International Journal of Phytomedicine 6 (2014) 425-432
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Isolation of 12 Bacterial endophytes from some mangrove plants and determination of, antimicrobial properties of the isolates and the plant extracts

Ibrahim.M.S.Eldeen[1]

*Corresponding author:

Ibrahim.M.S.Eldeen

[1]Institute of Marine Biotechnology,University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

Abstract

The mangrove designates a highly productive ecosystem with important economic and environmental functions. Endophytes are microorganisms that live in the intercellular spaces of plant tissue.

This study aimed to isolate and identify bacterial endophytes from five mangrove plants and to determine, antimicrobial properties of the isolates and the plant extracts against four pathogenic bacteria: *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium* using the deferred antagonism and the microdilution assays. Of the total 33 endophytic bacteria isolated, 18 strains showed antagonistic effects. Twelve of these inhibitors were identified using VITEK 2. Crude protein from each of the producer strains were precipitated and tested for minimum inhibitory concentration (MIC) against the pathogenic bacteria using the microdilution assay.

Best activities were recorded for *Staphylococcus intermedius* and *Bacillus licheniformis* (19 µl/ml) against *B. cereus.* The *S. intermedius* also inhibited growth of both *S. aureus* and *S. typhimurium* (39 µl/ml). *Staphylococcus lentus, Bacillus pumilus* and *Bacillus coagulans* possessed activities against *S.typhimurium* with an MIC value of 78 µg/ml.

For the plant extract, the lowest MIC value (9.7 µg/ml) was obtained by *Aviecenna lanata* and *Sonneratia caseolaris* against *B.cereus. S.caseolaris* also showed significant inhibitory effects against *E.coli* and *S.typhimurium* (19.5 µg/ml). Our results indicated the potentiality of the isolated bacterial endophytes as a producer of antimicrobial substances which could be developed for various applications. MIC values obtained for the plant extracts in this study showed the effective plant part and extracts to be further developed and profiled as antimicrobial agents.

Keywords: Bacterial endphytes, Bacteriocins, Mangrove plants,Antimicrobials.

Introduction

Endophytes are microorganisms including bacteria that live in the intercellular spaces of plant tissue with no apparent damage to their host. They represent a huge diversity of microbial adaptations that have developed in special sequestered environment [1]. Plant host interaction requires sustained and prolonged reactions against the defense mechanisms of the host by the endophyte. This could act as a selection pressure for the development of novel metabolic pathways and therefore, makes endophytes to be considered as a rich source of effective new bioactive substances [2].

Bacterial endophytes provide a broad variety of bioactive metabolites `including substances of peptide structure such as bacteriocins. These substances are a heterogeneous group of ribosomally synthesized peptides that inhibit or kill bacteria that are usually, but not always, closely related to the producer strain [3]. Example of such usefull metabolite is nisin, a bacteriocin produced by *Lactobacillus lactis* and exhibits antibacterial activity against a wide range of Gram-positive bacteria [4,5]. Bioactive metabolites from a variety of Gram-positive species including S*taphylococcus* and *Bacillus* species have been characterized [6,7].

The mangrove habit has proved to be a rich source of endophytes. They occupy the upper part of the trees as the bases of mangrove trunks and aerating roots are permanently or intermittently submerged. Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which make them suitable as biocontrol agents [8]. Interactions of endophytes under this environmental condition may lead to the production of wide group of chemicals of interesting pharmaceutical applications [9].

Mangroves are highly productive ecosystem with various important economic and environmental functions. Large number of mangrove plant species are used in traditional medicine [10]. Extracts from different mangrove plants such as *Avicenna* sp, *Rhizophora* sp, *Sonneratia* sp, and *Bruguiera* sp were reported to possess antimicrobial effects among other medicinal properties [10,11,12]. Methanolic bark extracts of *Ceriops tagal* showed higher antibacterial activity against *Vibrio alginolyticus*. This activity was reported as equivalent to the antibiotic drug Streptomycin [13]. Bark extract of *Xylocarpus granatum* was reported to possess a

significant antidiarrhoeal activity with dose-dependent effects on *in vivo* model [14].

Mangrove plants and their endophytes represent a rich source of effective metabolites to be investigated as an endless source of novel constituents. A systematic investigation of this source of natural products may lead to the discovery of novel bioactive agents with useful medicinal and pharmaceutical applications.

