining much importance and are found to be safe and effective eliminators of *H. pylori* infection (Singh et al., 2009; Song et al., 2014). Thus, to overcome these hurdles, we continued to explore for some herbal isolate that is having the potential to inhibit the growth of *H. pylori* with minimal or no side effects. In this study, we aim to determine the anti-*H. pylori* activity of berberine isolated from *Berberis aristata* stem.

Berberis aristata commonly called as *Daruharidra, Daruhaldi* and Chitra is a spinous shrub native to the northern Himalayan region. *Berberis aristata* is a well-known herb in Ayurveda medicines from a very long time (Berberine., 2000; Potdar et al., 2012). Traditionally it is used in inflammation, wound healing, skin diseases, menorrhagia, diarrhea, jaundice and infection of eyes (Komal et al., 2011; Singh and Kakkar, 2009). effective ayurvedic preparation 'Rashut' is made by this herb (Komal et al., 2011). Berberis decoctions and extracts have demonstrated significant antimicrobial activity against a variety of organisms including viruses, bacteria, fungi, protozoans, helminths, and chlamydia. Currently, the plant is used in different bacterial diarrhea, intestinal parasite infections, and ocular trachoma infections (Derosa et al., 2016; Komal et al., 2011; Unkeshwar et al., 2013). The aim of the current experimental study to isolate, characterized the phyto-constituent from the *Berberis aristata* DC and scrutinizes its efficacy against the *H. pylori*.

2. Methodology

2.1. Collection and authentication

The stem of *Berberis aristata* DC was collected from the forest area of Mussoorie, Dehradun, India on February 2008. The herbarium of *Berberis aristata* DC was prepared and submitted to National Bureau of Plant and Genetic Resources (NBPGR) for authentication. The specimen was authenticated by botanist Dr. K.C. Bhatt, Senior Scientist, NBPGR, Pusa Campus, New Delhi. Authentication number is NHCP/NBPGR/2008/4.

2.2. Extraction of Berberis aristata DC STEM

The stem of *Berberis aristata* DC was washed with water after dead cell scraping, chopped into small pieces for air-drying at room temperature for 7 days and coarsely powdered. 200 g of pulverized plant material was extracted by soxhlet extraction method with petroleum ether 60–80 °C for defatting and then extracted with 500 ml of methanol for 48 h. The obtained extract was filtered and evaporated to dryness using a rotary type evaporator (R-114, Buchi, Switzerland) at reduced pressure and temperature. The concentrate was further dried to obtain a yield of 12.8%w/w which was used in the preliminary phytochemical screening. The other chemicals and solvents used were of the analytical grade.

2.3. Preliminary phytochemical screening

Preliminary phytochemical tests were done for the confirmation of different kinds of secondary metabolites. As secondary metabolites are directly related to the therapeutic responses of any kind of drug, it has become very important to analyze the metabolites present in an extract (Kumar et al., 2014, 2013). Phytochemical screenings were performed by doing different qualitative chemical tests including tests for alkaloids, glycosides, tannins, carbohydrates, amino acids, saponins, proteins, lipids/fats, resins, phenolic compound, and flavonoids in methanol extract of stem of *Berberis aristata* DC (Price, 1985; Richardson and Harborne, 1990).

2.4. Isolation of compound from the methanol extract of stem of Berberis aristata DC (BAME)

Healthy stems were collected from the *in vivo* plants, shade dried and powdered mechanically. 100 g of the powdered stem was extracted for 48 h with methanol by using a Soxhlet apparatus.

The extract was concentrated under reduced pressure using rotary flash evaporator (Büchi, Switzerland); 2% HCl was used to acidify 10 g of extract. The acidic solution was then filtered and extracted with diethyl ether (Et_2O) to remove neutral materials and used sodium bicarbonate (Na_2CO_3) to make it basic (pH 8–9), then extracted with dichloromethane (CH_2Cl_2). The CH_2Cl_2 solution was concentrated and dried in a desiccator. The crystals of the isolated compound thus obtained was powdered and stored in the airtight amber coloured bottle away from light and moisture.

2.5. Preliminary phytochemical screening of the isolated compound from bame

Phytochemical screenings were performed by doing different qualitative chemical tests including tests for alkaloids, glycosides, tannins, carbohydrates, amino acids, saponins, proteins, lipids/fats, resins, phenolic compound, flavonoids in the isolated compound from methanol extract of stem of *Berberis aristata* DC (Evans, 2002).

2.6. Spectroscopy study

The characterization of the isolated compound was carried out by HPTLC, HPLC, FTIR, ^{1}HNMR, ^{13}CNMR and MASS spectral studies. HPTLC study of the isolated compound was done by Camag Linomat V HPTLC (Switzerland) equipped with 100 µl Camag syringe and scanner III by dissolving isolated compound in ethanol and then spotted on the pre-coated silica gel G aluminium plate 60F-254 (20 × 10 cm with 0.2 mm of thickness, E. Merck, Germany) with the help of capillary tubes. TLC plates were developed and scanned at 234 and 366 nm. A mixture of n-propanol: acetic acid: water (8:1:1 v/v/v) was used as solvent system. Anisaldehyde sulfuric acid was used as spraying reagent to detect the spots. Saturation of 30 ml of the solvent system was poured in Camag Twin trough glass chamber which was lined with filter paper. A total of 20 min saturation time was allowed.

