Indian Journal of Geo Marine Sciences
Vol. 52 (08), August 2023, pp. 381-389
DOI: 10.56042/ijms.v52i08.11021

Bactericidal properties of mangrove *Bruguiera cylindrica* (L.) Blume leaf and *Rhizophora mucronata* Poir. stilt root extracts on *Vibrio cholera,* MTCC 435 and *Escherichia coli* pathogens

M Gnanadesigan*[,a], A Dhass[b], V Nandagopalan[c], V Rajagopalan[d] & V Saravanan[e]

[a]Natural Products Research Laboratory, Department of Microbial Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu – 641 046, India

[b]Department of Microbiology, National College (Autonomous), Tiruchirapalli, Tamil Nadu – 620 001, India

[c]Department of Botany, National College (Autonomous), Tiruchirapalli, Tamil Nadu – 620 001, India

[d]PG and Research Department of Biotechnology, National College (Autonomous), Tiruchirapalli, Tamil Nadu – 620 001, India

[e]Department of Zoology, The M.D.T. Hindu College Pettai, Tirunelveli, Tamil Nadu – 627 010, India

*[E-mail: contactdrgd@buc.edu.in]

Received 23 October 2022; revised 29 July 2023

This research aimed to determine the bactericidal and cytotoxic properties of two mangrove plants commonly used in traditional medicine: *Bruguiera cylindrica* (leaf) and *Rhizophora mucronata* (stilt root). The selected plant parts were subjected to solvent polarity-based extraction. Of the solvent extracts, the acetone, ethyl acetate leaf extract of *B. cylindrica* and acetone stilt root extract of *R. mucronata* were chosen for further study as they exhibited minimum inhibitory and bactericidal concentrations between 0.5 and 1.0 $mg.ml^{-1}$ against MTCC 435, *Vibrio cholerae* and *Escherichia coli.* The well and disk diffusion analysis of these mangrove extracts showed inhibition zone between 6 – 16 mm diameter for the bacterial pathogens. Similarly, the mangrove extracts displayed > 98 % reduction of viable counts for MTCC 435, *E. coli* and *V. cholerae* at different time intervals between 8 – 16 h. Electron microscope analyses of the bacterial cells treated with mangrove extracts confirmed the bactericidal properties by cellular aggregation and leakage. Further, the cytotoxicity evaluation of mangrove extracts with Henrietta Lacks cell lines did not show any viability interference up to 200 $\mu g.ml^{-1}$, indicating their non-toxicity to human cells and biocompatibility for drug development. The research findings suggest that the acetone, ethyl acetate leaf extract of *B. cylindrica* and the acetone stilt root extract of *R. mucronata* could be used as a bactericidal resource against *V. cholerae*, MTCC 435 and *E. coli*.

[**Keywords**: *Bruguiera cylindrica*, Cytotoxicity, MBC, MIC, *Rhizophora mucronata*, SEM]

Introduction

According to the WHO, about 25 % of the currently available drugs used in the US market are herbal-derived medicines. In India, the Ministry of Health and Family Welfare has established the AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy) about 65 % of the Indians make use of traditional medicine for their health care needs[1]. The development of herbal medicine has gained top priority in recent years due to the emergence of new infectious diseases and the development of antibiotic resistance. Likewise, there is a continuing demand for anti-infective and restorative medicines. Studies on terrestrial plants and associated microbes revealed a wide variety of beneficial properties including antibiotic, antitumor, neurotrophic and immune-modulatory properties[2]. In order to investigate the possibility of developing drugs for the treatment of human ailments, studies relating to the bioactive potential of marine bioresources have been given top priority[3,4]. However, not much research has been done onto marine plants possible efficacy in treating infectious diseases.

Traditionally, many of the mangrove species such as *Avicennia* sp., *Bruguiera* sp., *Carapa* sp., *Ceriops* sp., *Derris* sp., *Excoecaria* sp., *Lumnitzera* sp., *Rhizophora* sp., etc., are used as a folk remedy for human and animal ailments[3]. Similarly, seaweeds and seagrasses have also been investigated for their potential medicinal uses. However, there is a severe lack of data concerning the traditional utilisation of mangroves[5-9]. In this context, in the current study two different mangrove plants, *viz*. *Bruguiera cylindrica* (leaf) and *Rhizophora mucronata* (stilt root) were selected to examine their bactericidal and cytotoxic properties. For long years, the leaf and bark of

Bruguiera cylindrica is used as a folk remedy for hepatitis[3]. Earlier, the plant has also been reported to have antioxidant, antibacterial and antiviral properties[10]. Likewise, *Rhizophora mucronata* is also reported as a folk remedy for hepatitis, febrifuge and ulcer. Scientifically, it has been proven to have brine shrimp lethal activity, analgesic activity, anti-diarrhoeal, hepatoprotective, antioxidant and diuretic activities[11-14]. The current study is undertaken as no/limited previous reports have been found on the antibacterial and cytotoxicity activity of *Bruguiera cylindrica* (leaf) and *Rhizophora mucronata* (stilt root) plants with polarity-based extraction method.