This report highlights isolation and identification of bacterial endophytes from five Malaysian mangrove plants. The report also highlights determination of antimicrobial properties of, the isolated bacterial endophytes and the mangrove plants.

Material and methods

Collection of plant material

Plant parts including leaves and roots from five mangrove plants were collected from a lagoon in the Setiu District of Terengganu State on the east coast of peninsular Malaysia. The plant spceies were: *Avicennia lanata*, *Rhizophora mucronata*, *Rhizophora apiculata*, *Sonneratia caseolaris*, and *Xylocarpus moluccensis*.

Culuring, isolation and identification of bacterial endophytes

For culturing of bacterial endophytes, a number of ten healthy leaves from each plants were collected and kept in sterile bags. All samples were transfered immediately to our laboratory and stored at 4 C for further process. The collected leaves were washed in running water to remove soil particles and then surface sterilized by sequential immersion in 70% ethanol for 5 min and sodium hypochlorite for 10 min. The samples then washed three times in sterile distilled water to remove surface sterilizing agents before being soaked in 10% sodium bicarbonate. Each of the samples was then cut into small fragments (2-5 mm). All the work was performed in the sterilized laminar air hood. Five fragments of each samples were plated on nutrient agar (Difco agar) and incubated at 37 C for five days and observed for the growth of bacterial colonies surrounding the leaf fragment. Pure cultures of bacterial endophytes were developed by dilution-streaking on the same media. Bacterial strains with potential antagonistic interactions were selected for further test.

Preparations of the palnt extracts

The collected samples including leaves and roots were dried in oven (55 C) for seven days, grounded and extracted sequentially using dichloromethane, ethyleacetate and methanol. Obtained residue were concentrated to dryness and kept on fridge (10 C) for further process.

Determination of potential antagonistic interactions and identification of the active bacterial strains

Potential antagonistic interactions of the isolated endophytic bacterial strains were obtained using the deferred-antagonism plate assay. Bacterial strains appeared to produce inhibitory substances were inoculated along a striaght line through the centre of Difco agar plates by using sterile cotton-tipped swabs dipped in overnight broth cultures. The plate was incubated for 24-h at 37 C to allow bacterial growth and production of inhibitory substances. Then the indicator strains were inoculated perpendicular to the producing bacteria with sterile cotton-tipped swabs dipped in overnight broth cultures. The plate contents were incubated for a further 24 h at 37 C before they were examined for the presence of inhibition zones at the intersections of the streak. Only bacterial strains with clear antagonistic interactions were stained, morphologically characterized and further identified using the fully automated VITEK 2 system.

Production and precipitation of Antimicrobial substances by the isolated bacterial endophytes

Bacterial endophytes were primarily screened for production of antimicrobial substances following cross-streak assay method using four test organisms: *Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium (data not shown)*.

To produce and extract the antimicrobial substances of the isolated endophytes, each strain was cultivated separately in tryptic soya broth (TSB) for 24h at 30 C, centrifuged at 16 000 x g for 15 min. The supernatant was then concentrated with ammonium sulfate to 80% saturation and precipitated at 4 C. The precipitated proteins were pelleted by centrifugation at 16000 x g for 20 min at 4 C and sucked up from the bottom of the centrifuge tube (4 ml) and re-centrifuged again for 10 min. The upper layer was carefully sucked up. The remaining crude protein extract (2 ml) was re-suspended in 2 ml of 50 mM phosphate buffer (pH 7.0). The stock solution (I ml of crude protein extract dissolved in I ml of 50 mM phosphate buffer) was tested for antimicrobial activity using the micro-dilution assay as described below for determination of the minimum inhibitory concentrations.

Determination of antimicrobial activity of the crude bacterial proteins and the plant extracts

The antibacterial activities of the crude proteins and the plant extracts were determined using the serial dilution technique described by Eloff [15], using 96-well micro-plates. Two ml cultures of four bacterial strains: Gram-positive *Bacillus cereus* and *Staphylococcus aureus* and two Gram-negative: *Escherichia coli* and *Salmonella typhimurium* were prepared and placed in a water bath overnight at 37 C. The overnight-cultures were diluted with sterile MH broth (1 ml bacteria/ 50 ml MH broth) and adjusted to an optical density of 0.4.

100 µl of the stock solution (initial concentration of 1 ml/ml) were two-fold serially diluted with 100 µl sterile distilled water in a sterile

96-well micro-plate from well A to H before adding 100 µl of each of the test bacterial strains .