The R_f value was calculated using the following formula:

R_f = Distance travelled by solute/ Distance travelled by solvent

HPLC of the isolated compound was done by Waters HPLC with 3 µl injection and 10 min run time. Area, height, % area was calculated.

FTIR spectrum was recorded with KBr pellets on a PerkinElmer 1710 FTIR spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were obtained by Bruker AMX (400MHZ) spectrophotometer. DMSO and $CDCl_3$ were used as a solvent in ^{1}HNMR and ^{13}CNMR respectively. The MASS spectrum was recorded on a Bruker micrOTOF-Q II 10262 ESI spectrophotometer. Source type was ESI, ion polarity was positive, the dry heater was set on 180 °C and dry gas was set on 4.0 L/min. The software used was Bruker Compass Data Analysis 4.0.

2.7. Anti helicobacter pylori activity

2.7.1. H. pylori strain and culture preparation of bacterial inoculum

H. pylori (HP1) strain was obtained from gastroesophageal reflux disease (GERD) patient with no previous antimicrobial therapy to eradicate *H. pylori* infection. The collection of the sample was approved by the ethical committee at Yashoda super specialty Hospital, Ghaziabad, Uttar Pradesh, India. *H. pylori* strain was isolated from antral mucosal biopsy specimen and was identified based on gram staining, colony appearance and positive reactions in biochemical tests (urease, catalase and oxidase). *H. pylori* strain was revived and cultured on brain-heart infusion (BHI) agar (Difco Laboratories, Detroit, MI) supplemented with 5% horse serum (Invitrogen, NY), 0.4% IsovitaleX (Becton Dickinson, MD), polymixin B (10 µg/ml), vancomycin (8 µg/ml) and trimethoprim (5 µg/ml). The plate was incubated at 37 °C in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) (Double gas incubator, Hera cell 150i) for 3–6 days. The stock culture was maintained until use at - 70 °C in brain heart infusion broth with 20% glycerol.

2.8. Suspension preparation

Direct colony method was used for the preparation of the bacterial suspension. The colonies were taken directly from the plate and were suspended in 5 ml of sterile 0.85% phosphate buffer saline (PBS). The turbidity was adjusted in the initial suspension by comparing with McFarland's standard number 2 (0.2 ml 1% w/v $BaCl_2 \times 2H_2O$ + 99.8 ml 1% w/v H_2SO_4). When adjusted to the turbidity of the McFarland's standard no. 2, the bacterium suspension contains about 3×10^8 colony forming units (CFU)/mL. 3 µl of the adjusted inoculum was spotted on the BHI media plates containing antibiotics. The range of concentration was obtained by two-folded dilution of antibiotics i.e for metronidazole (0.2 µg/ml – 64 µg/ml) and for clarithromycin (0.125 µg/ml – 2 µg/ml). After 72 h incubation under microaerophilic conditions, the minimal inhibitory concentration (MIC) was recorded as the lowest concentration of the drug that inhibited the visible growth of organisms. Minimal inhibitory concentration (MIC) for Metronidazole was defined as (>8 µg/ml) and for Clarithromycin was (>0.5 µg/ml) (The European Committee on Antimicrobial Susceptibility Testing, 2017).

2.9. Anti- H. pylori activity by disc diffusion assay

Preliminary screening of plant isolate, effective against *H. pylori* strain was done by Kirby Bauer method. Sterile Whatman paper discs (6 mm in diameter) were soaked with different concentrations of the isolated compound from BAME and placed on the inoculated plates with 1.2×10^9 colonies forming unit (CFU) of *H. pylori*. The plates were kept under observation for 2 days at 37 °C under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) (Mehrotra et al., 2011). The experiment was performed in triplicates (Kirby et al., 1966). Minimum inhibitory concentration (MIC) was calculated based on the lowest amount of isolated compound from BAME exhibiting hairline growth inhibition around the isolated compound containing a disc. DMSO was used as a negative control against the *H. pylori* (HP1) strain.

2.10. Micro-dilution method

Minimum inhibitory concentration (MIC) was also determined by the micro-dilution method. The 12-well plate was prepared by dispensing 80 µl of BHI broth into first well. A 20 µl from the stock solution of isolated compound (10 mg/ml) was added to the first well. Then, sixteen-fold serial dilution was performed till 8th well. The obtained drug concentration range was from 100 µg/ml to 0.2×10^{-6} µg/ml. To each well 150 µl of the diluted bacterial cells were added to give a final concentration of 3.3×10^8 CFU/ml. The inoculated plates were incubated at 37 °C for 2 days at microaerophilic conditions. MIC^{90} was defined as the minimum concentration of isolated compound from BAME that inhibited the 90% of *H. pylori* cells when compared to control i.e. *H. pylori* cells without the isolated compound. Readings were noted on Elisa plate reader (Erba Lisa Scan II).

