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ANTIBACTERIAL ACTIVITY OF *HALODULE UNINERVIS* LEAVES EXTRACTS AGAINST SOME BACTERIAL PATHOGENS STRAINS

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ABSTRACT

In this present investigation, the antibacterial activity of *Haloduleuninervis* against seven bacterial pathogens (*Bacillus subtilis*; Methicillin-Resistant Staphylococcus aureus (MRSA); *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*; *Klebsiellapneumoniae* and *Pseudomonas aeruginosa*) using different solvent extracts. The results showed the highest activity of ethanol leaves extract of *Haloduleuninervis* against *Pseudomonas aeruginosa* while displaying the lowest activity against *Staphylococcus aureus*. However, aqueous extract only showed activity against *P. aeruginosa*. RAPD analysis for *P. aeruginosa* treated with *H. uninervis* leaves extract. The obtained results showed that leaves extract affected on *P. aeruginosa*. RAPD analysis displayed two types of polymorphisms in either absence of amplified fragments in the treated strain of *P. aeruginosa* or induction of new fragments that were disappeared in the untreated bacteria (control).

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Introduction

Infectious diseases still remain a major cause of death due to the existence of antibiotic resistant microorganisms. Microbial organisms continue to be resistant at a significantly high rate globally [1]. Increased rate of resistance of pathogens coupled with antibiotics ineffectiveness has made it necessary to look for other options [2]. To deal with this issue, new drugs that have a higher effectiveness and reduced consequences need to be manufactured. In entirety, in order to develop antibiotics that are strong to kill fungi, viruses, bacteria as well as other dangerous microorganisms, marine plants, such as seagrasses, seaweeds, marine sponges and mangroves have been under extensive research [3, 4].

Medicinal plants contain medical elements or substances that have medical purposes either used as medicine or in making a drug [5]. Over time, a significant role is played by medicinal plants in curing human diseases and approximately three quarters of the world's population utilize plants to undertake health surveys [6, 7, 8]. Typically, the newest drugs, as well as natural products, are majorly made from plants and microbes [8, 9, 10, 11, 12, 13]. In drug synthesis from the natural products, bioassay-guided isolation is key and can be derived from traditional uses of local plants (ethnobotanical and ethnopharmacological applications) [14].

Seagrasses are marine plants found in large numbers in the tidal and sub-tidal parts most sear apart from those in the Polar Regions. The leafy part of seagrasses is widely used as food by people living in the coastal regions [15]. Various ailments, including skin problems, muscle pains, fiver, and stomach aches, and other ailments in folk medicine are treatable using seagrasses [16]. In India, seagrasses were famous for managing heart conditions, as fertilizer, nutritious purposes as well as animal feeds [17]. Seagrasses have been associated with antibacterial activities. Examples, *Cymodoceaserrulate*, *Halodulepinifolia* and *Halophilastipulacea* [18].

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The exploited genotoxicity assays based on molecular techniques. Random amplified polymorphic DNA (RAPD) assay based on PCR amplification of random DNA fragments of genomic DNA [19]. It has been utilized to detect DNA mutations and damage in various organisms [20, 21].

The present study was done to find out the antibacterial activity of seagrass, *Haloduleuninervis* from the Jeddah city (ObhurAljanoubiyah) in Saudi Arabia against some bacterial pathogenic strains.

Materials and Methods

Sample collection

Collection of fresh leaves from the intertidal region of Jeddah city (ObhurAljanoubiyah) 21º 42' 32.2" N 39º 05' 47.3" E (Lat.) and attended in the laboratory in sterile plastic bags with water to suppress the evaporation.

Extraction

Ten Grams of dried seagrasses leaves samples. Adding 100 ml of distilled water or organic solvents (Ethyl acetate, Ethanol, Chloroform and Petroleum ether) (1:10 W/V) to make the extraction by using separating funnel and shaking for 72 hours at room temperature. Whatman filter (No.1) paper is used to filter the solvent extract then the solvents are evaporating under low pressure to its dryness at 40ºC. All plant extracts were dissolved in DMSO and kept in small tight closed vials at low temperatures of 4ºC [22].