For the plant extracts, the residue obtained were resuspended to a concentration of 5 mg/ml with 50% ethanol. For each of the four bacteria used, 100 µl of the re-dissolved extract were two-fold serially diluted with 100 µl sterile distilled water in a sterile 96-well micro-plate. A similar two-fold serial dilution of gentamicine sulfate (Sigma) (0.1 mg /ml) was used as a positive control against each bacterium. One hundred µl of each bacterial culture were added to each well as mentioned earlier. The plates were covered and incubated overnight at 37 C. To indicate bacterial growth, 50 µl of 0.2 mg/ ml of Resazurin blue was added to each well and the plates incubated at 37 C for 30 min. Bacterial growth in the wells was indicated by a pink colour, whereas blue wells indicated inhibition by the tested substances.

Results and discussion

Isolation and identification of bacterial endophytes

A total of 33 bacterial endophytes were isolated and purified from the leaves of five Malaysian mangrove plants. The removal of surface microbial flora was achieved by the surface sterilization process including sequential immersion in 70% ethanol, 10 % sodium hypochlorite and sodium bicarbonate. The colonies were purified and subjected to morphological characterization prior to further identification process. Eighteen of the isolated strains showed positive antagonistic effects against the indicator strains when evaluated using the deferred-antagonism plate assay. Twelve of these producer strains were identified to species level suing VITEK2 (GP and CBC cards). The strains were identified to be: *Staphylococcus sciuri* 1_a, *Staphylococcus intermedius* 3_2, *Bacillus pumilus* 1_1, *Staphylococcus lentus* 2_1, *Bacillus licheniformis 7i, Bacillus pumilus 3 Bacillus coagulans* 1_{21}, *Staphylococcus lentus* 4_2, *Staphylococcus intermedius* 4_1, *Bacilus pumilus* 2_{i1}, *Bacillus pumilus* 7_{is}, *Staphylococcus lentus* 7_1, *and Bacillus pumilus* 1_0. Some of the relevant biochemical reactions are given in Table (1).Six strains were reported unidentified. The VITEK 2 is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a state-of-the-art technology plat form for phenotypic identification methods. The system was confirmed to provide accurate identification reports without the need for further assessment [16,17]. However, lack of information in the database in some cases and /or low reactivity of the strains within the incubation time may lead to failure of identification. This may justify that six of our isolated strains were unidentified.

Crude proteins from the identified isolates were produced and tested for minimum inhibitory concentration against the indicator strains. The twelve isolated strains possessed different antibacterial activities with an MIC values ranging from 19 to 250 µl/ml (Table 1).

Table: 1. Identification of the isolated endophytic bacterial strains using the VITEK 2 compact and their antimicrobial properties expressed as MIC values (µl/ml) as detected using the Microdilution assay.

Isolate	Source	Morphological characterisitc		VITEK 2 identification	Confidence	Probability %	Analysis time/h	MIC values µl/ml			
		Gram	Shape					Bacteria tested			
								Bc	Ec	Sa	St
1_{a1}	*Aviecenna lanata*	+	cocci	*Staphylococcus sciuri*	Excellent	96	6	250±3.1	250±3.0	39±2.1	39±1.8
1_1	*Aviecenna lanata*	+	bacili	*Bacillus pumilus*	Excellent	98	14	250±3.7	250±2.6	156±3.2	78±2.4
2_1	*Rhizophora apiculata*	+	cocci	*Staphylococcus lentus*	Good	92	5	250±4.1	250±5.0	156±3.1	78±1.7
2_i	*Rhizophora apiculata*	+	bacili	*Bacillus pumilus*	Excellent	99	14	156±1.8	62±2.4	156±3.1	78±2.1
3_2	*Rhizophora mucronata*	+	cocci	*Staphylococcus intermedius*	Good	92	5	19±2.2	250±3.2	39±2.1	39±2.2
3_i	*Rhizophora mucronaata*	+	bacili	*Bacillus pumilus*	Acceptable	88	5	156±2.2	62±3.1	156±4.1	78±1.3
4_1	*Sonneratia caseolaris*	+	cocci	*Staphylococcus intermedius*	Acceptable	87	5	19±2.3	250±4.1	39±2.0	39±1.9
4_2	*Sonneratia caseolaris*	+	cocci	*Staphylococcus lentus*	Good	92	5	250±1.9	250±3.8	156±2.3	78±1.4
7_1	*Xylocarpus moluccensis*	+	bacili	*Bacillus coagulans*	Good	94	18	156±3.1	62±2.0	156±2.0	78±2.1
7_i	*Xylocarpus moluccensis*	+	bacili	*Bacillus licheniformis*	Acceptable	87	6	19±3.4	250±3.4	39±2.5	156±3.1
7_{i1}	*Xylocarpus moluccensis*	+	bacili	*Bacillus pumilus*	Excellent	98		250±2.3	250±2.1	156±3.1	78±2.7
7_{11}	*Xylocarpus moluccensis*	+	bacili	*Bacillus coagulans*	Good	96	5	78±3.7	250±3.1	250±2.4	78±1.1
Gentamicin sulfate (positive control)								2.1.±1.3	8.3±1	1.9±2.4	1±0.4