3. Results

The extraction was done by hot percolation method by soxhlet apparatus. Petroleum ether 60–80 °C was used primarily for defatting and then extracted with methanol. The yield, colour and consistency of methanol extract of stem of *Berberis aristata* DC (BAME) and BAME were screened for different phytochemicals like alkaloid, glycoside, flavonoid, tannin, saponin, phenolic compounds, etc. and results revealed the strong presence of alkaloid, carbohydrate, phenolic compound, flavonoid and tannin.

3.1. Isolation of isoquinolone alkaloid from BAME

The isolated compound was obtained from the methanol extract of stem of *Berberis aristata* DC as a yellowish needle-shaped crystal (Table 1)

In TLC the single spot at R_f 0.55 clearly showed the presence of a single compound which is isolated from BAME by chemical method. The % yield of the isolated compound was calculated as 2.8%w/w (Table. 2). In this isolation process mixture of n-Butanol: Glacial acetic acid: water (12:3:4 v/v/v) was used as solvent system.

The preliminary phytochemical analysis had given a positive test for alkaloid by all methods and negative test for carbohydrate, tannin, phytosterol, saponin, and flavonoid. The results obtained indicated the strong presence of alkaloid and this confirmed the isolated compound obtained from BAME was alkaloidal (Table 3).

3.2. Spectroscopy study

3.2.1. HPTLC finger print and HPLC analysis of isolated compound from the methanol stem extract of Berberis aristata DC

The isolated compound from BAME solubilized in methanol. The mobile phase used for separation was a mixture of n-propanol: acetic acid: water (8: 1: 1 v/v/v). Saturation time for the solvent was 20 min (Fig. 1 and Fig. 2).

3.2.2. FTIR fingerprint analysis of isolated compound from the methanol stem extract of Berberis aristata DC

The FTIR spectra were interpreted as follows (Fig. 3).

3340 cm^{-1} *N*–H stretching, 2912 cm^{-1} C–H stretching, 1612 cm^{-1} C═C, C═N stretching, 1504 cm^{-1} *N*–H bending, 1381 cm^{-1} Asymmetric CH_3, 1041 cm^{-1} C–O stretching.

Table 1
Percentage yield of methanol extract of stem of *Berberis aristata* DC.

Solvent	Extract	Colour andConsistency	Percentage yield (w/w) on dry basis
Methanol	BAME	Brownish yellow Dried powder	12.8%

Table 2
TLC study of isolated compound from BAME.

Name of extract	% Yield	Solvent system	R_f Value	Inference
Isolated Compound	2.8% w/w stem of *Berberis aristata* DC on dry wt basis	n-Butanol: Glacial acetic acid: Water (12:3:4 v/v/v)	0.55	A single R_f value indicates a single compound may be isolated

Table 3
Preliminary phytochemical studies of methanol extract of stem of *Berberis aristata* DC.

S. No.	Phytochemical constituents	Methanol Extract
1	Alkaloid	+++
2	Carbohydrate	++
3	Glycoside	+
4	Saponin	-
5	Phenolic compound	++
6	Flavonoid	++
7	Phytosterol	+
8	Triterpenoid	-
9	Tannin	++

+++ Significantly Present, ++ Moderately Present, + Slightly Present, - Absent.

BAME was screened for different phytochemicals like alkaloid, glycoside, flavonoid, tannin, saponin, phenolic compounds etc. and results (Table 4) revealed the strong presence of alkaloid, carbohydrate, phenolic compound, flavonoid and tannin.

3.2.3. NMR spectral study of isolated compound from the methanol stem extract of Berberis aristata DC

3.2.3.1. ^{1}H NMR peak of isolated compound from the methanol stem extract of Berberis aristata DC (Fig. 4). ^{1}H NMR (DMSO-d_6): δ 8.19 (^{1}H, d, J = 9.2 Hz, H-11), 7.99 (^{1}H, d, J = 9.2 Hz, H-12), 7.80 (^{1}H, s, H-1), 7.09 (^{1}H, s, H-8), 6.79 (^{1}H, s, H-4), 6.17 (2H, brs, O–CH_2–O), 5.99 (^{1}H, s, H-13), 4.09 (3H, brs, OMe), 4.07 (3H, brs, OMe), 3.80 (2H, dd, J = 1.6, 2.0 Hz, H_2-6), 2.50 (2H, dd, J = 1.6, 2.0 Hz, H_2-5).

