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Research Article

MORINDA COREIA HAS AN ANTI-*HELICOBACTER PYLORI* EFFECT AGAINST THE MULTIDRUG-RESISTANT CLINICAL ISOLATE OF NORTH-EAST INDIA

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ABSTRACT

Objective: Antimicrobial resistance is a growing problem in *Helicobacter pylori* eradication which is a microaerophilic bacterium causing various gastroduodenal diseases. The present study has been designed to test the efficacy of *Morinda coreia* against the metronidazole clarithromycin and levofloxacin-resistant *H. pylori* strains isolated from the biopsy taken from the patient suffering from gastric erosion in Guwahati, Assam.

Method: The antimicrobial activity of n-hexane and chloroform extract of *M. coreia* was tested against multidrug-resistant *H. pylori* isolate of Guwahati, Assam, by agar well method and microdilution method.

Result: In the present study, the *H. pylori* strain resistant for metronidazole (minimal inhibitory concentration [MIC] >64 µg/mL), clarithromycin at (MIC >0.5 µg/mL), and levofloxacin at (MIC >1 µg/mL) was tested against the n-hexane and chloroform extract of *M. coreia.* Both the extracts of *M. coreia* showed good efficacy against the multidrug-resistant strain of *H. pylori* shown inhibition at 1.2 µg/mL with n-hexane extract and 2 µg/mL with chloroform extract of *M. coreia.*

Conclusion: The prevalence of metronidazole-resistant ranges between 50% and 90% in the developing countries, including India, clarithromycin ranges from 0% to 15% in India, and levofloxacin ranges between 50% and 70% in India, so there is a need of alternative therapy for the eradication of this bacterium from the stomach. Hence, this study suggests that *M. coreia*, which has been used traditionally as a folk medicine for the treatment of many gastric diseases, has also shown good efficacy against the multidrug-resistant *H. pylori* strain of North-east India.

Keywords: *Helicobacter pylori*, *Morinda coreia*, Resistance, Antibiogram.

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INTRODUCTION

The Gram-negative, microaerophilic gastric bacteria *Helicobacter pylori* is recognized as an important human pathogen and classified as a Group I carcinogen by the International Agency for Research on Cancer [1]. *H. pylori* has infected over half of the world's population, and its infection usually occurs in early childhood and can persist throughout a lifespan if it is untreated with a specific antibiotic regimen [2]. *H. pylori* infections are largely asymptomatic, but the studies have also shown that, if its association lasts for long term with the host, it can cause more serious disease such as gastritis, peptic ulcer, gastric cancer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma [3,4]. *H. pylori* eradication from the infected individuals is the best and only choice for an effective treatment of its associated diseases. Eradication by triple therapy regimen consists of a proton-pump inhibitor in combination with two antibiotics, such as clarithromycin and amoxicillin or metronidazole, which has been recommended as the first-line therapy and results in a high eradication rate [5]. The other antibiotics which are also used for the eradication of *H. pylori* as a second-line therapy or third-line therapy are levofloxacin, furazolidone, and tetracycline. Due to the use of these antibiotics for the treatment of other diseases like respiratory, anaerobic infection, dental infection, which have resulted in the emergence of antibiotic resistance which may be the major cause of treatment failure [6,7]. Metronidazole and clarithromycin are the most commonly used antibiotics in the treatment of *H. pylori* infection as compared to other antibiotics, but many studies from India and abroad have reported that the resistance for both the antibiotics has been increasing nowadays which may be the reason for the treatment failure [8-10]. The prevalence of dual drug resistance and multidrug resistance has been observed in many countries which has also become the major obstacle in the treatment for *H. pylori* infection. In the geographical areas where the resistance rate ranges between 15% and 90%, there is an urgent need to develop new treatment strategies for *H. pylori* infection. Unlike synthetic drugs, bioactive natural products which are benefical without any side effects as they are part of our day to day life and other culinary items [11]. In this search, *Morinda coreia* which is generally found in dry forests of the greater part of India and Sri Lanka belongs to the family *Rubiaceae*, which is one of the largest and the most widely distributed plants in approximately 400 genera in this family. *M. coreia* is also known by its Tamil name as "Nuna." The plant is small- to medium-sized tree with a straight cylindrical stem 3.6–4.2 m in length and 90 cm in width [12,13]. However, it has been shown that the beverage produced by fermenting the fruits was able to inhibit enteropathogenic bacteria and also had a high amount of potassium. It is also extensively used in Thai traditional medicine to cure stomach infection and blood stasis [14]. A dye obtained from the root bark is used for coloring linen and woolen goods [14]. Studies have conducted using extracts of *M. coreia* against various Gram-negative and Gram-positive pathogenic bacteria but not with *H. pylori* [15]. Therefore, in the present study, we tested the antimicrobial activity of *M. coreia* against the multidrug-resistant strain of *H. pylori* isolated from the patient suffering from gastric erosion in North-East India.