Staphylococcus sciuri1$_a$, Staphylococcus intermedius3$_2$, B.pumilus1$_1$, S.lentus 2$_1$, B.licheniformis7$_i$, Bacillus pumilus3 4$_1$, Bacillus pumilus2$_{i1}$, Bacillus pumilus7$_{i1}$, S.lentus 7$_1$, B. coagulans1$_{21}$, S.lentus 4$_2$, Staphylococcus intermedius 1$_0$.

Bacterial strains of *Staphylococcus intermedius* (3_2 and 4_1) isolated from *Rhizophora mucronata* and *Sonneratia caseolaris* respectively, showed wider spectra of activities against, *Bacillus* sp (19 µl/ml), *Staphylococcus aureus* and *Salmonella* sp (39 µl/ml). *Stahpylococcus sciuri* also possessed activities against *S.aureus* and *S.thyphimurium* with an MIC value of 39 µl/ml. *Staphylococcus lentus* showed activity against *S.typhimurium* with an MIC value of 78 µl/ml but appeared to be weaker against the other indicator strains. Interestingly, strain of *Bacillus pumilus* (2_1 and 3_1) isolated from *R.apiculata* and *R.mucronata* together with *Bacillus coagulans* (7_1) isolated from *Xylocarpus moluccensis*, exhibited activity against *E.coli* with an MIC value of 62 µl/ml.

Microbial antagonism can lead to the production of valuable secondary metabolites. In particular, growth inhibitors as one of the mechanisms of adaptation can provide advantages in competition for available nutrients and living space [18]. This is very much true with the bacterial endophytes regarding their special growth environment [19].

Bacillus species in this study showed broad spectrum of activities against the indicator strains. This is in agreement with previous report of antimicrobial properties of 29 *Bacillus* species isolated from the soil and found effective against Gram-positive and Gram-negative bacteria [20,21]. *Bacillus* species are also known to produce effective molecules during a biocontrol treatment of systemic *Staphylococcus aureus* infections [22]. Antimicrobial properties of *Bacillus* species against Gram-negative *E.coli* was also recorded previously for *B. thuringiensis*, *B. subtilis* and *B. megaterium* strains [23]. Antimicrobial agents isolated from *Lactobacillus plantarum* and identified as Plantaricin MG was reported to have broad inhibitory activity against Gram-positive and Gram-negative bacteria including *Salmonella typhimurium* [24].

The strain of *Bacillus licheniformis* in this study showed strong activities against *Bacillus cereus*. This is in agreement with several reports describing antimicrobial properties of *Bacillus licheniformis* [25,26,27].

Staphylococcus species possessed inhibitory effects against *B.cereus, S.aureus*, and *Salmonella typhimurium* in this study. The best activities observed were recorded for *S.intermedius*. A previous report on the antagonistic effects of *Staphylococcus* strains against closley related species indicated production of molecules with remarkable inhibitory effects against wide range of bacterial strains including Gram-negative [28]. These findings support our results of the antimicrobial properties observed by the isolated *Staphylococcus* species in this study.