3.2.3.2. ^{13}CNMR peak of isolated compound from the methanol stem extract of Berberis aristata DC (Fig. 5). ^{13}C NMR (DMSO-d_6): δ 105.40 (C-1), 147.63 (C-2), 149.77 (C-3), 108.37 (C-4), 130.62 (C-4a), 26.29 (C-5), 55.15 (C-6), 145.39 (C-8), 120.15 (C-8a), 143.65 (C-9), 150.34 (C-10), 126.73 (C-11), 123.49 (C-12), 132.97 (C-12a), 120.39 (C-13), 137.43 (C-13a), 121.36 (C-13 b), 102.02 (O–CH_2–O), 61.89 (OMe), 57.04 (OMe).

*3.2.3.3. **MASS spectra of the isolated compound from the methanol stem extract of Berberis aristata DC*** (Fig. 6). The bioactive isolated compound from BAME had given molecular ion peak [M+1] 336 in the ESI mass spectrum, which suggested molecular formula **$C_{20}H_{18}NO_4$**.

Based on phytochemical qualitative tests it was found as an alkaloid. The spectral data of FTIR, NMR and MASS were interpreted to predict the molecular structure, atomic stretching, possible

Molecular functional groups present, etc., and finally, the active constituent was suggested as an alkaloid, berberine from *Berberis aristata* DC stem. The chemical name is 9,10-dimethoxy-5,6-dihydro [1,3] dioxolo [4,5-g]isoquino [3,2-*a*] isoquinolin-7-ium.

3.3. Assessment of in vitro anti H. pylori activity

One clinical isolate HP1 was isolated from the patient suffering from GERD (Gastro-Esophageal Reflux Disease) and used for this research work. The strain (HP1) was resistant to both the antibiotics i.e. clarithromycin and metronidazole which is commonly used in triple therapy for the treatment of *H. pylori* infection.

3.4. Disc diffusion assay

Different concentrations of berberine from BAME in DMSO was loaded onto the disc and was air-dried. 100 µl of the suspended *H. pylori* strains in PBS having Mac Farland 2 was spread, plated onto BHI medium. The disc loaded with the different concentrations of the isolated compound from BAME were placed upside down in the *H. pylori* plates. After 72 h of incubation berberine showed anti-*H. pylori* activity. The MIC of the berberine was <50 µg. Among the different concentrations tested, 200 µg displayed maximum inhibition against the drug-resistant *H. pylori* (HP1) strain. The diameter of the zone of inhibition was 18 mm

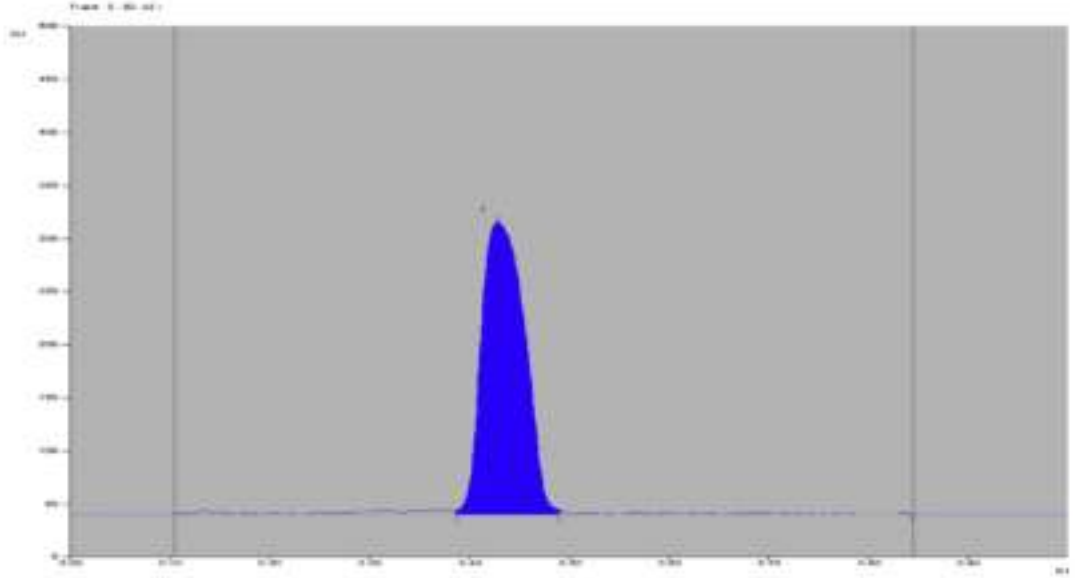
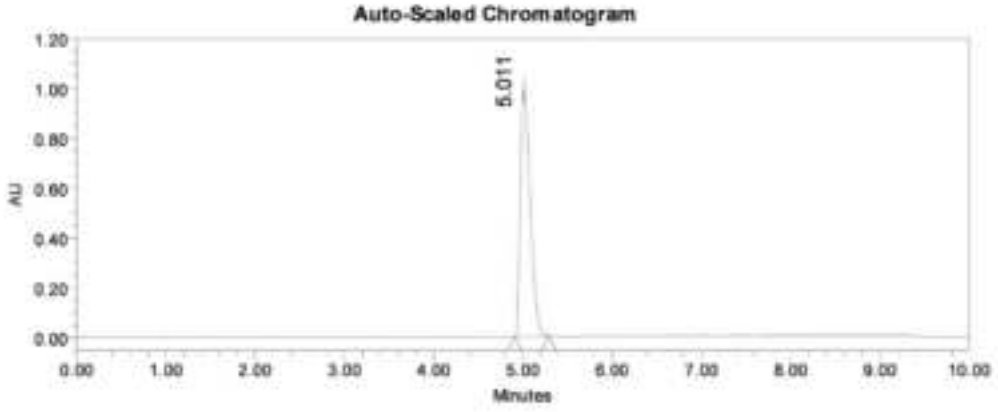


Fig. 1. HPTLC finger print analysis of isolated compound from the methanol stem extract of *Berberis aristata* DC.