METHODS

Source of plant material

Healthy leaves of *M. coreia* were collected from Sivakasi, Tamil Nadu, India. The leaves of *M. coreia* were authenticated at the Botany

Department of Amity University. The leaves were washed properly under running tap water and then rinsed in distilled water. Then, it was air dried in the shade and grinded into powder using mortar and pestle.

Extract preparation of *M. coreia*

To obtain the extract of *M. corea,* 100 g of leaves was crushed with mortar and pestle and sieved. The dried powder was then extracted with 400 mL of n-hexane, chloroform, methanol, water, and ethanol consecutively for 72 h solvent under constant stirring. The extract was then filtered and dried under pressure and resuspended in the solvents. The concentrated extract was then kept in the dark bottles at 4°C until used. The n-hexane and chloroform extracts of *M. coreia* were used in the present study to test the antimicrobial activity against multidrug-resistant *H. pylori* isolates from North-East India.

Isolation of *H. pylori* strains and culture

The patient suffering from gastric erosion was included in the study. The *H. pylori* strains were isolated from the antral biopsies of the patient who underwent endoscopy procedure in the Gastroenterology Department of Gauhati Medical College, Guwahati, Assam. The identification of the *H. pylori* was done on the basis of colony morphology, gram staining, and positive reaction in the biochemical test (catalase, urease, and oxidase). *H. pylori* strain was isolated 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), trimethoprim (5 μg/mL), vancomycin (8 μg/mL), and polymixin B (10 μg/mL). The plates were incubated at 37°C under microaerophilic condition (5% O_2, 10% CO_2, and 85% N_2) (Double gas incubator, Hera cell 150i) for 3–6 days. Stock cultures were maintained until use at –80°C in BHI broth with 20% glycerol.

Suspension preparation of *H. pylori* strain

The bacterial suspension of *H. pylori* was prepared by direct colony method [16]. The colonies were taken directly from the plate and were suspended in 5 mL of sterile 0.85% phosphate buffer saline (PBS). The turbidity of the initial suspension was adjusted by comparing with McFarland's standard number 2 (which contains about 1×10^8 colony-forming units (CFU)/mL) [17].

Antimicrobial susceptibility and resistance determination

Exponentially grown *H. pylori* cells on antibiotic-free BHI agar were further suspended in PBS buffer, 10-fold serial dilution of these cell suspensions was prepared, and 10 μL of each dilution was spotted on freshly prepared BHI agar containing various concentrations of different antibiotics in μg/mL, namely metronidazole (0.2, 0.5, 1.5, 3, 8, 16, 32, and 64), clarithromycin (0.125, 025, 1, and 2), furazolidone (0.2, 0.5, 1, and 2), amoxicillin (0.125, 0.25, 1, and 2), levofloxacin (0.2, 0.4, 1, and 2), and tetracycline (1, 2, 3, and 4).

Determination of minimum inhibitory concentration (MIC) by agar dilution method

The antibiotics plates with *H. pylori* cultures were incubated for 72 h under microaerophilic conditions; the MIC was defined as the lowest concentration that inhibited visible growth of organisms. The *H. pylori* isolates are considered to be resistance if the MIC for different antibiotics is metronidazole (>8 μg/mL) [18], clarithromycin (>0.5 μg/mL) [18], amoxicillin (>0.12 μg/mL) [18], tetracycline (>1 μg/mL) [18], levofloxacin (>1 μg/mL) [18], and furazolidone (>2 μg/mL) [17].

