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RESEARCH ARTICLE

An *in-vitro* study on antibacterial potential of few medicinal plants of Assam against Multidrug-Resistant Bacterial Isolates

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Abstract

Since time immemorial, plants have been used as medicine to cure various ailments. Plants contain different bioactive compounds which make them potential alternate as antimicrobial agents. The excessive use or misuse of antibiotics has been developing resistance in bacteria to almost all the available antibiotics and resulting in serious health issues throughout the world. The exploration of plant-based therapeutic strategies could be a great choice to mitigate emerging health issues. The present study aimed to screen the antibacterial properties of five locally available medicinal plants of Assam, *Garcinia pedunculata* Roxb. ex Buch.-Ham, *Garcinia lanceifolia* Roxb., *Garcinia morella* (Gaertn.) Desr., *Capsicum chinense* Jacq. and *Flacourtia jangomas* (Lour.) Raeusch. against multidrug-resistant bacteria. The plant extracts were prepared using solvents- petroleum ether, ethyl acetate, and methanol. The Kirby-Bauer disk diffusion method was performed for testing the antibiotic susceptibility of bacterial isolates. For screening the *in vitro* antibacterial activity of the plant extracts, the agar well diffusion method was carried out along with the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) respectively. In *in vitro* antibacterial screening, the ethyl acetate and methanol extracts of *G. lanceifolia* Roxb. showed the highest zone of inhibition followed by ethyl acetate extracts of *G. pedunculata* Roxb. ex Buch.-Ham. However, among all the selected plants *G. lanceifolia* Roxb. displayed the highest bactericidal effect (at 50 mg/mL concentration) against bacterial pathogens with MIC of 0.39 mg/mL. The result indicated that *G. lanceifolia* Roxb. could be a potent inhibitor of multidrug-resistant bacteria.

Keywords: Antibiotics; Antibiotic Susceptibility; Gram-negative Bacteria; Minimum Inhibitory Concentration; Minimum Bactericidal Concentration; Plant Extract.

1. Introduction

Plants have been known for their medicinal properties since the ancient period. Recently, the WHO (World Health Organization, 2022) estimated that 80 % of people rely on herbal medicines for some aspect of their primary health care, and around 21,000 plant species have the potential for being used as medicinal plants (National Health Portal India, 2019; Prasathkumar et al., 2021). Plants contain different bioactive compounds and secondary metabolites, viz., alkaloids, glycosides, terpenoids, saponin, flavonoids, tannins, quinones, and coumarins (Das et al., 2018) that make them efficient to treat various health problems (Fernebro, 2011). The geographical location, climatic conditions, and diverse topography have made Assam rich in tropical types of vegetation (ENVIS Centre Assam, 2022). The hills and forests of this region are the primary hubs for numerous medicinal plants. *Garcinia* is a commonly found species in the North-Eastern part of India including Assam and belongs to the family Clusiaceae. *Garcinia pedunculata*, *Garcinia lanceifolia*, *Garcinia morella*, and *Garcinia cowa* are the major species reported from the region and used for local consumption as folk medicine (Gogoi and Das, 2016). The ripened fruits of *Garcinia* are used by ethnic people as a remedy for fever, jaundice, urinary infections, dysentery, diabetes, indigestion, and so on (Baruah et al., 2021). Likewise, *Capsicum chinense* is native to Assam and is used to treat various health ailments. The anti-inflammatory and antioxidant properties of *Capsicum chinense* fruit have increased its demand in the field of pharmaceutical and medicinal research (Mena et al., 2018). *Flacourtia jangomas* is endemic to the forests of Assam and the fruit is known for its medicinal and nutritional value. The fruit pulps are traditionally used to treat diarrhea, diabetes, blood purification, fever, and digestion problems mainly (Rai and Mishra, 2020). The other parts of the plants such as roots and leaves are also reported to be used for various diseases in different parts of India.

Due to the arrival of various antibiotics in the market and lack of proper indigenous knowledge, people started to depend on antibiotics rather than folk medicinal plants. Most people use antibiotics without a doctor's prescription. Excessive use and misuse of antibiotics result in bacterial evolution and mutation in their antibiotic resistance genes (Sharma et al., 2016; Singh et al., 2016; Wei et al., 2018), thus they are gaining resistance against almost all the antibiotics available in the markets. The prevalence of antibiotic-resistant bacteria leads to a major hazard to human health as well as the ecosystem and environment (Masoumian and Zandi, 2017). WHO (2020) stated in a report that antibiotics are becoming more ineffective as drug resistance spreads globally which results in difficulties in treating infections and finally causes death. Diseases caused by multiple drug-resistant (MDR) Gram-positive and Gram-negative bacteria have become difficult to treat or sometimes untreatable with the use of conventional drugs (Frieri et al., 2017). Hence, research and development of alternate strategies are urgently needed for treating diseases caused by drug-resistant bacteria.