Bacterial Strains

Seven tested bacterial strains were (four Gram-positive: *Bacillus subtilis* (ATCC11774); Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC977); *Staphylococcus aureus* (ATCC29213) and *Micrococcus luteus* (ATCC4698) and three Gram-negative: *Escherichia coli* (ATCC8739); *Klebsiellapneumoniae* (ATCC700603) and *Pseudomonas aeruginosa* (ATCC27853). Microbiologics R USA provided the strains at King Abdul-aziz Hospital, Jeddah, Saudi Arabia providing the bacteria.

Antibacterial Activity

In accordance with agar well diffusion method, the antibacterial activities of the plant's extracts were tested against different test microorganisms. Into a Mueller-Hinton agar bacterium, a suspension of testing microorganisms was added. Making use of a sterile cork borer, three 5 mm diameter wells were made in the agar plate. Into each well, 50 ul of the tested leaves extracts was added to each well. Each of the plates was left for about an hour at 4ºC and thereafter incubated for 24 h at 37ºC. Inhibition zones which included the diameter of the discs were measured and results obtained compared to a negative control in DMSO as well as against a positive control with a different antibiotic [23].

DNA Extraction

QIAamp DNA Mini Kit (QIAGEN, USA) was used to extract Genomic DNA from bacterial samples extracted DNA was stored at -20ºC until further use, this in accordance to manufacturers.

Random amplified polymorphic DNA (RAPD) analysis

DNA (RAPD) analysis was applied according to [24].

Polymerase chain reaction (PCR)

Experiment Design

- *Pseudomonasaeruginosa*

Sample 1 (control) **PC**
Sample 2 (treated) **PT**

Table 1: Primer Sequence used for ***Pseudomonasaeruginosa***

Primer Name	Primer Sequence
UBC-208	ACGGCCGACC
UBC-228	GCTGGGCCGA
UBC-241	GCCCGAGCGG
UBC-270	TGCGCGCGGG
UBC-272	AGCGGGCCAA
UBC-275	CCGGGCAAGC
UBC-277	AGGAAGGTGC
UBC-287	CGAACGGCGG
UBC-325	TCATGATGCA
UBC-327	ATACGGCGTC
UBC-700	GGACTAAGGT
UBC-44	TTACCCCGGC

Results

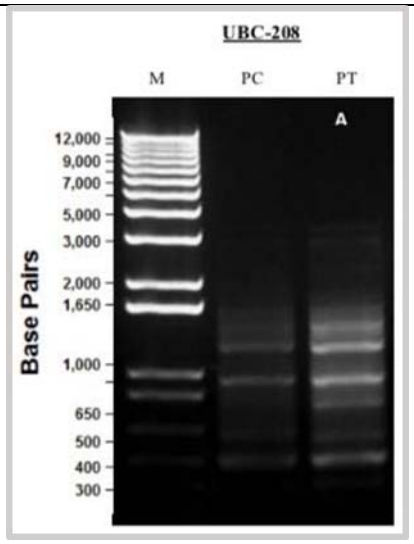
In all the four tested solvents extracts inhibited the growth of all bacterial pathogens strains by different zones. The inhibition at the highest level exhibited by ethanolic extract against *P. aeruginosa* showed (33.33 mm) followed by (24.67 mm) with *B. subtilis* in the same extract. Lowest zone of inhibition exhibited by petroleum ether extract against *S. aureus* was at (11.67mm). Aqueous extract exhibited no kind of inhibition with an exception to *P. aeruginosa* exhibiting at (14.67 mm) zone. In **Table 2,** all the antibacterial activity of *Haloduleuninervis* extracts on the seven bacterial pathogens are presented.

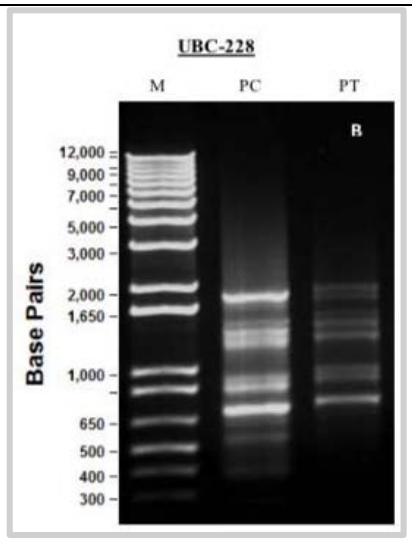
Table 2: Antibacterial activity of *Haloduleuninervis*seagrasses leaves extracts against tested bacteria using well diffusion assay.