Materials and Methods

Sample collection and extraction

Samples of *Bruguiera cylindrica* leaf and *Rhizophora mucronata* stilt roots were collected from the mangrove forest. After collecting fresh mangrove plant parts, the samples were washed three times in distilled water to get rid of any persisting soil particles or salts and microbes. About 500 g of each sample was subjected to a coarse powder for size reduction. Later, the polarity-based extraction was carried out using the solvents petroleum ether, dichloromethane, acetone, ethyl acetate, ethanol and methanol. The collected extracts were dried at an open (room temperature) temperature to get the residues. The residues obtained were further stored in polypropylene tubes and were kept in the refrigerator for further use.

Collection of bacterial pathogens

The human clinical pathogens such as *Staphylococcus aureus*, *Vibrio cholerae*, *V. alginolyticus*, *E. coli* and *P. aeruginosa* were procured from the Department of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai. Additionally, MTCC 3384 (*K. pneumoniae*), MTCC 97 (*S. marcescens*) and MTCC 435 (*S. epidermidis*) were procured from IMTECH, Chandigarh.

Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) was obtained with the filter-sterilized extracts. 0.5 ml of various concentrations (125, 250, 500, 1000, 1500, 2000 and 4000 $\mu g.mL^{-1}$) of the extracts were mixed with 0.5 ml sterile Mueller Hinton broth. Then, 50 µl of 12 h bacterial cultures (10^5 $cells.ml^{-1}$ obtained by adjusting to 0.1 OD at 600 nm with sterile nutrient broth) were added individually and incubated at 37 °C for 24 h Further, the MIC was calculated by visualizing turbidity in each concentration[15]. Sub-culturing the MIC dilutions onto sterile agar plates resulted in the Minimum Bactericidal Concentration (MBC). The minimal concentration of mangrove extracts shown to inhibit bacterial growth is tabulated.

Disk and well diffusion assay (Kirby Bauer method)

The antibacterial activity was performed by disk diffusion and well diffusion assays using standard protocols[16]. The assay was performed only with the extract, which showed inhibitory activity > = 2000 $\mu g.ml^{-1}$ concentration in MBC results. 0.1 ml of 12 h incubated clinical pathogens (10^5 $cells.ml^{-1}$ cultures adjusted to 0.1 OD at 600 nm with sterile nutrient broth) was overlaid on Muller Hinton agar medium. 1/2X, X and 2X concentrations of MBC values were used for the assays. Tetracycline (40 $\mu g.disk^{-1}$) was used as a positive control. The plates were incubated at 37±1 °C for 24 h. After incubation, the inhibition zone around the disk was calculated.

Determination of growth pattern

Twelve hour old cultures (10^5 $cells.ml^{-1}$) of pathogens were inoculated with 50 ml of sterile Mueller Hinton broth containing X and 2X strength of the mangrove extracts individually. Similarly, Tetracycline (40 $\mu g.ml^{-1}$) was also used as a positive control. Similar conditions were kept without the extracts serving as a control. All the above set ups were incubated at 37±1 °C for 24 h. Optical Density (OD) was measured at 600 nm for every 2 h for 24 h with respective blanks. The graph was plotted against time and OD values.

Determination of viable counts

Sample preparation and control used for the determination of viable counts is similar to the procedure followed for the determination of growth patterns. Post-incubation, 0.1 ml of the culture was withdrawn at different time intervals (2, 4, 8, 16 and 24 h, respectively) and serially diluted up to 10^{-3} dilutions and plated onto the nutrient agar plate. Emergents were counted after 24 h incubation.

The percentage reduction of the CFU (Colony Forming Unit) values was counted as follows:

(Total no. of CFU in control – Total no. of CFU in treatments)/ Total no. of CFU in control) × 100

The values showing > 99.5 % are considered as 100 % significant[17].

Scanning Electron Microscopy (SEM)

0.5 ml of 2X concentration of the mangrove extracts were mixed with 50 µl of 12 h old bacterial cultures (10^5 cells.ml^{-1}) with 0.5 ml sterile Mueller Hinton broth individually and incubated at 37 °C for 12 h. Cells without extracts were used as control. After incubation, the bacterial cells were centrifuged at 6000 rpm twice and washed with 0.01 M potassium phosphate buffer. The collected bacterial pellets were fixed with 2 % glutaraldehyde for 2 h at 4 °C. After that, gradient dehydration was carried out with the ethanol solution (10 – 100 %). Further, the slides were dried with desiccators and subjected to SEM (JEOL JSM–5610 series, Japan) observation.

Cytotoxicity analysis

The HeLa cell lines were procured from the NCCS Pune, India. Monolayer cultures of cells were established in RPMI 1640 medium (37 °C, 5 % of CO_2 atmosphere). Cell lines (100 µl) were seeded in 96 well plates at a concentration of 5×10^3 cells.ml^{-1} for 24 h. Afterwards, the culture medium was replaced with 100 µl serum-free medium containing various concentrations (12.5, 25, 50, 100 and 200 µg.ml^{-1}) of filter-sterilized acetone and ethyl acetate leaf extract of *B. cylindrica* and *R. mucronata* acetone stilt root extracts. Later, the medium was refreshed with 100 µl of serum-free medium (RPMI 1640) and 20 µl of MTT (5 µg.ml^{-1} of 3, 4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazo liumbromide. The microtitre plates were measured with an ELISA reader at 570 nm. The percentage of cell viability was calculated as follows:

$$\% \text{ Cell viability} = [A] \text{ Test} / [A] \text{ control} \times 100$$

Triplicates were maintained for each treatment. Inhibitory Concentration (IC_{50}) values were directly determined by linear regression analysis with Office XP (SDAS) software[13].