Numerous studies investigated the antibacterial properties of ethnic medicinal plants from different parts of the world and stated their high possibilities as alternative therapeutic strategies because of the active biomolecules present in them (Mahmoud et al., 2004; Masoumian and Zandi, 2017). This study focused on screening the *in vitro* antimicrobial properties of five locally available plant species of Assam, *Garcinia pedunculata* Roxb. ex Buch.-Ham, *Garcinia lanceifolia* Roxb., *Garcinia morella* (Gaertn.) Desr., *Capsicum chinense* Jacq. and *Flacourtia jangomas* (Lour.) Raeusch. against drug-resistant Gram-negative *Escherichia coli, Klebsiella pneumoniae, Yersinia* sp., *Acinetobacter* sp., and Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*.

Table 1. Results of antibiotic susceptibility test for Gram-positive and Gram-negative bacteria

Antibiotics	*E. coli* (ATCC 25922)	*K. pneumoniae*	*Yersinia* sp.	*Acinetobacter* sp.	*B. subtilis*	*S. aureus*
CB (100 mcg)	S	R	R	S	S	S
PI (100 mcg)	S	R	R	R	S	S
MET (5 mcg)	S	R	R	R	S	R
CX (30 mcg)	S	R	R	R	S	R
CTX (30 mcg)	S	R	I	R	S	S
CPM (30 mcg)	S	R	R	I	S	S
IMP (10 mcg)	S	S	S	S	S	S
VA (30 mcg)	S	R	S	S	S	S
AK (30 mcg)	I	I	R	R	S	S
TE (30 mcg)	S	R	S	R	S	S
TGC (15 mcg)	S	S	S	S	S	S
E (15 mcg)	S	R	I	R	S	S
CIP (5 mcg)	S	R	R	R	S	S
NA (30 mcg)	S	I	R	R	S	R
COT (25 mcg)	S	S	S	R	S	S

('I' indicates intermediate, 'R' indicates resistant, 'S' indicates susceptible as per zone size interpretative chart)