Conformation of antibiotic resistance by sequence analysis of *23S rRNA, gyraseA, rdx*, and *frx* genes of *H. pylori* isolate

Antibiotic-resistant strain by agar dilution method was further confirmed by polymerase chain reaction (PCR) followed by sequencing for the amplification of *23S rRNA*, *gyraseA*, *rdx,* and *frx* gene mutations responsible for the resistance of clarithromycin, levofloxacin, and metronidazole, respectively. Genomic DNA was isolated from the *H. pylori* strain by C-TAB method [19], and the total DNA concentration was determined by NanoDrop 1510 (IS10-03571C, Multiskan GO, Thermo Fisher Scientific). The PCR for *23S rRNA*, *gyraseA*, *rdx,* and *frx* gene was performed to detect the resistance for all the antibiotics. All the genes were amplified using the primer sets as listed in Table 1. The PCR was carried out in 20 μL reaction containing 10 pmol dNTP (Bangalore Genei, Bengaluru, India), 10 pmole primer sets (Sigma-Aldrich), 1.5U Taq DNA polymerase (Bangalore Genei), and 10 ng of *H. pylori*-positive genomic DNA Eppendorf Thermocycler (vapo.protect™) for 35 cycles under the following cycling conditions 94°C for 1 min, annealing for 1 min, and 72° for 1min. The product was finally analyzed in 2% agarose gel stained with ethidium bromide under standard procedure. All the amplified PCR products were further purified by QIAquick PCR Purification kit (QIAGEN, Hilden, Germany). The purified products were further sequenced by ABI sequencer 3100XL consisting of Big Dye® Terminator (PerkinElmer) with Ampli Taq FS. The sequence was edited after aligning with SeqMan program (DNASTAR Inc., Madison, WI). For the detection of point mutation in various genes of *H. pylori*, multiple sequence alignment was performed by CLUSTAL OMEGA taking 26695 strains of *H. pylori* as a reference strain.

Anti-*H. pylori* activity of *M. coreia* against multidrug-resistant strain

MIC determination by the agar well method

The different concentration of n-hexane and chloroform extract of *M. coreia* was loaded into the well of 6 mm in diameter in inoculated plates with 3×10^9 CFU of multidrug-resistant strain of *H. pylori* isolated from the patient of North-East India. The plates were kept under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) for 2 days at 37°C. All experiments were performed in triplicates. Pure n-hexane and chloroform were used as a negative control, and amoxicillin was used as positive control.

MIC determination by microdilution method

The 96-well microtiter plate was prepared by dispensing 80 μL of BHI broth into first well. A 20 μL from the stock solution of *M. coreia* extract (120 μg/mL) was added to the first well. Then, five-fold serial dilution was performed until 8th well. The obtained concentration range was from 12 μg/mL to 12×10^{-7} μg/mL. To each well, 150 μL of the diluted bacterial cells was added to give a final concentration of 3×10^9 CFU/mL. The inoculated plates were incubated at 37°C for 72 h under microaerophilic conditions. MIC_{90} was defined as the lowest concentration of extract sample that inhibited the 90% of *H. pylori* cells when compared to control, i.e., *H. pylori* cells without extract sample. Reading was noted on the Elisa reader (Erba Lisa Scan II).

Table 1: PCR primers of *23S rRNA, gyraseA, rdx,* and *frx* genes of *H. pylori*

Gene	Primer sequence	Size of the product	References
23S rRNA	F-5'GGCTCTTTGAGTCCTTTAGGACAA-3' forward sense positions 2020-2044 of U27270) R-3'CTCCATAAGAGCCAAAGCCCTTACT-5' reverse antisense position 2612-2636 of U27270)	613	[20]
gyraseA	F-5'-TTTRGCTTATTCMATGAGCGT-3' R-3' -GCAGACGGCTTGGTARAATA-5'	428	[21]
rdx	F-5'-GCAGGAGCATCAGATAGTTCT-3' F-3'-GGGATTTTATTGTATGCTACAA-5'	886	[22]
frx	F-5'-GGATATGGCAGCCGTTTATCATT -3' F-3'-GAATAGGCATCATTTTAAGAGATT -5'	780	[22]