Results

The MIC and MBC results are depicted in Tables 1 and 2. Of the two plant extracts, *R. mucronata* acetone stilt root extract showed a minimum (0.5 mg.ml^{-1}) range of MIC values with MTCC 435, *V. cholerae* and *E. coli*. Similarly, *B. cylindrica* ethyl acetate leaf extract showed a minimum (0.5 mg.ml^{-1}) range of MIC values with MTCC 435. The observations of the disk and well diffusion assays are tabulated in Table 3. The maximum zone of inhibition

Table 1 — Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of *R. mucronata* stilt root extracts against clinical pathogens

Pathogens	*R. mucronata* stilt root extract (mg.ml^{-1})					
	Petroleum ether	Dichloromethane	Acetone	Ethyl acetate	Ethanol	Methanol
MTCC 435	-	-	0.5 (1)	-	-	-
MTCC 97	-	-	-	-	4 (> 4)	-
MTCC 3384	-	4 (> 4)	-	-	-	-
S. aureus	-	-	-	-	-	-
Vibrio cholerae	-	-	0.5 (1)	-	-	-
V. alginolyticus	-	-	-	-	-	-
E. coli	-	-	0.5 (1)	-	-	4 (> 4)
P. aeruginosa	-	-	-	-	-	-

Values in the parenthesis are MBC values

Table 2 — Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the *B. cylindrica* leaf extract against clinical pathogens

Pathogens	*B. cylindrica* leaf extract (mg.ml^{-1})					
	Petroleum ether	Dichloromethane	Acetone	Ethyl acetate	Ethanol	Methanol
MTCC 435	-	-	1 (2)	-	-	-
MTCC 97	-	-	-	-	-	4 (> 4)
MTCC 3384	-	-	-	-	-	-
S. aureus	-	-	-	-	-	-
Vibrio cholerae	-	-	-	-	-	-
V. alginolyticus	-	-	-	-	-	-
E. coli	-	-	-	0.5 (1)	-	-
P. aeruginosa	-	-	-	-	-	-

Values in the parenthesis are MBC values

was observed in *R. mucronata* acetone-stilt root extract against *E. coli* (16 mm) with the well diffusion method. The growth pattern analysis showed enhanced OD (Optical Density) values with increased time intervals. But mangrove extracts (X MBC and 2X MBC) and tetracycline-treated bacterial pathogens revealed reduced OD values as compared to the control bacterial pathogens (Fig. 1).

All the mangrove-extract treated cultures (X and 2X MBC concentrations) showed decreased viable

Table 3 — Disk diffusion and well diffusion assay of the *R. mucronata* stilt root and *B. cylindrica* leaf extracts against clinical pathogens

Pathogens	Disk diffusion/Well diffusion methods (mm)								
	R. mucronata acetone- stilt root extract			*B. cylindrica* acetone- leaf extract			*B. cylindrica* ethyl acetate leaf extract		
	½ X	X	2X	½ X	X	2X	½ X	X	2X
MTCC 435	7/7.5	7/9	14/16	7/8	9/9	11/12	ND	ND	ND
E. coli	6/7	6/9	12/13	ND	ND	ND	6/7	7/9	13/15
Vibrio cholerae	7/8	6.8/8	13/14	ND	ND	ND	ND	ND	ND