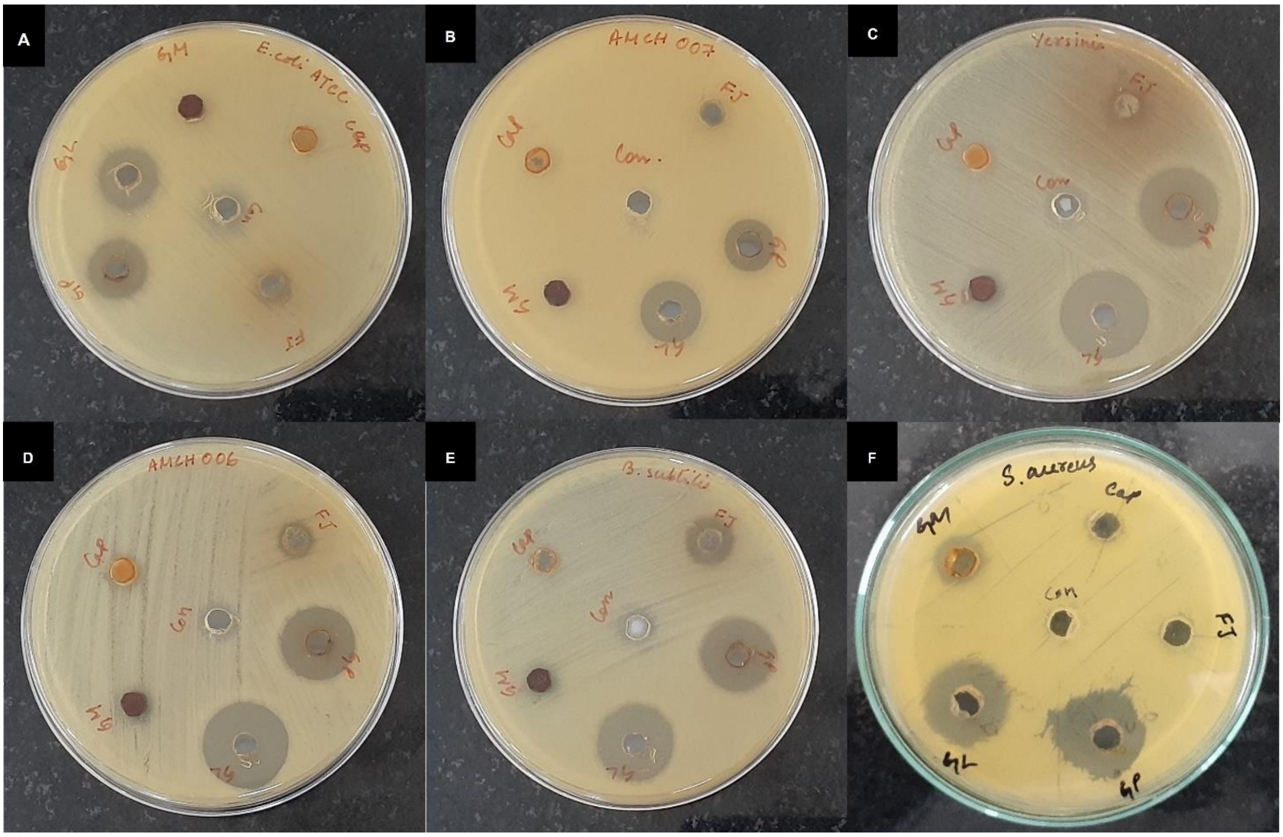

Figure 1. Screening of antibacterial activity of ethyl acetate extract of five plants (50 mg/mL) using Agar well diffusion method against (A) *E. coli* (ATCC-25922); (B) *Klebsiella pneumoniae*; (C) *Yersinia* sp.; (D) *Acinetobacter* sp.; (E) *B. subtilis;* and (F) *S. aureus* respectively

2. Materials and methods

2.1. Preparation of plant extract

The healthy and uninfected plant samples were rinsed with water to remove all the debris, shed dried, and grounded into fine powder by an electric mixer grinder. Approximately, 100 g of plant powder was extracted with 750 mL of solvents- petroleum ether, ethyl acetate, and methanol, having polarity indexes of 0.1, 4.4, and 5.1 respectively. The crude extracts obtained were stored at 4° C until further use.

2.2. Confirmation of bacterial isolates

This study was a laboratory-based, which involved clinical bacterial isolates obtained from the repository of Department of Life Sciences, Dibrugarh University (DBT-NER Twinning project ID-BT/PR16669/NER/95/239/2015 dated 18/10/2016 collection., ethical clearance No. DU/Dib/ECBHR(Human)/2021-22/02). The bacterial isolates were confirmed by Gram staining (Smith and Hussey, 2005) and biochemical tests as per Bergey's Manual of Determinative Bacteriology (Sharma et al., 2021). For Gram staining, the Gram stain kit of HiMedia was taken. All the media used for biochemical tests were from HiMedia and performed in a level II biosafety cabinet.

2.3. Antibiotic Susceptibility Test

Antibiotic Susceptibility Test was performed by Kirby- Bauer disc diffusion method using Muller Hinton Agar (HiMedia) against different antibiotics- Carbenicillin (CB), Piperacillin (PI), Methicillin (MET), Cefoxitin (CX), Cefotaxime (CTX), Cefepime (CPM), Imipenem (IMP), Vancomycin (VA), Amikacin (AK), Tetracycline (TE), Tigecycline (TGC), Erythromycin (E), Ciprofloxacin (CIP), Nalidixic acid (NA), and Co-Trimoxazole (COT). *Escherichia coli* ATCC 25922 was used as a control strain. The zones of inhibition obtained from the test were interpreted as per CLSI guidelines (Sharma et al., 2021).

2.4. Antibacterial activity of plant extracts

The antibacterial activity of plant extracts was screened using the Agar Well Diffusion method (Balouiri et al., 2016) using Muller Hinton Agar. The entire agar surface was inoculated by spreading the microbial inoculum using a sterile swab (HiMedia). The microbial inoculum was incubated overnight in Muller Hinton Broth and the turbidity was matched with 0.5 McFarland standard which provides the final inoculum size of 5×10^5 CFU/mL. The stock solutions for each plant extract were made in a 1.5 mL centrifuge tube by dissolving in DMSO. 50 µL of the plant extract solution was introduced into the well prepared with a sterile cork borer of a diameter of 6 mm. DMSO was considered a negative control. The