F: Forward primer; R: Reverse primer; bp: Base pairs, PCR: Polymerase chain reaction

Rapid urease test (RUT)

For the microbial growth visualization, RUT was done. The basis of the test is the ability of *H. pylori* to secrete urease enzyme which hydrolyzes the urea to ammonia and carbon dioxide and raises the pH of the medium from yellow (*H. pylori* negative) to a pink color (*H. pylori* positive). MIC was defined as the lowest concentration of extract sample that prevented RUT medium color change from yellow to pink.

RESULT

One clinical isolate was included in the study from the patient suffering from gastric erosion. The MIC for various antibiotics, namely metronidazole, clarithromycin, amoxicillin, tetracycline, levofloxacin, and furazolidone, was determined by agar dilution method against *H. pylori* isolate of North-East India. The strain which was selected to test the efficacy of *M. corea* was multidrug resistant showing resistance against metronidazole at the MIC of >64 µg/mL, clarithromycin at MIC >0.5 µg/mL, and levofloxacin at MIC >1 µg/mL but sensitive to other drugs, namely furazolidone, amoxicillin, and tetracycline. The genomic DNA was further PCR amplified for *23S rRNA*, *gyraseA*, *rdx,* and *frx* gene of *H. pylori* for sequencing and sequence analysis (Fig. 1a-c).

Sequence analysis of various genes of *H. pylori* for the conformation of resistance pattern

Mutation analysis of 23S rRNA gene of H. pylori for clarithromycin resistance

The sequence of *23S rRNA* gene was aligned with the reference sequence U27270 using CLUSTAL OMEGA for the presence of mutation at A to G at 2142, A to G at 2143, and T to C at 2182 positions in the V domain of *23S rRNA* gene of *H. pylori* (Fig. 2). The strong association was observed in the agar dilution method, and sequence analysis method showed that the *H. pylori* strain resistant for clarithromycin was having a mutation at T to C at the 2182 position and MIC of >0.5 µg/mL.

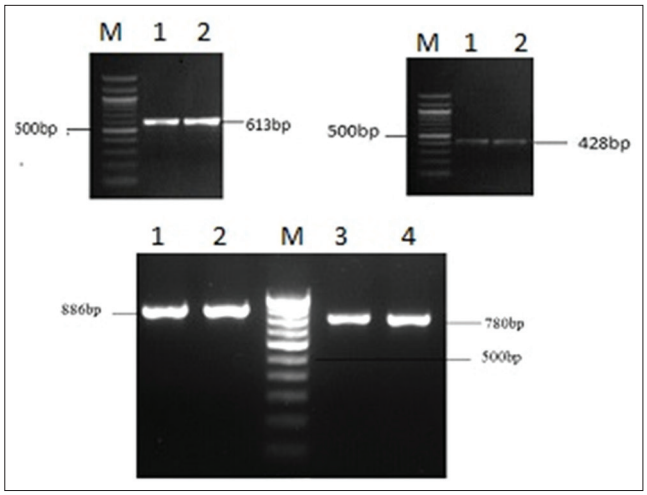

Fig. 1: DNA amplification of *Helicobacter pylori* gene: (a) *23S rRNA* gene of 613bp. Lane 1 - positive control, 26695 Lane 2 *H. pylori* clinical isolate,and Lane M - Marker (100bp). (b) *gyraseA* gene of 428 bp. Lane 1 - positive control, 26695 Lane 2 - *H. pylori* clinical isolate, and Lane M - Marker (100bp). (c) *rdx* and *frx* gene of 886bp and 780bp. Lane1 - positive control of *rdx* gene, 26695 Lane 2 - *H. pylori* clinical isolate, Lane M - Marker (100bp), Lane 3 - positive control of *frx* gene, 26695 and Lane 4 - *H. pylori* clinical isolate

Mutation analysis of *gyraseA* gene of *H. pylori* for Levofloxacin resistance:

The resistance for levofloxacin was observed due to a mutation in *gyraseA* gene of *H. pylori* at various amino acid positions, namely asparagine (N) to lysine (K) at 87 position, alanine (A) to valine (V) at 88 position, and aspartic acid (D) to glycine (G)/tyrosine (Y)/ asparagine (N) at 91 position. The *H. pylori* nucleotide sequence was converted to protein sequence by EXPASY TRANSLATE TOOL and was aligned with the reference sequence HP0701 using CLUSTAL OMEGA for mutation analysis. Point mutation was observed from aspartic acid (D) to glycine(G) at 91 position in *gyraseA* gene of *H. pylori* (Fig. 3). The strong association was observed in the sequence analysis method and agar dilution method showing MIC at >1 ug/mL for levofloxacin.

Mutation analysis of *rdx* and *frx* gene of *H. pylori* for metronidazole resistance

The resistance for metronidazole was observed by the mutation in *rdx* and *frx* gene of *H. pylori* at various amino acid positions as these gene are highly conserved genes of *H. pylori* [22]. The *H. pylori* nucleotide sequence was converted to protein sequence by EXPASY TRANSLATE TOOL and was aligned with the reference sequence HP0954 for *rdx and* HP0642 for *frx* using Clustal Omega. Point mutation was observed as various amino acid positions of *rdx and frx* gene of *H. Pylori* (Fig. 4a and b). Hence, the strong association was observed in the sequence analysis method and agar dilution method which has shown MIC at >64 ug/mL for metronidazole.

Agar well method to test the efficacy of *M. coreia*

Different concentration of n-hexane and chloroform extract of *M. coreia* was loaded onto the well and was air dried. 100 µL of the suspended *H. pylori* strain in PBS having McFarland 2 (1×10^8 CFU/mL) was spreaded and plated onto BHI medium. The well was made in the plate using well puncher and the wells were loaded with different concentrations of the extract. After 72 h of incubation, we found that the n-hexane and chloroform extract of *M. coreia* has anti-*H. pylori* activity showing the zone of inhibition of 7 mm at 0.4 mg/mL for n-hexane extract (Fig. 5a and b) and 7 mm for 2.4 mg/mL chloroform extract (Fig. 6a and b) by agar well method, and the MIC of *M. coreia* was <12 µg/mL for n-hexane extract and <2 µg/mL for chloroform extract against multidrug-resistance *H. pylori* strain of North-East India by microdilution method (Fig. 7 and Table 2).

DISCUSSION

H. pylori have acquired antibiotic resistance for several antibiotics in the past two decades, which is the major cause of treatment failure. The antibiotic resistance varies geographically due to its varied use in other diseases for the treatment of the local population. The geographical resistance scenario of antibiotic resistance pattern provides a guideline to all the clinicians for the treatment regimen. Previous studies have shown that resistance toward metronidazole varies geographically in India such as Chandigarh (38.2%) [23], Delhi NCR (48.5%) [24], Lucknow (68%) [23], Hyderabad (100%) [23], Chennai (82.2%), [23], Kolkata (85%) [8], Gangetic belt of North India (100%) [25], and Gujarat (83.2%) [26]. High prevalence of metronidazole was also found in other developing countries such as Pakistan (97.8%) [27], China (78.8%), Nepal (88.1%), Bangladesh (77.8%), Bhutan (83%), Vietnam (72%), and Mexico (76.3%) [28,29]. The resistance to metronidazole is

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U27270    2137GGAAAGACCCCGTGGACCTTTACTACAACTTAGCACTGCTAATGGGAATATCATGCGCA 2197
GMC 48    454 GGAAAGACCCCGTGGACCTTTACTACAACTTAGCACTGCTAACGGGAATACCATGCGCA 514
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Fig. 2: Multiple sequence alignment of domain V of *23S rRNA* gene of clarithromycin of *Helicobacter pylori* strains. The strain number is indicated on the left-hand side of each sequence. U27270 is a representative sequence showing T to C point mutations marked in yellow at positions 2182 of the *23S rRNA* gene. Numbering of nucleotide position followed the proposed system by Taylor *et al.* (position [2515 373] + 1 = position 2143)