Peak Results

RT	Area	Height	% Area
5.011	7655353	987158	100

Fig. 2. HPLC chromatogram of isolated bioactive compound from the methanol stem extract of *Berberis aristata* DC.

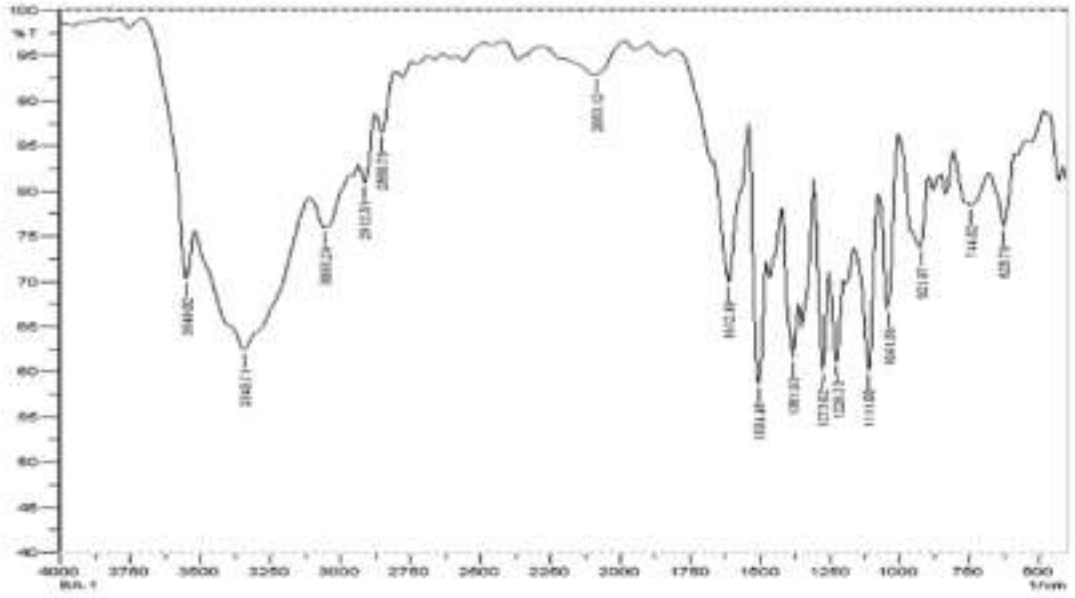


Fig. 3. FTIR finger print analysis of isolated compound from the methanol stem extract of *Berberis aristata* DC.

in the 200 μg of the berberine from the BAME in DMSO.

3.5. Microdilution method

The antimicrobial activity was determined in dual drug-resistant clinical *H. pylori* to isolate (HP1). MIC values ranged between 100 μg/ml to 0.2×10^{-5} μg/ml. MIC^{90} value of the isolated compound from the BAME in DMSO and methanol were 0.02 μg/ml and 0.000075 μg/ml respectively when observed at 405 nm[4] (Fig. 7).

Anti *H. pylori* activity of berberine in DMSO against dual drug-resistant (clarithromycin and metronidazole) *H. pylori* strain (HP1) was estimated. DMSO was used as the negative control.

Zone of inhibition was observed between 15 and 18 mm diameter against berberine at 50–200 μg (Table 4).

In Fig. 8, lane 1 to 8 showed the sixteen-fold serial dilution of the berberine with a constant cell count of *H. pylori*. Lane 9 showed media control without cells. Lane 10 showed cell control without berberine, Lane 11 showed the berberine control without cells. Lane 12 showed the control in which the berberine was dissolved with cells.

MIC^{90} was observed at lane 4 for sample 1 having berberine in a concentration of 0.02 μg/ml and at lane 6 for sample 2 having berberine in a concentration of 0.000075 μg/ml at OD, 405 nm.

The concentration of the stock solution of the berberine was 10 mg/ml.

4. Discussion

Plant used in the Indian Traditional System having long story. Plants having advantage due to its antioxidant in nature, they showed the various pharmacological effects against various diseases. Berberine (a natural isoquinoline) quaternary alkaloids and is the active phyto-constituent of *Berberis aristata* DC. Barberine already exhibited its pharmacological importance via its anti-microbial, anti-inflammatory, anti-oxidant and anti-cancer effects.