Table 2. Diameter of Zone of Inhibition (ZOI) formed by different plant extracts

Plant species	Solvent	Zone of Inhibition in diameter (mm)					
		E. coli (ATCC 25922)	*K. pneumoniae*	*Yersinia* sp.	*Acinetobacter* sp.	*B. subtilis*	*S. aureus*
Garcinia pedunculata Roxb. ex Buch.-Ham	PE	-	-	-	-	-	-
	EA	13.33±0.57	12.33±0.57	16.50±0.50	13.88±0.78	17.77±0.97	-
	M	-	-	12.66±0.86	14.66±0.71	16.88±0.78	-
Garcinia lanceifolia Roxb.	PE	-	-	-	-	-	-
	EA	14.11±0.60	14.11±0.78	16.77±0.68	19.00± 0.86	20.00±0.71	
	M	10.22±0.67	10.44±0.88	12.55±0.73	11.44±0.73	12.22±0.97	-
Garcinia morella (Gaertn.) Desr.	PE	-	-	-	-	-	-
	EA	-	-	-	-	-	-
	M	-	-	13.22±0.67	12.77±0.83	16.44±0.73	-
Capsicum chinense Jacq.	PE	-	-	-	-	-	-
	EA	-	-	-	-	-	-
	M	-	-	-	-	-	-
Flacourtia jangomas (Lour.) Raeusch.	PE	-	-	-	-	-	-
	EA	-	-	-	-	11.77±0.83	-
	M	-	-	-	-	-	-

('PE' indicates petroleum ether extract, 'EA' indicates ethyl acetate extract, 'M' indicates methanol extract, and the negative sign indicates no zone of inhibition. Values are the average of the triplicates (n) ± standard error)

agar plates were incubated under a bacteriological incubator at 37° C for 24 hrs and the Zones of Inhibition (ZOI) were measured using the HiMedia scale. The extracts showing inhibition were tested two more times.

2.5. Determination of Minimal Inhibitory Concentration (MIC)

After screening the *in vitro* antibacterial activity of plant extracts, those which showed maximum inhibitory effects were further tested for determining MIC and MBC. For determining MIC, the micro broth dilution method using a 96-well plate was applied (Mogana et al., 2020). The two-fold serial dilution of plant extracts (50 mg/mL) was performed in 96- well plates using Mueller Hinton Broth (HiMedia), where concentrations ranged from 25 mg/mL-0.09 mg/mL. In the first well 100 µL of the MHB was dispensed, while in wells 2-11, 50 µL of MHB was taken. 100 µL of stock solution was loaded in well 2 and 50 µL was transferred to each subsequent well (up to well 11) to get expected diluted concentrations, followed by dispensing 100 µL of bacterial suspension (5×10^5 CFU/mL) in each well giving a final volume of 200 µL. The first well with no plant extracts was considered a positive control and for the negative control, only DMSO was taken. The 96-well plate was incubated for 24 hrs at 37° C and the bacterial growth was determined by observing turbidity. The MIC value of the extract was the lowest concentration (in mg/mL) with no visible growth of the bacterial culture.

2.6. Determination of Minimal Bactericidal Concentration (MBC)

To determine the Minimal Bactericidal Concentration (MBC), 10 µL of 2-3 test dilution concentrations higher than the MIC value was spread on Nutrient Agar plates and incubated for 24 hrs at 37° C (Ozturk and Ercisli, 2006). The concentration where no bacterial colony growth was observed from the directly plated culture of the wells was considered as the MBC value (Elshikh et al., 2016). After calculating MBC/MIC value (Mogana et al., 2020), if the ratio ≤4, then the effect was considered bactericidal while the ratio >4, was considered bacteriostatic. Each test was performed at least three times.

3. Results

3.1. Confirmation of Bacterial Isolates

From the results obtained from the Gram staining and biochemical tests, three bacterial strains were confirmed as Gram-negative *Klebsiella pneumoniae*, *Yersinia* sp., and *Acinetobacter* sp. whereas two were confirmed as Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* respectively as per Bergey's Manual of Determinative Bacteriology.

3.2. Antibiotic Susceptibility Test

Antibiotic susceptibility tests for antibiotics showed different effects against tested bacterial strains. As per the interpretation of CLSI guidelines, *Klebsiella pneumoniae* and *Acinetobacter* sp. showed resistance against 15 different antibiotics, which was found to be the highest among all the test isolates. On the other hand, Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* were found to be susceptible to almost all the antibiotics used in the test. The interpreting results obtained from measuring the diameter of the zone of inhibition were enlisted in Table 1.