Tested Bacteria	**Diameter of the inhibition zone (mm) Mean ± SD**				
	Ethanol	**Chloroform**	**Ethyl acetate**	**Petroleum Ether**	**Distilled water**
***Bacillus subtilis* ATCC11774**	24.67 ± 0.58	17 ± 0.0	15.67 ± 0.58	15.67 ± 0.58	0 ± 0.0
Methicillin-Resistant *Staphylococcus aureus* ATCC977	20 ± 0.0	18.33 ± 0.58	16.67 ± 0.58	16 ± 0.58	0 ± 0.0
***Micrococcus luteus* ATCC4698**	17.33 ± 0.58	15.67 ± 0.58	15 ± 0.0	14.33 ± 0.58	0 ± 0.0
***Staphylococcus aureus* ATCC29213**	15.67 ± 0.58	13.67 ± 0.58	12.67 ± 0.58	11.67 ± 0.58	0 ± 0.0
***Escherichia coli* ATCC8739**	17.33 ± 0.58	16.33 ± 0.58	15.33 ± 0.58	14.33 ± 0.58	0± 0.0
***Klebsiellapneumoniae* ATCC700603**	18.67 ± 0.58	17.67 ± 0.58	16 ± 0.0	14.67 ± 0.58	0± 0.0
***Pseudomonas aeruginosa* ATCC27853**	33.33 ± 1.15	18.33 ± 0.58	16.67±0.58	15.67 ± 0.58	14.67 ± 0.58

Molecular RAPD-PCR of *Pseudomonasaeruginosa*

Polymorphism was exhibited in twelve of the preselected random primers (RAPD) obtained from the DNAs of seven samples of PC: *Pseudomonas aeruginosa* Control, PT: *Pseudomonas aeruginosa* Treatment. Study *P. aeruginosa* under investigation was the DNA based analysis of plant extraction treatment. PCR products with varied fragments numbers were resultant from the use of all the primers as exhibited in (**Figure 1**). In all the twelve random primers, a total of 102 DNA fragments were detected, in total 37 were polymorphic representing a 36% of the total (**Table 3**).


UBC-208
M
PC
PT
A
Base Pairs
12,000
9,000
7,000
5,000
3,000
2,000
1,650
1,000
650
500
400
300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M
PC
PT
B
Base Pairs
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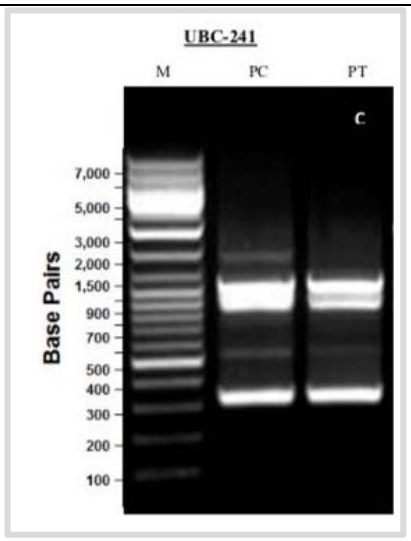
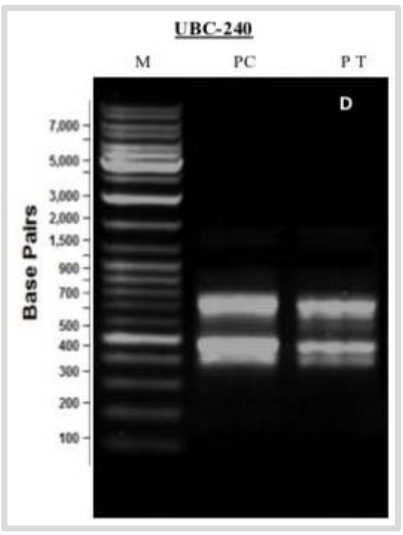
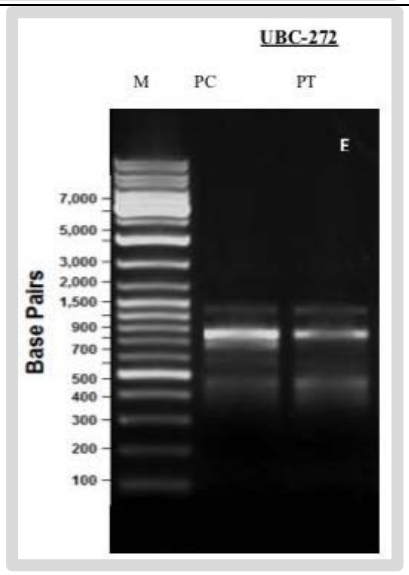
9,000
7,000
5,000
3,000
2,000
1,650
1,000
650
500
400
300