Eradication of *H. pylori* is a challenge for the treatment strategies worldwide. Unfortunately, none of them have been successfully able to eradicate *H. pylori* related complications (Alba et al., 2017; Ghotaslou, 2015). The main reason behind the treatment failure could be the

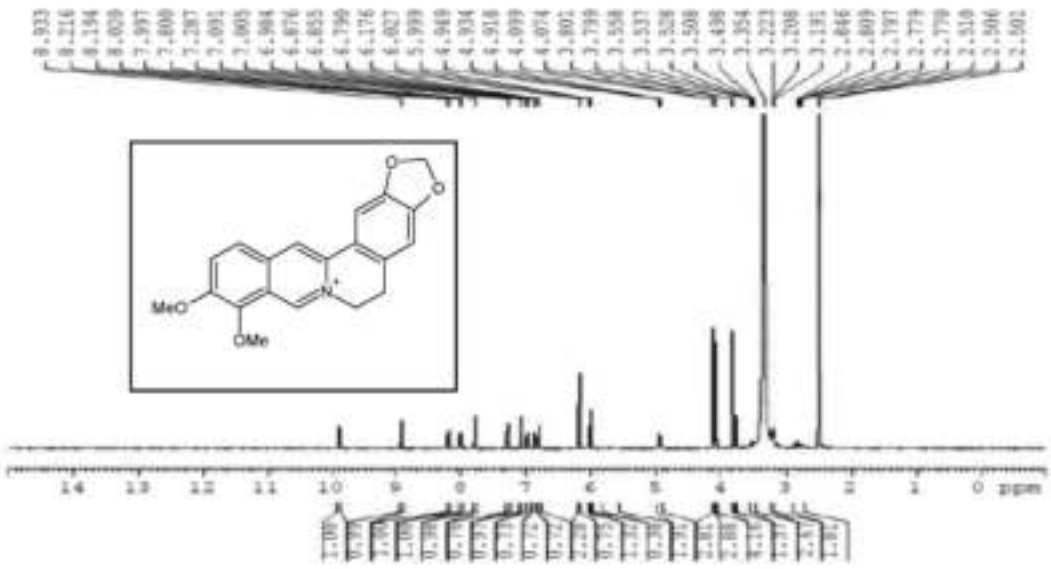


Fig. 4. ^{1}H NMR peak of isolated bioactive compound from the methanol stem extract of *Berberis aristata* DC.

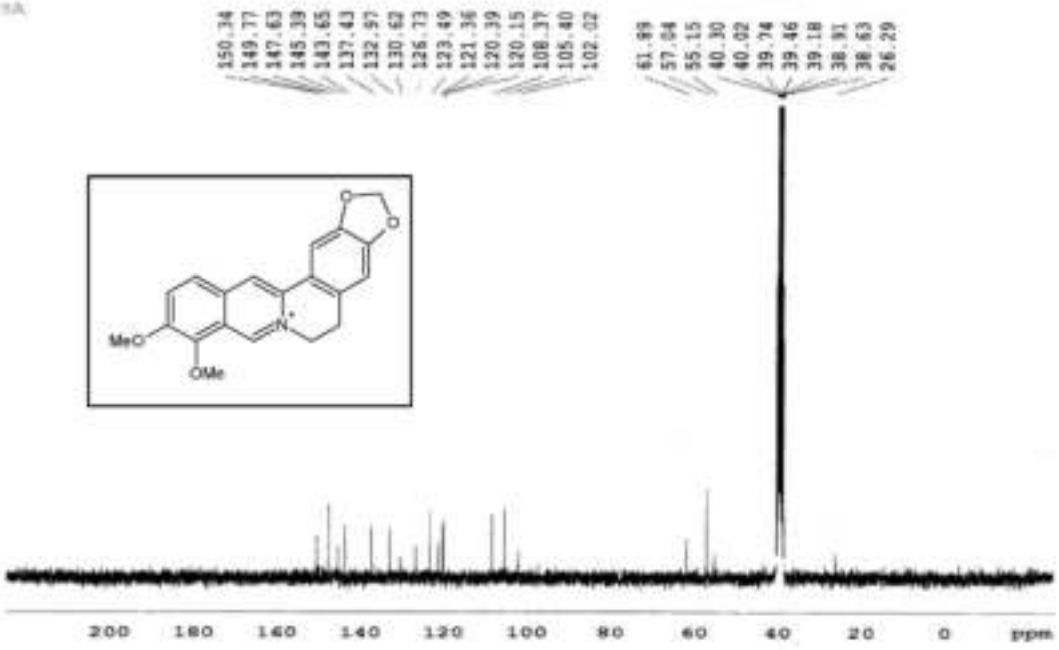


Fig. 5. ^{13}CNMR peak of isolated bioactive compound from the methanol stem extract of *Berberis aristata* DC.

rapidly growing resistance in many strains of *H. pylori* towards various antibiotics (Mégraud, 2004; Mégraud, 2004). Recently, Gehlot et al., reported that the antibiotic resistances to various commonly used antibiotics against *H. pylori* are variable in different parts of India (Seck et al., 2013). Besides, the prevalence of multidrug-resistant strain was also reported in various studies done in North India, 8.8%; Multicentric study in India, 43.2%; Gujarat, 85% respectively (Savoldi et al., 2018; Seck et al., 2013). Such a high prevalence of dual drug and multi-drug resistant *H. pylori* isolates has prompted us to look into the natural herbal products which can be effective against *H. pylori*.