ND: Not determined; ½ X, X and 2X are the concentration of MBC values

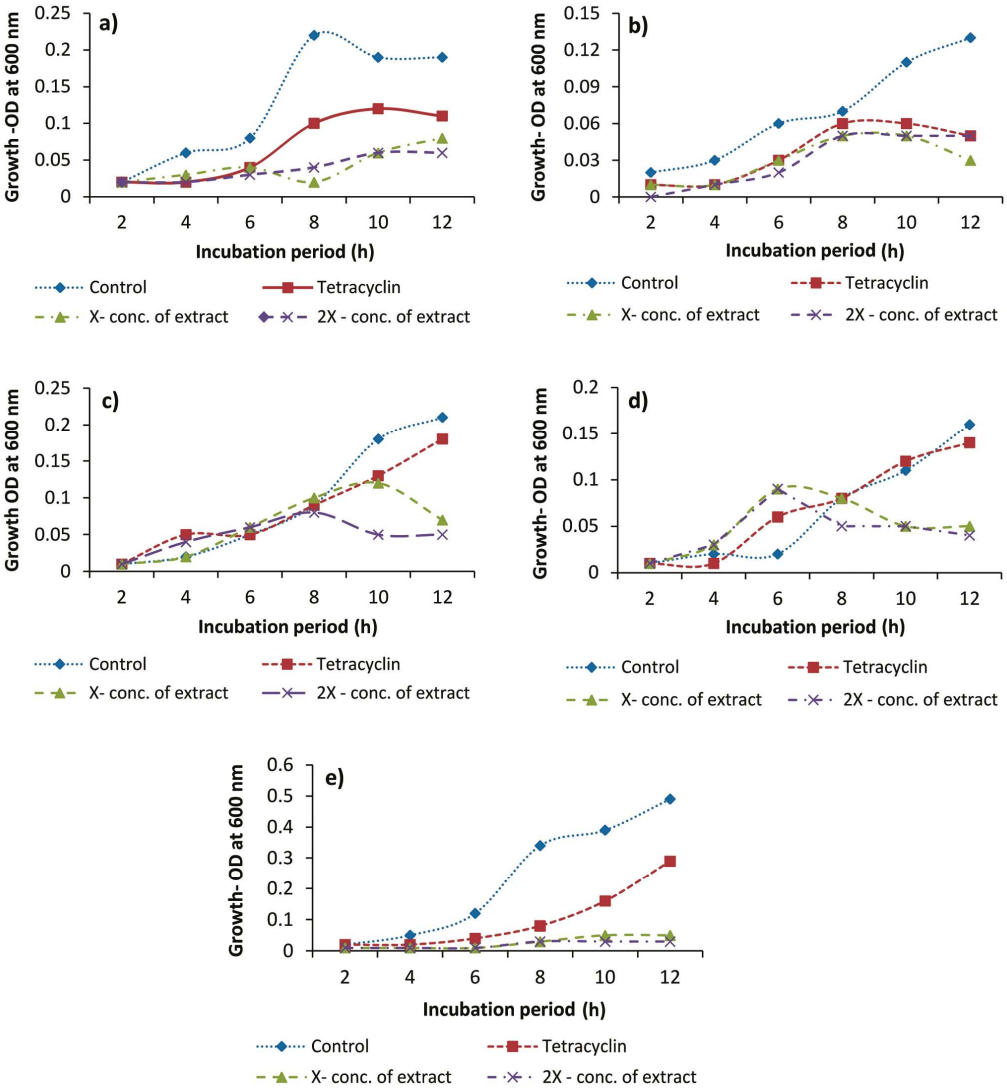

Fig. 1 — Effect of various extracts on growth pattern of clinical pathogens: a) *R. mucronata* acetone stilt root extract with *MTCC 435*; b) *B. cylindrica* acetone leaf extract with *MTCC 435*; c) *R. mucronata* acetone stilt root extract with *E. coli*; d) *B. cylindrica* acetone leaf extract with *E. coli*; and e) *B. cylindrica* ethyl acetate leaf extract with *V. cholera*

Table 4 — Effect of various extracts on viable counts of pathogens (10^3 CFU.ml^{-1})

	Incubation (h)	Control	X- MBC	2X- MBC	Tetracycline (40 µg.ml^{-1})
R. mucronata acetone stilt root extract with *MTCC 435*	4	156±1.5	16±1.0 (86.9)	0 (100)	6±3 (96.1)
	8	696±5.0	34±2.0 (95.1)	7±2.64 (98.9)	27±8 (96.1)
	16	> 60000	121±9.0 (100)	25±6.0 (100)	145±34.1 (100)
	24	> 60000	145±11.9 (100)	36±6.4 (100)	228±40.2 (100)
B. cylindrica acetone leaf extract with *MTCC 435*	4	317±20.0	73±19.0 (76.9)	17±11.0 (99.4)	5±2.6 (98.4)
	8	734±60.4	99±27.5 (86.5)	29±17.3 (96.0)	15±11.5 (97.9)
	16	> 70,000	397±70.7 (99.4)	61±18.3 (100)	126±20.3 (100)
	24	> 70,000	408±48.1 (99.4)	114±20.5 (100)	183±20.5 (100)
R. mucronata acetone stilt root extract with *E. coli*	4	327±23.9	67±13.5 (79.5)	12±3.5 (96.3)	0 (100)
	8	782±18.5	156±32.0 (80.1)	27±11.09 (97.0)	0 (100)
	16	> 70000	495±35.64 (99.2)	97±18.5 (100)	12±7.0 (100)
	24	> 70000	720±33.5 (100)	135 ± 19.0 (100)	73±24.5 (100)
B. cylindrica acetone leaf extract with *E. coli*	4	298±80.5	72 ±17.1 (75.8)	18±13.5 (93.9)	0 (100)
	8	489±43.7	239±26.6 (51.1)	39±17.5 (92.0)	4±2.0 (99.2)
	16	> 50000	478±31.13 (99.0)	67±19.1 (99.9)	16±8.0 (100)
	24	>50000	730±39.9 (98.5)	195±21.1 (100)	39 ±14.6 (100)
R. mucronata acetone stilt root extract with *V. cholerae*	4	353±42.1	67±26.5 (81.0)	12±7.5 (96.6)	0 (100)
	8	698±49.9	127±26.2 (81.8)	32±13.0 (95.4)	8±4.5 (100)
	16	> 60000	382±33.5 (99.3)	72±17.8 (100)	13±5.8 (99.9)
	24	> 60000	410±44.73 (99.3)	197±22.5 (100)	54±9.4 (99.9)

Values in the parenthesis are the percentage reduction of CFU in growth pattern of clinical pathogens; values are mean of triplicate reading (mean±SD)