UBC-241
M
PC
PT
C
Base Pairs
7,000
5,000
3,000
2,000
1,500
900
700
500
400
300
200
100


UBC-240
M
PC
P T
D
Base Pairs
7,000
5,000
3,000
2,000
1,500
900
700
500
400
300
200
100


UBC-272
M
PC
PT
E
Base Pairs
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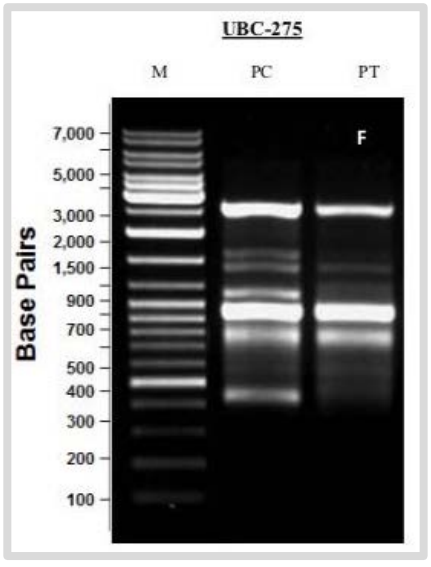
5,000
3,000
2,000
1,500
900
700
500
400
300
200
100


UBC-275
M
PC
PT
F
Base Pairs
7,000
5,000
3,000
2,000
1,500
900
700
500
400
300
200
100