Earlier studies have shown that ethanol and methanol extracts of *Parmelia perlata* can effectively inhibit *H. pylori in vitro* (Seck et al., 2013). Similarly, methanol extract of *Paederia foetida* was effective in inhibiting the *in vitro* growth of metronidazole-resistant *H. pylori* strain (Mégraud, 2004). De et al., has mentioned the therapeutic potential of curcumin against *H. pylori*-associated gastroduodenal diseases (De et al., 2009).

Berberis aristata DC (Daruharidra) is one of the herbs mentioned in ancient scriptures of Ayurveda (Alamgir, 2017). The root and wood part of the herb is rich in Berberine, a bitter yellow coloured alkaloid, which dissolves in acids and forms salts.("Berberine: Alkaloid with wide spectrum of pharmacological activities," Berberine, 2010) Berberine alkaloid has been effective against intestinal infections such as *Shigella dysenteriae, Salmonella paratyphi*, various Klebsiella species (Sun et al., 1988). It has also been reported to inhibit the intestinal secretory response of *Vibrio cholera* and *Escherichia coli* (Sack and Froehlich, 1982). Berberine from stem, root and leaf extracts of various species of *Berberis aristata* also reported possessing *in vitro* antimicrobial property against Gram-negative and Gram-positive bacteria and fungi (Cernakova and Kostálová, 2002) Published review articles have also described the antimicrobial effects of Berberis species have been attributed to their various secondary metabolites, especially alkaloids.("Berberine:

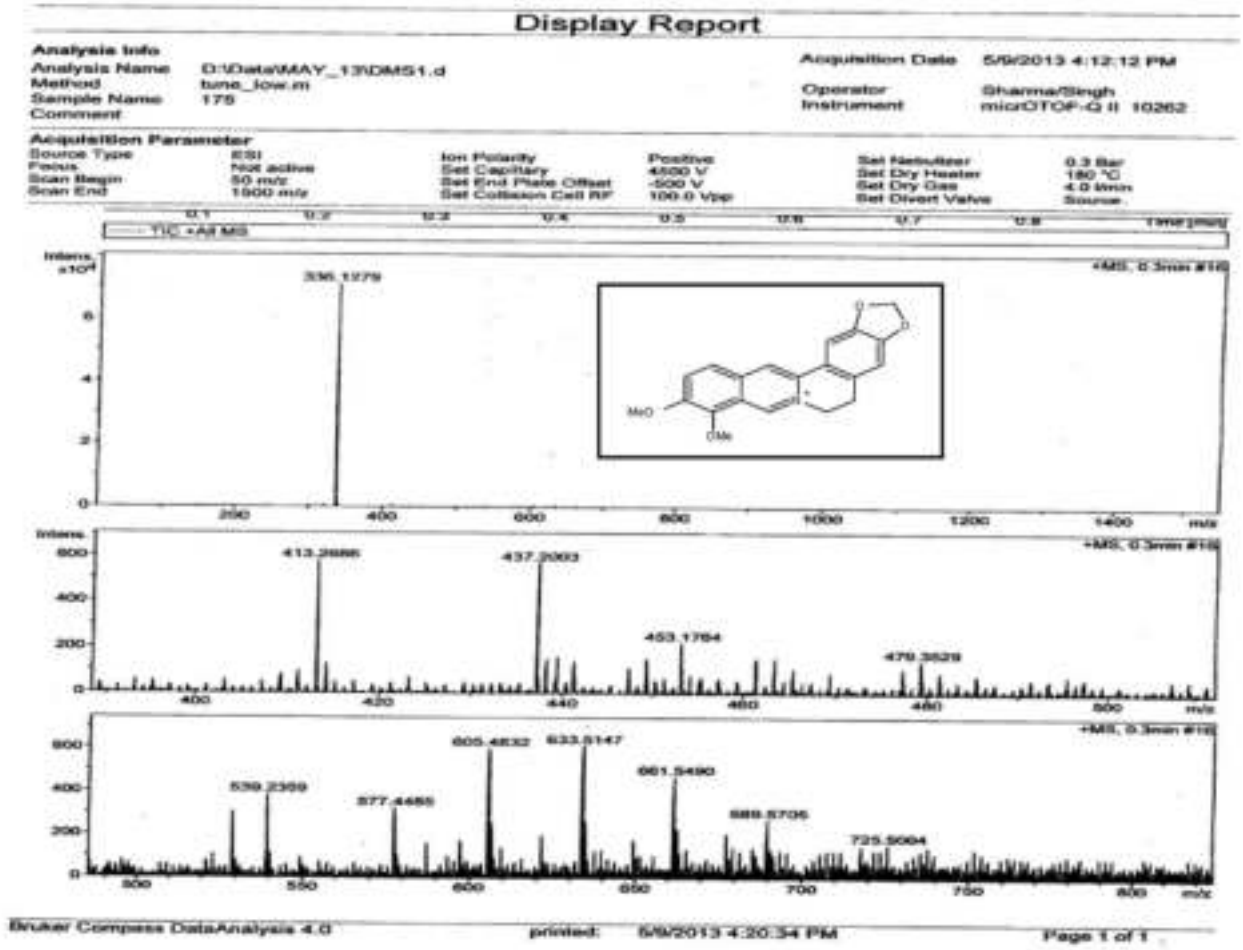


Fig. 6. MASS spectra of isolated bioactive compound from the methanol stem extract of *Berberis aristata* DC.

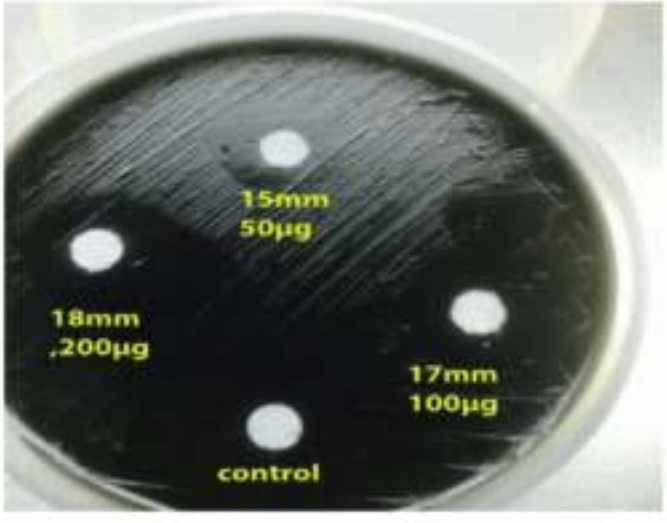


Fig. 7. Determination of zone of inhibition of berberine from the methanol extract of stem of *Berberis aristata* DC from disc diffusion method.

Alkaloid with wide spectrum of pharmacological activities," 2010).

In present study, anti-*H. pylori* activity of berberine from BAME showed the zone of inhibition. The maximum zone of inhibition found in 200 µg solution was 8 mm. The MIC[90] value was found from the microtiter well plate using ELISA plate reader at Lane 4 was 0.02 µg/ml; and Lane 2 was 0.000075 µg/ml, at 405 nm. Due to the incomplete therapeutic effect achieved with the triple therapies and their possible side effects, herbal systems of medicine have become popular in recent years. Nearly 70% of synthetic drugs are synthesized from medicinal herbs and they have been figured high in pharmaceutical research because of their high therapeutic activity and diversified therapeutic efficacy. Herbal therapies have shown better efficiency in inhibiting *H. pylori* at both *in vitro* and *in vivo* levels (O'Gara et al., 2000).

Table 4
Zone of inhibition with different concentrations of berberine against the drug resistant *H. pylori* (HP1).

Bacteria	Concentration of Berberine from BAME extract (Stock solution concentration: 10 mg/ml)		Zone of Inhibition (Diameter of Disc, 6 mm)
H. pylori strain (HP1)	Isolated compound from the methanol extract of stem of *Berberis aristata* DC. in DMSO	50 µg	15 mm
		100 µg	17 mm
		200 µg	18 mm
	Control (DMSO)	200 µg	6 mm

5. Conclusion

It was observed in the above study that the isolated compound (berberine) from BAME showed significant anti *H. pylori* activity. This

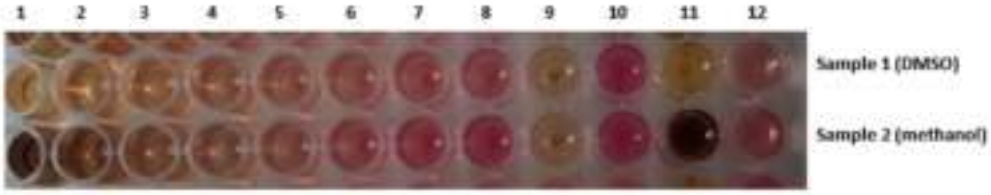


Fig. 8. 12 Microtiter well plated showing Minimal Inhibitory Concentration of berberine.

anti *H. pylori* activity may be beneficial for the treatment of ulcer mediated by *H. pylori*. Future studies will assess the mechanism by which berberine affect the survival of *H. pylori*.

Declaration of competing interest

All authors hereby declare that there is no financial conflict of interest.

Acknowledgement

The authors are very thankful to BIT, Mesra, India and Amity University, Noida, India for providing the infrastructure and support to carry out the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2020.101622.

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