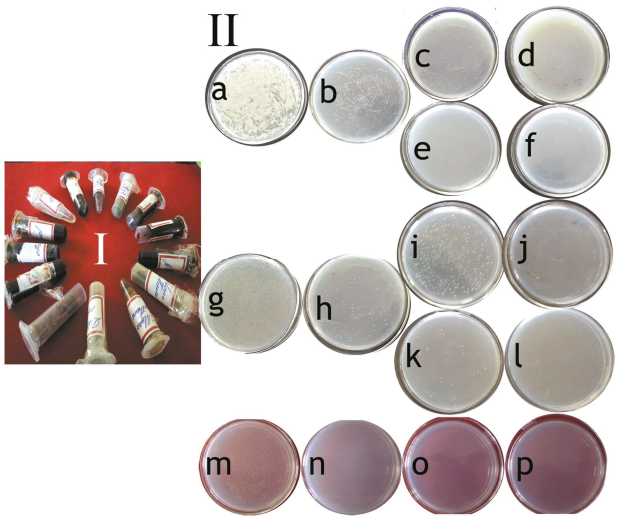

Fig. 2 — Viable count analysis of the bacterial pathogens - I: Collected solvent extract; II: Control plates [a) *MTCC 435*, g) *E. coli*, m) *V. cholerae*; b, h & n: Tetracycline (40 µg.ml^{-1}) treated plates - (b) *MTCC 435*, h) *E. coli*, and n) *V. cholerae*); c, e, i, k & o: X- MBC concentration treated plates - (c) *MTCC 435* with *R. mucronata* acetone stilt root extract, e) *MTCC 435* with *B. cylindrica* acetone leaf extract, i) *E. coli* with *R. mucronata* acetone stilt root extract, k) *E. coli* with *B. cylindrica* acetone leaf extract, and o) *V. cholerae* with *B. cylindrica* ethyl acetate leaf extract); d, f, j, l & p: 2X-MBC concentration treated plates - (d) *MTCC 435* with *R. mucronata* acetone stilt root extract, f) *MTCC 435* with *B. cylindrica* acetone leaf extract, j) *E. coli* with *R. mucronata* acetone stilt root extract, l) *E. coli* with *B. cylindrica* acetone leaf extract, and p) *V. cholerae* with *B. cylindrica* ethyl acetate leaf extract)]

counts when compared with the control. MTCC 435 showed maximum (36±6.4×10^3 CFU.ml^{-1}) viable count reduction with *R. mucronata* acetone root extract at 24 h incubation. Similarly, *R. mucronata* acetone stilt root extract also displayed minimum viable counts (135±19.0×103 CFU.ml^{-1}) of *E. coli* cells at 24 h incubation (Table 4). The percentage reduction of CFU denotes bactericidal activity of *R. mucronata* acetone stilt root extract with MTCC 435 and *E. coli* isolates at 16 and 24 h incubation with 1000 µg.ml^{-1} concentration. Further, the *B. cylindrica* acetone leaf extract showed bactericidal activity with MTCC 435 and *E. coli* at 24 h incubation with 4000 and 1000 µg.ml^{-1} concentration. *Bruguiera cylindrica* leaf ethyl acetate extract showed bactericidal activity against *V. cholerae* at 16 h incubation with 2000 µg.ml^{-1} concentration (Table 4). Cytotoxicity effect of acetone and ethyl acetate leaf extract of *B. cylindrica* and acetone stilt root extracts of *R. mucronata* on HeLa cell lines did not show any significant morphological changes up to 200 µg.ml^{-1} concentration and the IC_{50} value was identified as > 200 µg.ml^{-1} for all the three extracts (Fig. 2). SEM images of all the untreated bacterial pathogens (MTCC435, *E. coli* and *V. cholerae*) exhibited smooth cell surfaces without morphological changes (Fig. 3a, d & g). But the mangrove extracts-treated