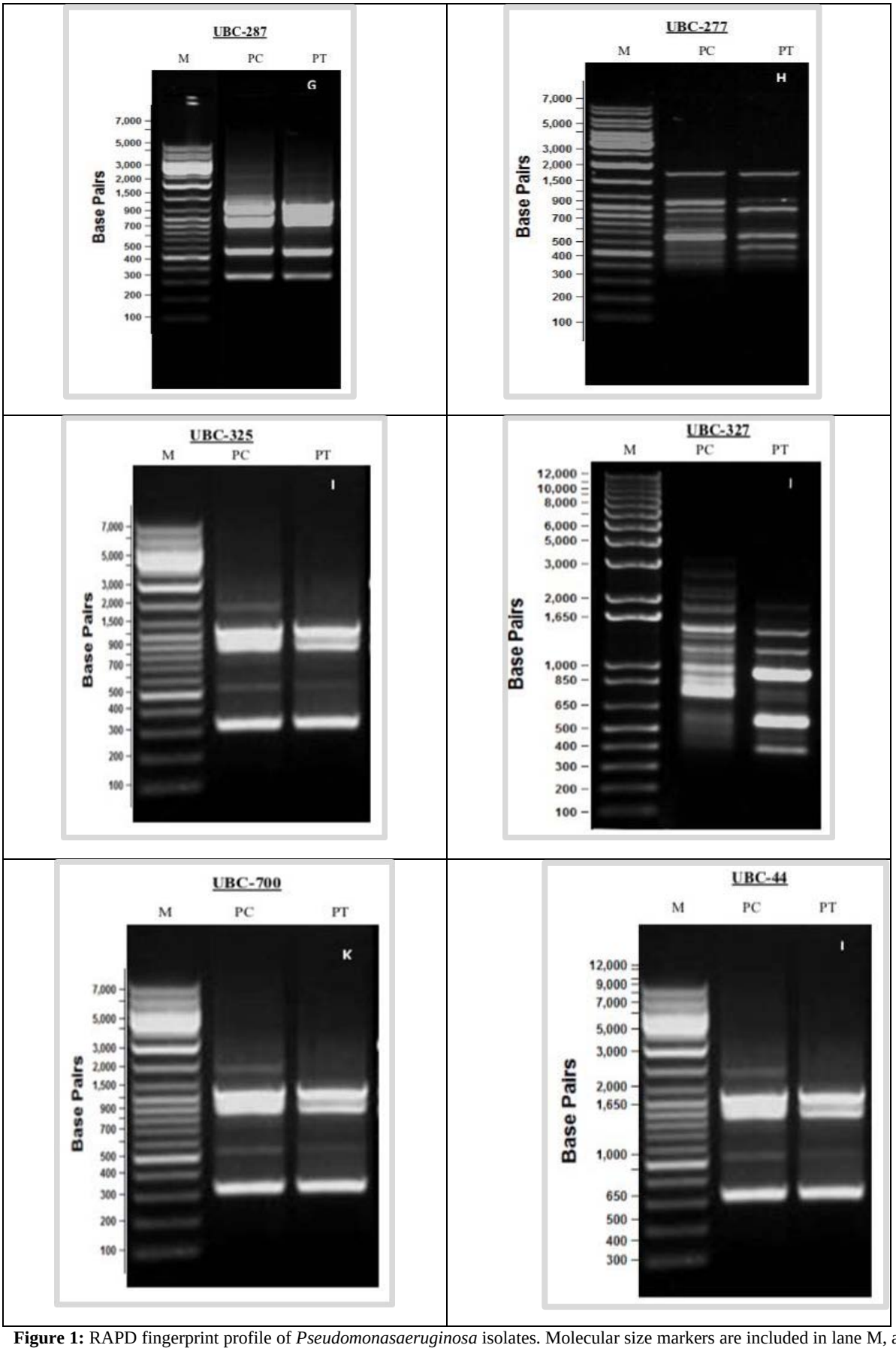


Figure 1: RAPD fingerprint profile of *Pseudomonasaeruginosa* isolates. Molecular size markers are included in lane M, and their sizes (in bases pairs) are indicated to the left of the gel. The key is indicated above each lane as: **PC:***Pseudomonasaeruginosa* Control and **PT:***Pseudomonasaeruginosa* Treatment.

Primer UBC-208 has 8 amplicons which included 3 polymorphic amplicons and 5 monomorphic fragments that shows 60% percent of polymorphic. The fragment sizes ranged from 500 to 1500bp (Figure 1.A). Primer UBC-228 has 12 amplicons which included 7 polymorphic amplicons and 5 monomorphic fragments that shows 71% percent of polymorphic. The fragment sizes ranged from 400 to 2000bp (Figure 1.B). Primer UBC-241 has 12 amplicons which included 6 polymorphic amplicons and 6 monomorphic fragments that shows 100% percent of polymorphic. The fragment sizes ranged from 300 to 2000bp (Figure 1.C). Primer UBC-270 has 3 amplicons which included 0 polymorphic amplicons and 3 monomorphic fragments. The fragment sizes ranged from 300 to 900bp (Figure 1.D).
Primer UBC-272 has 5 amplicons which included 2 polymorphic amplicons and 3 monomorphic fragments that shows 66% percent of polymorphic. The fragment sizes ranged from 400 to 1500bp (Figure 1.E). Primer UBC-275 has 8 amplicons which included 3 polymorphic amplicons and 5 monomorphic fragments that shows 60% percent of polymorphic. The fragment sizes ranged from 400 to 6000bp (Figure 1.F). Primer UBC-277 has 8 amplicons which included 2 polymorphic amplicons and 6 monomorphic fragments that shows 33% percent of polymorphic. The fragment sizes ranged from 400 to 4000bp (Figure 1.G). Primer UBC-287 has 6 amplicons which included 1 polymorphic amplicons and 5 monomorphic fragments that shows 20% percent of polymorphic. The fragment sizes ranged from 300 to 3000bp (Figure 1.H).
Primer UBC-325 has 6 amplicons which included 2 polymorphic amplicons and 4 monomorphic fragments that shows 50% percent of polymorphic. The fragment sizes ranged from 300 to 3000bp (Figure 1.I). Primer UBC-327 has 15 amplicons which included 9 polymorphic amplicons and 6 monomorphic fragments that shows 60% percent of polymorphic. The fragment sizes ranged from 300 to 2500bp (Figure 1.J). Primer UBC-700 has 13 amplicons which included 0 polymorphic amplicons and 13 monomorphic fragments. The fragment sizes ranged from 300 to 2500bp (Figure 1.K). Primer UBC-44 has 6 amplicons which included 2 polymorphic amplicons and 4 monomorphic fragments that shows 50% percent of polymorphic. The fragment sizes ranged from 300 to 2000bp (Figure 1.L).