Antibacterial properties of the mangrove plant extracts

The antibacterial activities of the plant extracts was determined using the serial dilution technique. Four bacterial strains were used and the antibacterial results obtained are presented in Table 2. The lowest MIC value (9.7 µg/ml) was obtained by dichloromethane leaf extract of *A.lantana* and methanolic leaf extract of *S.caseolaris* against the Gram-positive bacteria *Bacillus* sp. Dichloromethane extract of *S.caseolaris* and methanolic leaf extract of *A. lanata* showed inhibitory effects against *E.coli* with an MIC value of 19.5 µg/ml. The lowest MIC values against *S.typhimurium* was 19.5 µg/ml obtained by methanolic extract of *S.caseolaris* followed by dichloromethane, root and leaf extracts of *R. mucronata* and *A.lanata* respectively (MIC value of 39 µg/ml). The lowest MIC value obtained against *S.aureus* was 78 µg/ml. This was recorded for methanolic leaf extracts of *A.lanata*, methanolic, root and leaf extracts of *R. mucronata* and *S.caseolaris* respectively. MIC values recorded for the positive control was 1.9 µg/ml against *B. cereus*, 7.8 µg/ml, against *E.coli*, 1.9 µg/ml, against *S.aureus* and 0.5 µg/ml against *S.typhimiurium*.

Table 2. Ethnobotany and Minimum inhibitory concentration (MIC) values (µg/ml) of the extracts obtained from the five mangrove plants as detected using the micro-dilution assay

Plant name	Ethnobotanical uses	Plant extract tested	MIC values (µg/ml)			
			Bacteria tested			
			Bc	Ec	Sa	St
Aviecenna lanata	*Aviecenna* sp are used for stomach problems inccluding diarrhoe, small pox, leprosy, diuretic [30].	Meth- leaf extract	15.6±2.7	19.5±1.8	78±2.1	312±3.4
		Dich-leaf extract	9.7±1.2	312±4.3	156±2.1	39±1.8
Rhizophora apiculata	Used for diarrhea, nausea, vomiting, typhoid and microbial related infections [9,30].	Meth-root extract	312±3.6	312±3.7	625±7.8	312±3.2
		Dich-root extract	562±3.2	281±1.6	562±2.5	562±3.1
Rhizophora mucronata	Hemorrhage, hepatitis, ulcer and haematuria [31].	Meth-leaf extract	281±2.3	281±2.5	562±1.6	562±4.2
		Dich-leaf extract	19.5±2.4	156±3.0	156±2.7	312±3.1
		Meth-root extract	156±1.8	312±4.1	78±1.5	312±1.7
		Dich-root extract	19.5±2.4	156±2.5	156±2.4	39±2.3
Sonneratia caseolaris	Different parts are used to relieve cough, as astringent and antiseptic, also used for haemorrhage and anthelmintic [7,10].	Meth-leaf extract	9.7±1.1	78±2.1	78±1.9	19.5±2.0
		Dich-leaf extract	312±3.3	19.5±3.2	312±2.9	156±2.8
Xylocarpus moluccensis	used as an astringent and for fever, dysentery, diarrhoea and other abdominal troubles [31].	Meth-root extract	140±1.7	281±3.6	562±4.1	140±2.8
		Dich-root extract	312±1.6	312±2.6	625±3.1	625±3.4
Gentamicin sulfate			1.9±0.9	7.8±1.0	1.9±0.9	0.5±0.9

Results expressed as means ± SD. Bc=*Bacillus cereus;* Ec=*Escherichia coli,* Sa=*Staphylococcus aureus.* St=*Salmonella typhimurium.*

Methanolic leaf extract of *S. caseolaris* in this study possessed the best activities against all the tested bacterial strains with an MIC values ranging between 9.7 and 78 µg/ml. During previous investigation of antimicrobial effects of *Sonneratia* species, a methanolic extract was found to be effective against *E.coli* and *S.aurus* and *B.cereus* [12]. Thus , it is possible to observe similar activities with the related species *S.caseolaris* suggesting that extract of the plant could be used as antimicrobial agents.

Dichloromethane leaf and root extract of *R. mucronata* also showed wide range of activities against *B. cereus, E.coli, S.aureus* and *S.typhimurium* .This may be due to the presence of mixture of various antimicrobial agents in these extract [29].

Dichloromethane leaf extract of *A. lanata* showed interesting activity against *B. cereus*, and *S.typhimurium*. Antimicrobial properties of the closely related species *A.marina* was intensively studied and clearly highlighted [9]. The Antibacterial effects observed by the extract of *A.lanata* in this study may be due to the presence of similar or related active agents.

Mangrove plants thrive under extreme environment which lead to the development of special metabolic pathways to produce unique chemicals that enable them to tolerate such stressful growth conditions. Some of these chemicals are confirmed to be of great potential as a source of novel agents for various pharmaceutical and other industerial applications.