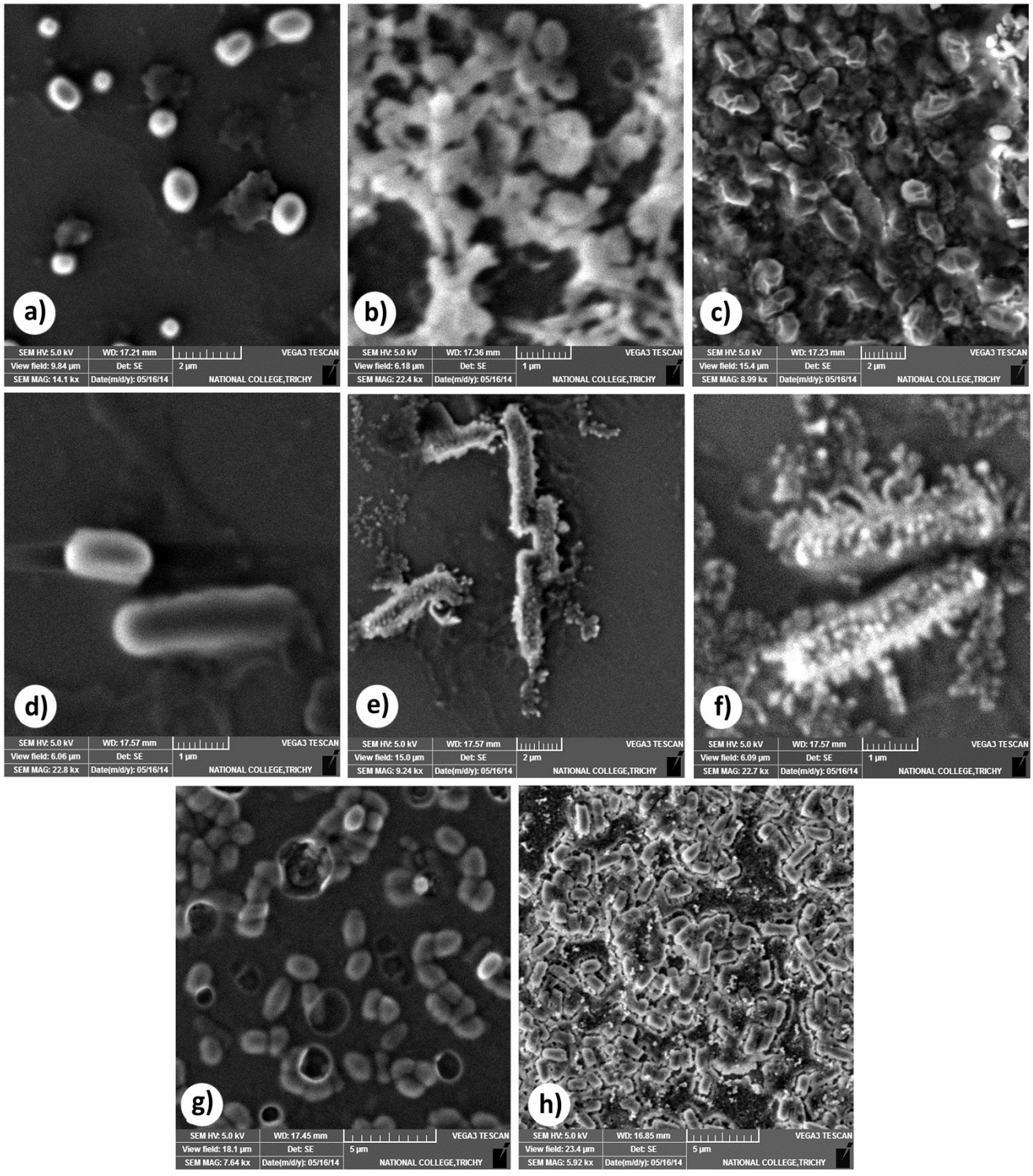

Fig. 3 — SEM images of mangrove extracts with treated and un-treated bacterial cells: (a) Un-treated; (b) *MTCC 435* treated with *R. mucronata* acetone stilt root extract; (c) *MTCC 435* treated with *B. cylindrica* acetone leaf extract; (d) Un-treated *Escherichia coli*; (e) *Escherichia coli* treated with *R. mucronata* acetone stilt root extract; (f) *Escherichia coli* treated with *B. cylindrica* ethyl acetate leaf extract; (g) Un-treated *Vibrio cholera*; and (h) *Vibrio cholerae* treated with *R. mucronata* acetone stilt root extract

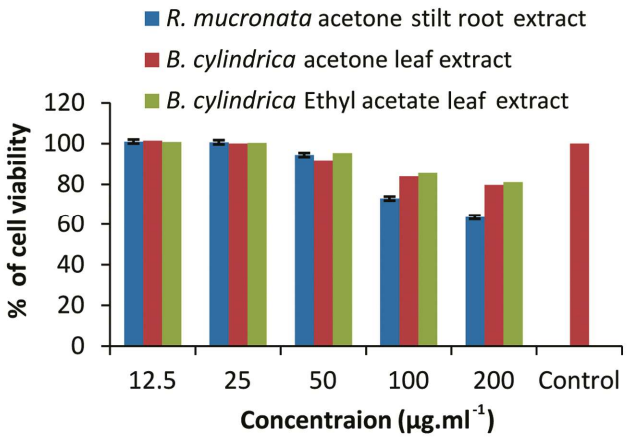

Fig. 4 — Percentage viability of HeLa cells with different mangrove extracts. Values are mean of triplicate reading (mean±SD)

bacterial cells showed cell aggregation (Fig. 3b) and morphological changes (Fig. 3c, h). Likewise, *E. coli* demonstrated leakage of cell contents (Fig. 3e, f). Cytotoxicity effects of mangrove extracts with HeLa cell lines did not reveal any significant morphological changes and cell viability between the control and extract-treated cell lines (Fig. 4).

Discussion

Plant extracts have long served as a fertile ground for new pharmaceutical compounds[18-19]. The current work on antibacterial properties of traditionally used mangrove plants indicated that the solvent extracts (dichloromethane, methanol, ethanol and ethyl acetate) of the *R. mucronata* stilt root and *B. cylindrica* leaf extracts possessed a wide range of MIC and MBC values (500 to 4000 µg.ml^{-1}) with the clinical and MTCC pathogens. It has been well documented that mangrove plants contain secondary metabolites like alkaloids, tannins, flavonoids, terpenoids and sugars[12,20]. The inhibitory properties of the leaf and stilt root extract of the *B. cylindrica* and *R. mucronata* may be a result of the secondary metabolites being present[21]. Here, a wide range of inhibitory (MIC and MBC) variations between the Gram-positive and Gram-negative bacterial pathogens was seen which might be due to the higher peptidoglycan layer present in Gram-positive bacterial isolates and outer phospholipidic membrane carrying the structural lipopolysaccharides in the Gram-negative bacteria. The presence of different layers in the cell makes the flow of phytochemicals resulting in inhibitory variations among the Gram-positive and Gram-negative bacteria[22].