Table 3: RAPD-PCR analysis of *Pseudomonas aeruginosa*

Primer name	Total amplicons	Monomorphic amplicons	Polymorphic amplicons	Percent of polymorphic	Range of amplicons size
UBC-208	8	5	3	60%	500 to 1500bp
UBC-228	12	5	7	71%	400 to 2000bp
UBC-241	12	6	6	100%	300 to 2000bp
UBC-270	3	3	0	0%	300 to 900bp
UBC-272	5	3	2	66%	400 to 1500bp
UBC-275	8	5	3	60%	400 to 6000bp
UBC-277	8	6	2	33%	400 to 4000bp
UBC-287	6	5	1	20%	300 to 3000bp
UBC-325	6	4	2	50%	300 to 3000bp
UBC-327	15	6	9	60%	300 to 2500bp
UBC-700	13	13	0	0%	300 to 2500bp
UBC-44	6	4	2	50%	300 to 2000bp

Discussion

In the recent past, the field of clinical treatment for infectious diseases has faced big problems due to the increasing resistance rate of microorganisms to the antibiotic drugs [25]. Various reports on the ability of mangrove, seaweeds and other marine plants to kill microorganism activities have been prepared with little written about the global seagrasses and there is also insufficient information about them [18]. This study aims at assessing and making a comparison on the potential of seagrass extracts in the synthesis of bioactive substance which can be utilized for therapeutic purposes. With the exhibition of antimicrobial activities seagrasses show an ability to produce bioactive secondary metabolites.
Three different leaves extracts of *H. uninervis* is against seven bacterial pathogens strains had an antibacterial activity that was effective. Of these, ethanol extract proved more effective against *P. aeruginosa* than evidenced with the other extracts. This demonstrated that ethanol has a higher suitability for extracting active compounds from seagrass. The earlier studies which suggested that the methanoic extract of *Enhalusacoroides* produced a stronger effect against *P. aeruginosa, S. aureus* and *K. pneumoniae* when compared to hexane extract supported the findings [26]. From this study, it was demonstrated that the ethanoic extract had the best antimicrobial activity which was affirmed earlier reports [27] and the methanolic and ethanoic extractions of the seagrasses *Halophilaovalis* and *Halodulepinafolia* with a better inhibition zones against tested bacteria were preferable to the other tested extracts [28].

Our most recent study revealed that Gram-positive bacteria were much stronger compared to the Gram-negative bacteria. This concurred with the findings of a certain report that there was an anti-fouling of various marine organisms against *Bacillus* and *Pseudomonas* sp. [29]. The inconsistencies of the extracts in the antibacterial activity can be as a result of the variations of antimicrobial agents from plant to plant [30].

The lowest antimicrobial effect was observed in the extracts of ethyl acetate of *H. uninervis*. The findings also revealed that *P. aeruginosa* had lesser sensitivity rate when the aqueous extract were used against them, as compared to other microorganisms. The findings confirm an earlier report that aqueous extracts of *C. rotundata* did not show any activity which showed very poor activity with all test bacteria [28].

Also, from the reported study of phytochemical analysis of chloroform, ethyl acetate ethanol and aqueous extracts of three glycosides have tannins and saponins. Quinine and sugars were absent in all the three seagrasses (*Cymodoceaserrulate, Haloduepinifolia*and*Halophilaovalis*) [31]. The presence of saponins, proteins, tannins, reducing sugars, resins, alkaloids, acidic compounds terpenoids and cardiac glycosides in the phytochemical analysis of *C. rotundata*.Glycoside, flavonoids, saponins, alkaloids, terpenoids and tannins which considered phytochemical compounds [32].

In the RAPD-PCR profile, the variation in intensity and the change of fragments number due to the genetic material change. The extent of damaged DNA and DNA efficiency to repair and replicate is referred to as the stability of genomic template (GTS %) [33]. For example, the stability of genomic template does not reduce as a result of the high level of damaged DNA from the inhibition of DNA replication and repair resulting from the lethal action of the plant extract.

From the results obtained it gives evidence to the ability of plant extract to mutate with an appearance of many genetic fragments in comparison to the untreated bacteria which serves as the control. The genetic techniques were obtained by [34, 35, 36].