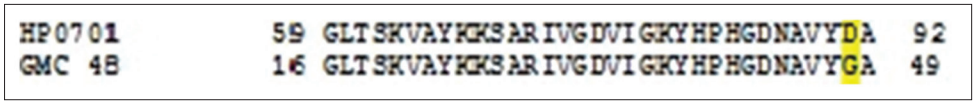

Fig. 3: Multiple sequence alignment of *gyraseA* gene of *Helicobacter pylori* for levofloxacin resistance pattern. The strain number is indicated on the left-hand side of each sequence. HP0701 is a representative sequence showing D to G point mutations marked in yellow at amino acid positions 91 of the *gyraseA* gene

Fig. 4: (a) Multiple sequence alignment of *rdx* gene of *Helicobacter pylori* for metronidazole resistance pattern. The strain number is indicated on the left-hand side of each sequence. HP0954 is a representative sequence showing multiple point mutations marked in yellow at amino acid positions, (b) multiple sequence alignment of *rdx* gene of *H. pylori* for metronidazole resistance pattern. The strain number is indicated on the left-hand side of each sequence. HP0642 is a representative sequence showing multiple point mutations marked in yellow at amino acid positions

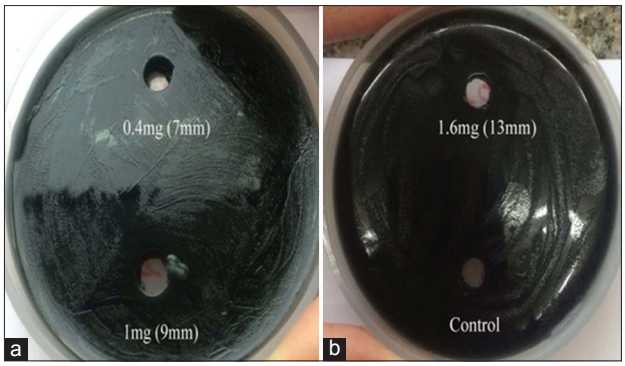

Fig. 5: (a and b) Agar well method: Antimicrobial activity of n-hexane extract of *Morinda coreia* against the multidrug-resistant strains of *Helicobacter pylori*

due to the mutation in oxygen-insensitive NADPH nitroreductase (*rdx)* and NADPH flavin oxidoreductase (*frx)* gene of *H. pylori* [22].

The resistance for levofloxacin was also reported high in many countries such as Delhi NCR (73.2%) [30], Gujarat (13.8%) [26], Bangladesh (66%), Bhutan (3%), and Vietnam (25%) [28,29]. The resistance for levofloxacin is due to the mutation in the amino acid chain of *gyraseA* gene of *H. pylori* at various positions [21]. The resistance to clarithromycin also observed and it is considered as one of the main drugs in the treatment regime in combination with proton-pump inhibitor and amoxicillin or metronidazole. The resistance to clarithromycin also varies geographically such as Kolkata (0%) [8], Gujarat (58.8%) [26], Delhi NCR (11.8%) [31], Lucknow (4%) [23], Pakistan (5.4%) [27], China (12.8–23.8%), Spain (35.6), and USA (10–15%) [32]. Hence, the emergence of resistance to various key antibiotics, which are mainly used as a first-line or a second-line therapy in the eradication of *H. pylori,* has prompted us to look into the non-antibiotic compound that inhibits *H. pylori* growth.

There are many plant and plant products which are having anti-*H. pylori* effect and showed good efficacy against the drug-resistant *H. pylori* strain. De *et al*. showed the good efficacy of curcumin in *H. pylori*-infected mouse [33]. Our other studies have shown that the methanolic extract of *Paederia foetida* [34], methanolic and ethanolic extract *Parmelia perlata* [35], *Embilica officinalis* [36], and methanolic and n-hexane extract o*f Brassica capitata* is effective against dual and multidrug resistance *H. pylori* stain [37]. Our various studies prompted us to explore the efficacy of *M. coreia*, which is being extensively cultivated in India for the dye which is obtained from the root bark and it is a popular medicinal plant in Thailand. It has been used to

Table 2: Antimicrobial activity of n-hexane and chloroform extract of *M. coreia* against multidrug-resistant *H. pylori* strain