Ravikumar *et al.*[23] identified the inhibitory properties (MIC) between 500 to 1000 µg.ml^{-1} concentrations with different Gram-positive and Gram-negative bacterial pathogens in marine plants. In this study, disk diffusion and well diffusion assay methods demonstrated a wide range of zone of inhibition properties. The maximum zone inhibition of the acetone stilt root extract of *R. mucronata* and leaf extract (acetone and ethyl acetate) of *B. cylindrica* could be due to the synergistic interaction of a heterogeneous mixture of mangrove metabolites with the antagonistic property. The pharmaceutical activity was reported with the mixtures of active constituents[24]. The growth curve analysis showed greater inhibitory activity in 2X MBC concentrations with the acetone stilt root extract of *R. mucronata* and leaf (acetone and ethyl acetate) extract of *B. cylindrica*. This inhibitory property further corroborates with the agar and well diffusion experiments. Similarly, Anas *et al.*[17] reported 70 % inhibitory properties with the leaf extract of *Psidium guajava* in *S. aureus.* Moreover, the viable count analysis decreased with the increased time intervals in the acetone stilt root extract of *R. mucronata* and leaf extract (acetone and ethyl acetate) of *B. cylindrica.* 100% CFU reduction was identified at 24 h incubation, thus indicating the bactericidal properties of the extract[17].

The SEM images showed the morphological alterations in mangrove extract-treated bacterial cells. It could be because mangrove secondary metabolites cross the phospholipids bi-layer and target intracellular components like the PMF (Proton Motive Force), the respiratory chain, or the electron transfer chain[25], thus causing the extensive loss of cell contents and death[26]. Similarly to this, *B. orellana* plant extract treated with *P. aeruginosa* showed cell lysis and bacterial aggregation[27]. In the present study, mangrove extract also exhibited the $IC_{50} > 200$ µg.ml^{-1} on the HeLa cell line indicating that the extracts did not show any cytotoxic effects on normal mammalian cell lines. The non-toxic effect of extracts are related to the traditional medicine consumed in the early years. Similar results have also been reported with the leaf extracts of the *Dendrophthoe pentandra* plant[28].

Conclusion

The study conducted to examine bactericidal and cytotoxic properties of *B. cylindrica* (acetone and ethyl acetate leaf extract) and *R. mucronata* (acetone stilt root extract) showed *in vitro* bactericidal

properties with > 98 % reduction of viable counts against MTCC 435, *E. coli* and *V. cholera* pathogens at different time intervals between 8 – 16 h.

Acknowledgements

Authors gratefully acknowledges the infrastructure support received from the DST-FIST, Government of India, New Delhi (Grant No: SR/FST/LS29 1/2017/10 Dt: 16.01.2018). The authors would like to express their gratitude to the University Grants Commission (UGC), New Delhi, for providing support through the Major Research Project (MRP-MAJOR-MICRO-2013-29259; F. No.-43-477/2014 (SR) dated 09.09.2015).

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Statement

This is to certify that the reported work in the paper entitled "Bactericidal properties of mangrove *Bruguiera cylindrica* (L.) Blume leaf and *Rhizophora mucronata* Poir. stilt root extracts on *Vibrio cholera*, MTCC 435 and *Escherichia coli* pathogens" submitted for publication is an original one and has not been submitted for publication elsewhere. I/we further certify that proper citations to the previously reported work have been given and no data/table/figure has been quoted verbatim from other publications without giving due acknowledgement and without the permission of the author(s). The consent of all the authors of this paper has been obtained for submitting the paper to the "Indian Journal of Geo-Marine Sciences".

Author Contributions

MG: Conceptualization, methodology, sample analyses, and writing - original draft; AD: Designing of work, resources, review & editing; VN: Sample analyses, and writing; VR: Editing the draft, statistical analyses, plotting graph; and VS: Sample and data analyses.

References

1 Parveen B, Parveen A, Parveen R, Ahmad S, Ahmad M, *et al*., Challenges and opportunities for traditional herbal medicine today, with special reference to its status in India, *Ann Phytomed*, 9 (2) (2020) 97-112. http://dx.doi.org/10.21276/ap.2020.9.2.8

2 Kanchanapoom T, Kamel M S, Kasai R, Picheansoonthon C, Hiraga Y, *et al*., Benzoxazinoid glucosides from *Acanthus ilicifolius*, *Phytochemistry*, 58 (4) (2001) 637–640. https://doi.org/10.1016/s0031-9422(01)00267-9

3 Bandaranayake W M, Bioactivities, bioactive compounds and chemical constituents of mangrove plants, *Wetl Ecol Manag*, 10 (6) (2002) 421–452. https://doi.org/10.1023/A:1021397624349

4 Xu N, Fan X, Yan X, Li X, Niu R, *et al*., Antibacterial bromophenols from the marine red alga *Rhodomela confervoides*, *Phytochemistry*, 62 (8) (2003) 1221–1224. https://doi.org/10.1016/s0031-9422(03)00004-9

5 Ravikumar S, Kathiresan K, Ignatiammal S T M, Babu Selvam M & Shanthy S, Nitrogen-fixing azotobacters from mangrove habitat and their utility as marine biofertilizers, *J Exp Mar Biol Ecol*, 312 (1) (2004) 5–17. http://dx.doi.org/10.1016/j.jembe.2004.05.020

6 Ravikumar S, Ramanathan G, Suba N, Jeyaseeli L & Sukumaran M, Quantification of halophilic *Azospirillum* from mangroves, *Indian J Geo-Mar Sci*, 31 (2) (2002) 157–160.

7 Bolotin A, Quinquis B, Sorokin A & Ehrlich S D, Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin, *Microbiology*, 151 (8) (2005) 2551–2561. https://doi.org/10.1099/mic.0.28048-0

8 Kumari N, Pandey S & Menghani E, Evaluation of actinomycetes isolated antimicrobial metabolites as potent inhibitor of multidrug resistant organisms, *Indian J Geo-Mar Sci*, 50 (01) (2022) 29–36.