Conclusion

Our study showed that the ethanolic leaves extract of seagrass *H. uninervis* gave better performance than other solvents extracts. Hence, the seagrass *H. uninervis* will be used as a source of extraction of new antibiotic compounds in the future. The result from this study form a basis for further studies of the potent seagrass so as to isolate the compounds responsible for the antibacterial activity. The treatments of *H. uninervis* extract caused genomic DNA alternation *in P .aeruginosa* cells and revealed that effected on *P .aeurginosa*. Furthermore, the present results demonstrate that RAPD technique as an investigation tool for detection of alterations in DNA produced by different extracts of medicinal plants.

References

1. Schmitz, F. J., Verhoef, J. and Fluit, A. C. (1999). Prevalence of resistance to MLS antibiotics in 20 European University hospitals participating in the European SENTRY surveillance program. J AntimicrobChemother; 43: 783-792.
2. Ravikumar, S., Thajuddin, N., Suganthi, P., Jacob Inbaneson, S. and Vinodkumar, T. (2010a). Bioactive potential of seagrass bacteria against human bacterial pathogens. J Environ Biol; 31: 387-389.
3. Ravikumar, S., Ramanathan, G., Subakaran, M. and Jacob Inbaneson, S. (2009). Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int J Med MedSci; 1(5): 184-191.
4. Ravikumar, S., Jacob Inbaneson, S., Suganthi, P. and Gnanadesigan, M. (2011). In vitro antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquinesensitive*Plasmodium falciparum*. Parasitol Res; 108: 873-878.
5. Sofowora, A. (1982). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd. Ibadan, Nigeria.
6. Farnsworth, N. R. (1994). Ethnobotany and the search for new drugs. John Wiley and Sons, New York.
7. Joy, P. P., Thomas, J., Mathew, S. and Skaria, B. P. (1998). Medicinal plants. Kerala Agricultural University, Kerala.
8. Harvey, A. (2000). Strategies for discovering drugs from previously unexplored natural products. Drug Discov Today 5 (7): 294-300.
9. Hayashi, K., Hayashi, T., Otsuka, H. et al. (1997). Antiviral activity of 5,6,7- trimethoxyflavone and its potential of the antiherpes activity of acyclovir. J AntimicrobChemother 39: 821-824.
10. Armaka, M., Papanikolau, E., Sivropoulou, A. and Arsenakis, M. (1999). Antiviral properties of isoborneol, a potent inhibitor of herpes simplex virus type 1. *Antiviral Research* 43**:**79–92.
11. Lin, Y. M., Flavin, M. T., Schure, R., Chen, F. C., Sidwell, R., Barnard, D. L., Huffman, J. H. and Kern, E. R. (1999a). Antiviral activities of bioflavonoids. Planta Med 65: 120-125.
12. Lin, Y. M., Flavin, M. T., Schure, R., Zembower, D. E. and Zhao, G. X. (1999b). Biflavanoids and derivatives there of as antiviral agents. United States Patent 5948918.
13. Basso, L. A., da Silva, L. H., Fett-Neto, A. G., de Azevedo, Jr. WF., de Moreira, I. S., Palma, M. S., Calixto, J. B., Astolfi-Filho, S., dos Santos, R. R., Soares, M. B. and Santos, D. S. (2005). The use of biodiversity as source of

new chemical entities against defined molecular targets for treatment of malaria, tuberculosis, and T-cell mediated diseases; A Review. Mem Inst Oswaldo Cruz 100: 475-506.