3.3. Antibacterial activity of plant extracts

Among the five selected plant species, all three species of *Garcinia* showed better antibacterial activity against pathogenic bacterial isolates compared to the other species. The activity was varied with the solvent extract. The ethyl acetate extract of *Garcinia lanceifolia* Roxb. was found to be exhibited a better inhibitory effect range from 14-20 mm in diameter than *Garcinia pedunculata* Roxb. ex Buch. Ham. at 50 mg/mL (Figure 1). A similar result was also found for methanolic extract. Methanolic extracts of *Garcinia lanceifolia* Roxb. inhibited the growth of four bacterial isolates, as compared to *Garcinia pedunculata* Roxb. ex Buch. Ham. and *Garcinia morella* (Gaertn.) Desr. *Flacourtia jangomas* (Lour.) Raeusch. showed activity in only one solvent extract and *Capsicum chinense* Jacq. showed no activity in any of the solvents. Different zones of inhibition formed by different plant extracts were listed in Table 2.

3.4. Determination of Minimal Inhibitory Concentration (MIC)

The MIC and MBC values were determined for ethyl acetate extracts of *Garcinia pedunculata* Roxb. ex Buch.-Ham and *Garcinia lanceifolia* Roxb. as they showed the highest inhibition against drug-resistant bacteria in the Agar Well Diffusion test. In the result, it was found that both extracts could inhibit the growth of all the tested bacteria in different concentrations (Table 3). Among the Gram-negative bacteria, *Yersinia* sp. was found to be the most susceptible to *Garcinia lanceifolia* Roxb. with the MIC 0.39 mg/mL. For other Gram-negative bacteria, *E. coli* (ATCC 25922), *Klebsiella pneumoniae* and *Acinetobacter* sp. the MIC values were found to be 3.12 mg/mL and 6.25 mg/mL respectively. Whereas the Gram-positive bacteria *B. subtilis* and *S. aureus* were found to be inhibited at 0.78 mg/mL using both plant extracts. For *Garcinia pedunculata* Roxb. ex Buch.-Ham., Gram-negative *E. coli* (ATCC 25922), *Klebsiella pneumoniae*, and *Yersinia* sp. were found to be inhibited at 6.25 mg/mL followed by *Acinetobacter* sp. at 12.5 mg/mL.

3.5. Determination of Minimal Bactericidal Concentration (MBC)

The MBC values were determined on agar plates after incubation and no growth of bacterial colonies in particular concentration of plant extracts confirmed it as bactericidal. *Garcinia pedunculata* Roxb. ex Buch.-Ham. displayed the highest bactericidal activity of MBC 1.56 mg/mL against *B. subtilis* compared to the rest of the isolates. While *Garcinia lanceifolia* Roxb. showed a promising bactericidal effect against *Yersinia* sp. with an MBC value of 0.39 mg/mL. The obtained MBC/MIC value (Table 3) confirmed the antagonistic effect of ethyl acetate extracts of both plants for multidrug resistance Gram-positive and Gram-negative bacteria in different concentrations.

4. Discussion

Increasing resistance to multiple drugs and most of the available antibiotics turns bacteria into an emerging health concern worldwide. In a recent report, CDC stated that about 2 million people of the United States are severely infected with one or more antibiotic-resistant bacteria each year, for which $20 billion have

Table 3. MIC (in mg/mL), MBC (in mg/mL) and MBC/MIC ratio of *Garcinia pedunculata* Roxb. ex Buch. -Ham. and *Garcinia lanceifolia* Roxb.

Bacteria	*G. pedunculata* Roxb. ex Buch. -Ham.			*G. lanceifolia* Roxb.		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
E. coli (ATCC 25922)	6.25	6.25	2	3.12	12.5	2
K. pneumoniae	6.25	6.25	1	6.25	12.5	2
Yersinia sp.	6.25	12.5	2	0.39	0.78	2
Acinetobacter sp.	12.5	12.5	1	6.25	12.5	2
B. subtilis	0.78	1.56	2	0.78	1.56	2
S. aureus	0.78	3.12	4	0.78	1.56	2

to be estimated for spending on direct health care (Kebede et al., 2021). Being the largest consumer of antibiotics, India have been battling with continuous increase of antimicrobial resistance since last two decades. More than 50 % of the research article indexed in PubMed are concerned with antimicrobial resistance in India. The infection rate of MDR Gram-negative bacteria such as, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus* spp. etc. are as high as 57 % with mortality rates up to 32 % - 50 % in Indian health care system reveals the burden of antibiotic resistance in the country (Gandra et al., 2019). To combat this effect on the socio-economic and health sector, the search for potent antimicrobial products from natural products could be a complementary and promising option to overcome untreatable infections caused by multi-drug resistant bacterial strains (Bakal et al., 2017; Kebede et al., 2021).