9 Madathil D & Chidambaram R, In-silico analysis of bioactive compounds extracted from seaweed *Amphiroa anceps* on the pathogenicity of bacteria, *Indian J Geo-Mar Sci*, 51 (05) (2022) 379–387.

10 Ravikumar S, Gnanadesigan M, Jacob Inbaneson S & Kalaiarasi A, Hepatoprotective and antioxidant properties of *Suaeda maritima* (L.) Dumort ethanolic extract on concanavalin-A induced hepatotoxicity in rats, *Indian J Exp Biol*, 49 (6) (2011) 455–460.

11 Ravikumar S, Ramanathan G, Subhakaran M & Inbaneson S J, Antimicrobial compounds from marine halophytes for silkworm disease treatment, *Int J Med Med Sci*, 1 (5) (2009) 184–191.

12 Sadeer N B, Zengin G & Mahomoodally M F, Biotechnological applications of mangrove plants and their isolated compounds in medicine-a mechanistic overview, *Crit Rev Biotechnol*, 43 (3) (2023) 393-414. https://doi.org/10.1080/07388551.2022.2033682

13 Krishnamoorthy M, Sasikumar J M, Shamna R, Pandiarajan C, Sofia P, *et al*., Antioxidant activities of bark extract from mangroves, *Bruguiera cylindrica* (L.) Blume and *Ceriops decandra* Perr, *Indian J Pharmacol*, 43 (5) (2011) 557–562.

14 Ravikumar S & Gnanadesigan M, Hepatoprotective and Antioxidant Properties of *Rhizophora mucronata* Mangrove Plant in CCl 4 Intoxicated Rats, *J Exp Clin Med*, 4 (1) (2012) 66–72. https://doi.org/10.1016/j.jecm.2011.11.012

15 Ravikumar S, Thajuddin N, Suganthi P, Lnbaneson S J & Vinodkumar T, Bioactive potential of seagrass bacteria against human bacterial pathogens, *J Environ Biol*, 31 (3) (2010) 387–389.

16 Krithiga N, Rajalakshmi A & Jayachitra A, Green Synthesis of Silver Nanoparticles Using Leaf Extracts of *Clitoria ternatea* and *Solanum nigrum* and Study of its Antibacterial Effect against Common Nosocomial Pathogens, *J Nanosci*, 2015 (2015) 1–8. http://dx.doi.org/10.1155/2015/928204

17 Anas K, Jayasree P R, Vijayakumar T & Kumar P R M, In vitro antibacterial activity of *Psidium guajava* Linn, leaf extract on clinical isolates of multidrug resistant *Staphylococcus aureus*, *Indian J Exp Biol*, 46 (1) (2008) 41–46.

18 Asiamah I, Obiri S A, Tamekloe W, Armah F A & Borquaye L S, Applications of molecular docking in natural products-based drug discovery, *Scientific African*, 20 (2023) p. e01593. https://doi.org/10.1016/j.sciaf.2023.e01593

19 Shi Y, Si D, Chen D, Zhang X, Han Z, *et al*., Bioactive compounds from Polygonatum genus as anti-diabetic agents with future perspectives, *Food Chem*, 408, p. 135183. https://doi.org/10.1016/j.foodchem.2022.135183

20 Lichota A & Gwozdzinski K, Anticancer Activity of Natural Compounds from Plant and Marine Environment, *Int J Mol Sci*, 19 (11) (2018) p. 3533. https://doi.org/10.3390/ijms19113533

21 Kumar M K & Pola S, Mangrove species as a potential source of bioactive compounds for diverse therapeutic applications, In: *Marine Antioxidants: Preparations, Syntheses, and Applications*, edited by Kim S-K, Shin K-H & Venkatesan J, (Academic Press, US), 2023, pp. 249-263.

22 Ravikumar S, Muthuraja M, Sivaperumal P & Gnanadesigan M, Antibacterial Activity of the Mangrove Leaves *Exoecaria agallocha* Against Selected Fish Pathogens, *Asian J Med Sci*, 2 (5) (2010) 211–213.

23 Ravikumar S, Gnanadesigan M, Suganthi P & Ramalakshmi A, Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens, *Int J Med Med Sci*, 2 (3) (2010) 94–99.

24 Atindehou K K, Koné M, Terreaux C, Traore D, Hostettmann K, *et al*., Evaluation of the antimicrobial potential of medicinal plants from the ivory coast, *Phytother Res*, 16 (5) (2002) 497–502. https://doi.org/10.1002/ptr.970

25 Zengin H & Baysal A H, Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy, *Molecules*, 19 (11) (2014) 17773–17798. https://doi.org/10.3390/molecules 191117773

26 Denyer S P, Mechanisms of action of biocides, *Int Biodeter*, 26 (2–4) (1990) 89–100. https://doi.org/10.1016/0265-3036(90)90050-H

27 Selvi T A, Satyan R S, Murugan D & Chandrasekaran B, Leaf and Seed extracts of *Bixa orellana* L, exert anti-microbial activity against bacterial pathogens, *J Appl Pharm Sci*, 1 (9) (2011) 116–120.

28 Zainuddin N A S N & Sul'ain M D, Phytochemical Analysis, Toxicity and Cytotoxicity Evaluation of Dendrophtoe Pentandra Leaves Extracts, *Int J Appl Biol Pharm Technol*, 6 (1) (2015) 108–117.