Several reports have clearly indicated the isolation of agents proven to be antimicrobial effective such as tannins, flavonoids, sterols, coumarins, glycosides, fatty acids, organic acids, alkaloids and saponins from mangrove and mangrove associated plants [9]. The observed antibacterial activities in this study support these findings and therefore require further study to isolate and develop these agents as potential antimicrobial drugs.

Conclusion

In this study, we investigated antimicrobial properties of extracts from five mangrove plants and their isolated bacterial endophytes. Obtained results indicated the presence of antimicrobial agents produced by the bacterial endophytes. These agents might be or related to bacteriocin-like substances. Bacteriocins are specific and highly potent proteins with antimicrobial properties that have

gained more attention recently as new molecules with interesting pharmacological properties. Efforts have been made in recent years to unravel the production of bacteriocins- like inhibitory substances from different bacterial groups including bacterial endophytes.

Our results also confirmed the antimicrobial properties of extracts from the investigated plants However, no correlation could be made between the activities of endophytes and the plant extracts. This suggested that biological activities observed in this study may be due to the effects of different metabolites that acting independently through different mechanism of actions in separate pathways.

This is the first report describing isolation and identification of these bacterial endophytes from the selected five mangrove plants in this study using the VITEK 2 system. The novelty of the work also includes determination of the antagonistic effects of the isolated strains against the pathogenic bacteria using the minimum inhibitory concentration techniques. MIC values of the plant extracts obtained in this study, indicated the best active extract from each of the investigated plants to be utilized as crude antimicrobial agents and/or to be further studied for isolation of the active principles.

Our research group in the Institute of Marine Biotechnology (IMB), University Malaysia Terengganu, are currently focusing on isolation and identification of bacteriocin like inhibitory substances from the isolated bacterial endophytes. Isolation of active agents from the plant extracts showing the best activities are also in progress.

Aknowledgement

This work was funded by an internal research grant from Research and Innovation Affairs University Malaysia Terengganu, and arranged by The Research Management Centre (RMC).

References

[1]. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol biol R.2002; 67:491–502.

[2]. Bhimba BV, Franco,AD, Mathew JM, Jose GM, Joel EL, Thangaraj M. Anticancer and antimicrobial activity of mangrove derived fungi *Hypocrea lixii* VB1. Chinese.J.of N.Med. 2012; 10 (Suppl 1): 0077-0080.

[3]. Kayalvizhi N, Gunasekaran P. Purification and characterization of a novel broad-spectrum bacteriocin from *Bacillus licheniformis* MKU3. Biotechnol and Biop.Eng.2010;15:365-370.

[4]. Molina G, Pimentel MR, Bertucci TCP, Pastore GM. Application of fungal endophytes in biotechnological processes. Chem Eng.Transaction. 2012;27:289-294.

[5]. Altuntas E. Bacteriocins: A natural way to combat with pathogens In: Méndez-Vilas Ed. Microbial pathogens and Strategies for Combating them: Science, Technology and Education. FORMATEX Microbiology Book Series.Formatex Research Centre: Badajoz, Spain.; 2013. P.1005-1015.

[6]. Premanathan M, Arakaki R, Izumi H, Kathiresan K, Nakano M, Yamamoto N, Nakashima, H. Antiviral properties of a mangrove plant, Rhizophora apiculata against immunodeficiency virus. Antiviral Research. 1999;44:113-122.

[7]. Bandaranayake WM. Traditional and medicinal uses of mangroves Mangroves and Salt Marshes. 1998; 2: 133–148.

[8]. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications.FEMS Microbial Letter. 2008; 278: 1-9.

[9]. Eldeen I MS, Effendy MAW. Antimicrobial agents from mangrove plants and their ndophytes. In: Méndez-Vilas Ed. Microbial pathogens and Strategies for Combating them: Science, Technology and Education. FORMATEX Microbiology Book Series.Formatex Research Centre: Badajoz, Spain.; 2013. P.872-882.

[10]. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management. 2002; 10: 421–452.

[11]. Sittiwet C. *In vitro* antimicrobial activity of *Pulchea indica* aqueous extract: The potential for urinary infection treatment. J Pharmacol and Toxicol.2009;4:87-90.

[12]. 12.Saad S, Taher M, Susanti D, Qaralleh H, Awang AF. *In vitro* antimicrobial activity of mangrove plant *Sonneratia alba*. Asian Pacific J Tropical Biomed. 2012; 427-429.