Plants have been proven as the renowned natural remedies for most health problems since ancient times. The plant-derived pure compounds and the plant extracts have an almost similar effect and hence in many cases, it has been substituted for medicines (Houghton, 2001). During 2006-2020, 12 countries from Asia, Africa, Europe, North America, and South America reported a total of 100 plants showing potent antimicrobial activity against ESKAPE (*Enterococcus* spp., *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) pathogens. Among these 12 countries, India reported the highest number i.e., 35 plants with significant antimicrobial activity which means our country has enormous scope for utilizing locally available plants to combat drug-resistant pathogens (Subramani et al., 2017).

In the current study, we investigated the antibacterial activity of the five medicinally important plants of Assam, against drug-resistant bacterial isolates. The study revealed that among those five plants, only two plant species i.e., *Garcinia lanceifolia* Roxb. and *Garcinia pedunculata* Roxb. ex Buch.-Ham showed prominent inhibitory action against selected drug-resistant bacterial isolates. Another report revealed that the hexane and chloroform extracts of *Garcinia cowa* and *G. pedunculata* showed antibacterial activity against pathogenic bacteria- *Bacillus cereus*, *B. coagulans*, *B. subtilis*, *S. aureus,* and *E. coli* (Negi et al., 2008). The extracts were prepared from the bark as well as fruit and the inhibition concentration was found to be 15-500 µg/mL and 30-1250 µg/mL for *G. cowa* and *G. pedunculata* respectively (Naves et al., 2019). The fruit extract of *Garcinia lanceifolia* Roxb. showed higher effectiveness against Gram-positive bacteria than Gram-negative bacteria in a study (Chowdhury and Handique, 2017) which corroborates our findings also. In previous studies, the antimicrobial activity of different species of *Garcinia* was screened using various solvent extracts. In another study, only aqueous and ethanolic solvents of *Garcinia cola* were analyzed for their antibacterial property, and resulted in the ethanolic extracts showed some satisfactory results against *Staphylococcus aureus* and *Klebsiella pneumoniae* at 0.5 mg/mL and 0.25 mg/mL respectively (Duro et al., 2015).

The methanolic leaf extracts of nine different *Garcinia* species of the Western Ghat region were studied for antibacterial activity and it was found that *G. morella* showed antibacterial activity against *Bacillus cereus* (MTCC430) and *Staphylococcus aureus* (MTCC7433) (Aravind et al., 2016; Murthy et al., 2020). Antimicrobial silver nanoparticles were synthesized using fruit extract of *G. morella* (Gaertn) Desr and resulted that *G. morella* (Gaertn) Desr mediated nanoparticles exhibit antibacterial effects against *Bacillus subtilis* (MTCC121) and *Pseudomonas aeruginosa* (MTCC4673) (Borah and Yadav, 2015). The studies support the current findings where *G. morella* (Gaertn) Desr. showed inhibitory activity against *Bacillus subtilis* along with Gram-negative antibiotic-resistant *Yersinia* sp. and *Acinetobacter* sp.
In this study, only the ethyl acetate solvent of *Flacourtia jangomas* showed some antibacterial activity (12 mm in diameter) against Gram-positive *Bacillus subtilis* whereas a study conducted on the inhibitory potential of three different extracts- methanol, ethyl acetate, and N-butanol revealed that all the extract possessed better antibacterial activity against Gram-positive bacteria in compared to Gram-negative bacteria (Merr and Raghavendra, 2020). Numerous studies were carried out with *Capsicum chinense* and found its antibacterial activity against *Staphylococcus aureus* and *E. coli* in acetonitrile extract (Das et al., 2018), *Klebsiella pneumoniae,* and *Staphylococcus aureus* in acetone extract and acetonitrile extract respectively (Gayathri et al., 2016). Interestingly, *Capsicum chinense* did not exhibit an inhibitory effect against any of the bacterial isolates in the current study.

Regarding antibiotic-resistant pathogenic bacteria in humans, a lot of work has been done using different plant extracts, but most of the previous reports focused on *E. coli* as well as *K. pneumoniae*. This paper reports the first published work on *in vitro* antimicrobial activity of five traditionally important medicinal plants of Assam against Gram-negative MDR *Yersinia* sp. and *Acinetobacter* sp. along with *E. coli*, *Klebsiella* sp., *B. subtilis*, and *S. aureus*. However, this study mainly focused on *in vitro* antimicrobial activity, further investigation is required to establish the potentiality of those plant extracts as antimicrobial drugs for treating infections caused by pathogenic bacterial strains.