Strain No.	MIC for MTZ, CLR, LEV (agar dilution method)	n-Hexane extract of *M. coreia* (Agar well method)		MIC_{90} for *M. coreia* (Microdilution method)	Chloroform extract of *M. coreia* (Agar well method)		MIC_{90} for *M. coreia* (Microdilution method)
		Amount (in mg)	Zone of inhibition (in mm)		Amount (in mg)	Zone of inhibition (in mm)	
Strain 1	MTZ=64 µg/mL CLR=0.5 µg/mL LEV=1 µg/mL	0.4	7	1.2 µg/mL	2.4	7	2 µg/mL

H. pylori: *Helicobacter pylori*, *M. coreia*: *Morinda coreia*, MIC: Minimal inhibitory concentration

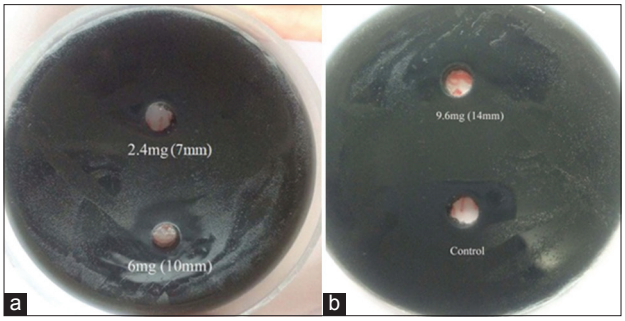

Fig. 6: (a and b) Agar well method: Antimicrobial activity of chloroform extract of *Morinda coreia* against the multidrug-resistant strains of *Helicobacter pylori*

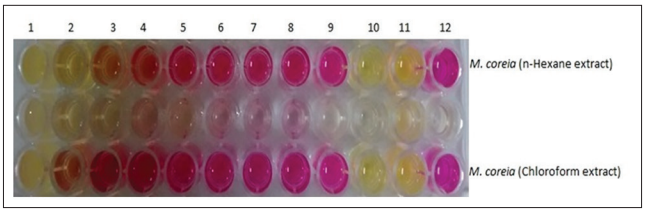

Fig. 7: Microdilution method: Lanes 1–8 show the 5-fold serial dilution of *Morinda coreia* extract from Lanes 1–8 with a constant cell count of *Helicobacter pylori*. Lane 9 shows the cell control without the extract. Lane 10 shows media control without cells. Lane 11 shows extract control without cells. Lane 12 shows the *H. pylori* cell control. Minimal inhibitory concentration 90 was observed at Lane 2 and Lane 1 for n-hexane and chloroform extract of *M. coreia*, respectively

treat menstrual disorders, as a tonic for stomach infection and blood stasis, and has shown antibacterial properties against Gram-negative and Gram-positive bacteria [15,38]. In our study, we found that the n-hexane and chloroform extract of *M. coreia* has shown good efficacy against the multidrug-resistant *H. pylori* stain of North-East India by agar well method and microdilution method.

However, further research needs to be done to determine the compounds that are responsible for antibacterial activity against multidrug-resistant *H. pylori* strain of North-East India.

CONCLUSION

In our present study, we have primarily shown that *M. coreia*, which is a traditional plant used for the treatment of various diseases, have potentially inhibited the growth of multidrug-resistant *H. pylori in vitro* that were isolated from the patient suffering from gastric erosion. It is noteworthy that the strain was resistant for metronidazole at MIC > 64 µg/mL, clarithromycin at MIC >0.5 µg/mL, and levofloxacin >1 µg/mL. Overall, this study provides novel insights in the therapeutic potential of n- hexane and chloroform extract of *M. coreia* against multidrug-resistant *H. pylori* strain, although further studies are required to determine the pathway of its mechanism and its active compounds.

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AUTHORS' CONTRIBUTION

RD has given the concept of antibiogram profiling of *H. pylori* strains. RD and PV have finalized the manuscript. SM has performed the experiments, analyzed the data, and drafted the manuscript. WS and NT have provided the *M. coreia* sample. VG and SH have performed some of the experiments.

CONFLICTS OF INTEREST

The authors declared they have no conflicts of interest.

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