[13]. Arvuselvan N, Jagadeesan D, Govindan T, Kathiresan K, Anantharaman P. *In vitro* antibacterial activity of leaf and bark extracts of selected mangrove against fish and shrimp pathogens. Global J of Pharmacol 2011; 5:112-116.

[14]. Rouf R, Uddin, SJ, Shilpi JA, Alamgir M. Assessment of antidiarrhoeal activity of the methanol extract of *Xylocarpus granatum* bark in mice model. J Ethnopharmacol. 2007; 109: 539–542.

[15]. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med.1998; 64:711-713.

[16]. Graf B, Adam T, Zill E, Göbel U.Evaluation of VITEK2 system for rapid identification of yeast and yeast-like organisms.J Clin Microbiol.2000; 38(Suppl 5):1782-1785.

[17]. Ling TKW, Liu Z K, Cheng AFB. Evaluation of the VITEK 2 system for rapid direct identification and

susceptibility testing of Gram-negative *Bacilli* from positive blood cultures. J Clin Microbiol.2003; 41(Suppl 10): 4705-4707.

[18]. Alekseevna BI, Danilovich, KA, Valerievna KU. Antimicrobial activity of heterotrophic bacterial strains of marine origin. *Jundishapur J of Microbiol.*2013;2:166-175.

[19]. Riely MA, Chavan, MA. Bacteriocins:Ecology and Evolution. Berlin Heidelberg New York: Springer – Verlag; 2007:P.1-3.

[20]. Yilmaza M, Sorana H, Beyatlib, Y. Antimicrobial activities of some *Bacillus* sp. strains isolated from the soil. Microbiological Res.2006; 161: 127-131.

[21]. Béri T, Koji M, Stankovi S, Topisirovi L, Degrassi G, Myers M, Venturi V, Djord et al. Antimicrobial Activity of *Bacillus* sp. Natural isolates and their potential use in the biocontrol of phytopathogenic Bacteria. Food Technology Biotechnol. 2012;50 (Suppl 1): 25–31 .

[22]. Heng NC, Wescombe PA, Burton JP, Jack RW, Tagg JR.The Diversity of Bacteriocins in ram-Positive Bacteria. In: Riley MA, Chavan MA Ed: Bacteriocins: Ecology and Evolution. Springer-Verlag Berlin Heidelberg. . 2007; P.54-92.

[23]. Aslim B, Sa lamn N, Beyatli Y. Determination of some properties of *Bacillus* isolated from Soil. Turk J Biol. 2002; 26:41-48.

[24]. Gong H-S, Meng X-C, Wang H. Mode of action of plantaricin MG, a bacteriocin active against *Salmonella typhimurium*. J Basic Microbiol. 2010; 50:37- 45.

[25]. Kayalvizhi N, Gunasekaran P. Purification and characterization of a novel broad-spectrum bacteriocin from *Bacillus licheniformis* MKU3. Biotechnol Bioprocess Engineering.2010;15:365-370.

[26]. Smith S, Bhat SG. Thermostable bacteriocin BL8 from *Bacillus licheniformis* isolated from marine sediment. J App Microbiol. 2013;114:688-94.

[27]. Gray EJ, Lee KD, Souleimanov AM, Di Falco MR. et al. A novel bacteriocin, thuricin 17, produced by plant growth promoting Rhizobacteria strain *Bacillus thuringiensis* NEB17: Isolation and classification. J Appl Microbiol. 2006;100:545-554.

[28]. Skalka B. Bacteriocin typing of Staphylococci. Acta Veterniaria Brno. 1992; 61: 179-187.

[29]. 29. Joel EL, Bhimba BV. A antibacterial activity and GCMS analysis of secondary metabolites produced by the mangrove plant *Rhizophora mucronata* against MRSA. [Quick Edit] Intal J Appl Bioengineering. 2010; 4: 25-28.

[30]. 30.Patra JK, Thatoi,NH. Metabolic diversity and bioactivity screening of mangrove plants: a review. Acta Physiol Plant. 2011; 33:1051–1061.

[31]. Wangensteen H, Klarpås L, Alamgir M, Samuelsen,ABC, Malterud KE**.** Can scientific evidence support using Bangladeshi traditional medicinal plants in the treatment of diarrhoea? A Review on seven plants. Nutrients. 2013**;** 5: 1757-1800.