Based on our study, it was found that ethyl acetate extracts of *Garcinia pedunculata* Roxb. ex Buch.-Ham and *Garcinia lanceifolia* Roxb. exhibit potent antibacterial activity against both Gram-negative and Gram-positive bacteria. *Garcinia pedunculata* Roxb. ex Buch.-Ham showed the highest inhibitory activity against Gram-negative *Yersinia* sp. at MIC 0.39 mg/mL while *Garcinia lanceifolia* Roxb. was found to be a potent antibacterial extract against Gram-positive *B. subtilis* and *S. aureus* at MIC 0.78 mg/mL. This also validates the traditional medicinal practices of the region for the use of these plants against different bacterial infections. The fruit extract of *Garcinia pedunculata* Roxb. ex Buch.-Ham could be an alternative therapeutic solution for the future development of antibacterial compounds to treat MDR *Yersinia* sp. causing infections. This study will also provide a better scope for understanding the pharmacological importance of commonly available medicinal plants and help to emphasize doing more clinical experimental trials on them.

5. Conclusion

In the present study, it was observed that extracts of *Garcinia lanceifolia* Roxb. exhibit the highest antibacterial activity against multidrug-resistant bacterial isolates and could be used as potent inhibitors against bacterial infections. This can lead to the formulation of a new plant-based drug to treat health problems caused by bacteria. The identification of bioactive compounds presents in the plant and the *in-silico* studies might provide great scope in the discovery of alternate therapeutic strategies to fight against the most harmful drug-resistant bacterial strains.

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Author's contribution

IG: Conceptualization, methodology, data curation, investigation, writing original draft. SS: Methodology, investigation, data curation. MP: Methodology, writing-review & editing. PC: Conceptualization, supervision, writing-review & editing.

Conflict of interest

The author declares no competing interests that are relevant to the content of this article.

References

Aravind APA, Nandu TG, Shiburaj S and Rameshkumar KB. 2016. Antioxidant and antibacterial activities of *Garcinia* species in the Western Ghats. Diversity of *Garcinia* Species in the Western Ghats : Pythochemical Perspective. Jawaharlal Nehru Tropical Botanic Garden and Research Institute Publishing. Pp: 179–186

Gogoi B, Das RP, Baruah U and Boruah R. 2016. Ethno-botanical Survey of *Garcinia* Species of Assam. International Journal of Bio-resources and Stress Management 7(4): 752–755.

Bakal SN, Bereswill S and Heimesaat MM. 2017. Finding novel antibiotic substances from medicinal plants — Antimicrobial properties of *Nigella sativa* directed against multidrug resistant bacteria. European Journal Microbiology and Immunology 7(1):92–98.

Balouiri M, Sadiki M and Ibnsouda SK. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 6(2):71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Baruah S, Barman P, Basumatary S and Bhuyan B. 2021. Diversity and ethnobotany of genus *Garcinia* l. (Clusiaceae) in Assam, eastern himalaya. Ethnobotany Research and Applications 21:1-14.

Borah D and Yadav A. 2015 A Novel 'Green' Synthesis of Antimicrobial Silver Nanoparticles (AgNPs) by using *Garcinia morella* (Gaertn) Desr. Fruit Extract. Nanoscience and Nanotechnology 5(3):25-31.

Chowdhury T and Handique P. 2017. Evaluation of antibacterial activity and phytochemical activity of *Garcinia lancifolia* roxb. International Journal of Pharmaceutical Science Research 3(6):1663-1667.

Das J, Deka M and Gogoi K. 2018. Antimicrobial Activity of Chilli Extracts (*Capsicum chinense*) Against Food Borne Pathogens *Escherichia coli* and *Staphylococcus aureus*. International Journal Research and Analytical Reviews 5(04):717–720.

Duro D, Hafsat B, Emmanuel OO, Yahaya TA and Adeola SO. 2015. Investigating the toxicity and antimicrobial activity of *Garcinia kola* extract. World Journal of Pharmaceutical Research 4(4):57–67.

ENVIS centre, Assam. 2022. https://astec.assam.gov.in/frontimpotentdata/envis-centre.

Elshikh M, Ahmed S, Mcgaw M, Marchant R, Funston S, Dunlop P. and Banat IM. 2016. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letter 38(6):1015–1019.

Fernebro J. 2011. Fighting bacterial infections - Future treatment options. Drug Resistance Updates 14(2):125–139.

Frieri M, Kumar K and Boutin A. 2017. Antibiotic resistance. Journal of Infection and Public Health 10(4):369–378.