14. Atta-ur-Rahman and Choudhary, M. I. (1999). Recent studies on bioactive natural products. Pure ApplChem 71 (6): 1079-1081.
15. Hemminga, M. A. and Duarte, C. M. (2000). Seagrass ecology. New York. pp: Cambridge University Press; 298.
16. de la Torre-Castro, M. and Rönnbäck, P. (2004). Links between humans and seagrasses – An example from tropical East Africa. Ocean and Coastal Management, 47, 361–387.
17. Newmaster, A. F., Berg, K. J., Ragupathy, S., Palanisamy, M., Sambandan, K., and Newmaster, S. G. (2011). Local knowledge and conservation of seagrasses in the Tamil Nadu state of India. Journal of Ethnobiology and Ethnomedicine, 7, 37.
18. Kannan, R. R., Arumugam, R. and Anantharaman, P. (2010). Antibacterial potential of three seagrasses against human pathogens. Asian Pacific Journal of Tropical Medicine; 890-893.
19. Atienzar, F. A., Conradi, M., Evenden, A. J., Jha, A. N. and Depledge, M. H. (1999). Qualititative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a] pyrene. Environmental Toxicology and Chemistry. 18: 2275-2282.
20. Savva, D. (1998). Use of DNA fingerprinting to detect genotoxic effects. Ecotoxicol, Environ. Safety, 41, 103-106.
21. Atienzar, F. A., Cheung, V. V., Jha, A. N. and Depledge, M. H. (2001). Fitness parameters and DNA effects are sensitive indicators of copper induced toxicity in *Daphnia magna*. Journal of Toxicological Sciences. 59: 241-250.
22. Boeru, V. and Derevici, A. (1978). Some chemical and physical data on Romania propolis. Apimondia "propolis" Bucharest, 19-26.
23. Egorove, N. (1985). Antibiotics scientific approach. Mirpublishers Moscow.
24. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Research, 6531-6535.
25. Ravikumar, S., Ramanathan, G., Jacob Inbaneson, S. and Ramu, A. (2010). Antiplasmodial activity of two marine polyherbal preparations from *Cheatomorphaantennina* and *Aegicerascorniculatum* against *Plasmodium falciparum*. Parasitol Res. doi: 10. 1007/ s00436-010-2041-5.
26. Alam, K., Agua, T., Maven, H., Taie, R., Rao, K. S., Burrows, I. (1994). Preliminary screening of seaweeds, seagrass and lemongrass oil from Papua New Guinea for antimicrobial and antifungal activity. Pharmaceutical Biol; 32(4): 396-9.
27. Umamaheshwari, R., Thirumaran, G. and Anantharaman, P. (2009). Potential antibacterial activities of sea grasses from Vellar Estuary; Southeast coast of India. *Advances in Biological Research*,3(3-4): 140-143.
28. Mani, A. E., Bharathi, V. and Patterson, J. (2012). Antibacterial Activity and Preliminary Phytochemical Analysis of Sea Grass *Cymodocearotundata*. International Journal of Microbiological Research; 3 (2): 99-103.
29. Bhosale, S. H., Nagle, V. L. and Jagtab, T. G. (2002). Antifouling potential of some marine organisms from India against species of *Bacillus* and *Pseudomonas*. Marine Biotechnol; 4: 111-8.
30. Lustigman, B. and Brown, C. (1991). Antibiotic production by marine algae isolated from the New York/New Jersey Coast. Bull Environ ContamToxicol; 46: 329-35.
31. Sangeetha, J. and Asokan, S. (2016). Phytochemical analysis and antibacterial activity of the three different seagrass extracts. International Journal of Advanced Research. Volume 4, Issue 5, 1451-1457.
32. Ergene, A., Guler, P., Tans, S., Miric, S., Hamzaoglu, E. and Duran, A. (2006). Antibacterial and antifungal activity of *Heracleumsphondylium* subsp. artvinense, Afirican J. Biotech., 5: 1087-1089.
33. Rocco, L., Izzo, A., Zito, G., Peluso, C. and Stingo, V. (2011). Genotoxicity in zebrafish (Danio rerio) exposed to two pharmacological products from an impacted Italian river. J Environ Anal Toxicol, 1(103), pp.2161-0525.
34. Adam, S. E., AL–Farhan, A. H., AL–Yahya, A. (2000). Effect of combined Citrulluscolocynthis and Rhazgastricta use in Najdi sheep. Am. J. Chin. Med. 28:385-390.
35. Morita, H., Awang, K., Hadi, A. H., Takeya, K., Itokawa, H. and Kobayashi, J. (2005). Conformational analysis of rhazinilam and three-dimensional quantitative structure-activity relationships of rhazinilam analogues. Bioorg. Med. Chem. Lett. 15 (4):1045-50.
36. Gilani, S. A., Kikuchi, A., Shinwari, Z. K., Khattak, Z. I. and Watanab, K. N. (2006). Phytochemical, pharmacological and ethnobotanical studies of RhazyastrictaDecne. Phytother. Res. 21:301-307.