Gandra S, Tseng KK, Arora A, Bhowmik B, Robinson ML, Panigrahi B, Laxminarayan R and Klein EY. 2019. The Mortality Burden of Multidrug-resistant Pathogens in India: A Retrospective, Observational Study. Clinical Infectious Diseases 69(4):563–570.

Gayathri N, Gopalakrishnan M and Sekar T. 2016. Phytochemical screening and antimicrobial activity of *Capsicum chinense* Jacq. International Journal of Advances in Pharmacy and Biotechnology 5(1):12-20.

Houghton PJ. 2001. Old yet new—pharmaceuticals from plants. Journal of Chemical Education 78(2):175.

Kebede T, Gadisa E and Tufa A. 2021. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLoS ONE 16:1–16. https://doi.org/10.1371/journal.pone.0249253

Mahmoud YAG, Ebrahim MKH and Aly MM. 2004. Influence of plant extracts and microbioagents on physiological traits of faba bean infected with *Botrytis fabae*. Journal of Plant Biology 47(3):194–202.

Masoumian M and Zandi M. 2017. Antimicrobial Activity of Some Medicinal Plant Extracts against Multidrug Resistant Bacteria. Zahedan Journal of Research In Medical Sciences 19(11): e10080. https://doi.org/10.5812/zjrms.10080

Mena E, Warade SD and Ansari MT. 2018. Evaluation of capsaicin , Ascorbic acid , α - Carotene and ß-Carotene in Bhut Jolokia (*Capsicum chinense* Jacq .) genotypes from North East India. The Pharma Innovation Journal 7(7):93–97.

Merr B and Raghavendra HL. 2020. Radical Scavenging, Cytotoxic and Antimicrobial Activity *Flacourtia indica*. Medical Health Sciences Research Journal 1(1): 76-82.

Mogana R, Adhikari A, Tzar MN, Ramliza R and Wiart C. 2020. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* miq. against bacterial clinical isolates. BMC Complementary Medicine and Therapies 20(1):1–11.

Murthy HN, Dalawai D, Dewir YH and Ibrahim A. 2020. Phytochemicals and biological activities of *Garcinia morella* (Gaertn.) desr.: A review. Molecules 25(23):1–15.

National Health Portal., India. 2019. https://www.india.gov.in

Naves VML, dos Santos MH, Ribeiro IS, da Silva CA, Silva NC, da Silva MA, da Silva G A, Dias ALT, Ionta M and Dias DF. 2019. Antimicrobial and antioxidant activity of Garcinia brasiliensis extracts. South African Journal of Botany 124:244–250.

Negi PS, Jayaprakasha GK and Jena BS. 2008. Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria. LWT - Food Science and Technology 41(10):1857–1861.

Ozturk S and Ercisli S. 2006. Chemical composition and in vitro antibacterial activity of *Seseli libanotis*. World Journal of Microbiology and Biotechnology 22:261–265.

Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. 2021. Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. Phytomedicine Plus 1(2):100029.

Rai A and Mishra T. 2020. Ethnomedicinal and therapeutic values of *Flacourtia jangomas*. The Journal of Indian Botanical Society 100(3&4):169–176.

Sharma M, Chetia P, Puzari M, Neog N, Phukan U and Borah A. 2021. Carbapenem resistance among common Enterobacteriaceae clinical isolates in part of North-East India. Anti-Infective Agents 19(4):72-78.

Sharma VK, Johnson N, Cizmas L, McDonald TJ and Kim H. 2016. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150:702–714.

Singh N, Sit MT, Chung DM, Lopez AA, Weerackoon R and Yeh PJ. 2016. How often are antibiotic-resistant bacteria said to "evolve" in the news? PLoS ONE 11(3):1–12. https://doi.org/10.1371/journal.pone.0150396

Smith AC and Hussey MA. 2005. Gram stain protocols. American Society for Microbiology 1(14):113-144.

Subramani R, Narayanasamy M and Feussner KD. 2017. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. 3 Biotechnology 7(3), 1–15. https://doi.org/10.1007/s13205-017-0848-9

Wei J, Wenjie Y, Ping L, Na W, Haixia R and Xuequn Z. 2018. Antibiotic resistance of *Klebsiella pneumoniae* through β-arrestin recruitment-induced β-lactamase signaling pathway. Experimental Therapeutic Medicine 15(3):2247–2254. https://doi.org/10.3892/etm.2018.5728

WHO. 2022. World Health Organization, Who global centre for traditional medicine. https://www.who.int.