ISSN: 2287-6898
International
Journal of Bio-Pharma Research
Volume 8, Issue 3 (2019) pp.2494-22503
Research Article Open Access

Bioactive potential of *Pseudomonas alcaliphila* isolated from a marine sponge against human pathogens

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Abstract: Metabolite extraction is considered as one of the important steps in metabolomics, the marine metabolite are the new source of the most antimicrobial agents used in both pharmacological and biological applications. In the present study, sponge associated bacterial metabolites was investigated. A total of 20 bacterial strains were isolated from the sponge *Haliclona* sp., All the strains were screened primarily with cross streaking method against human bacterial pathogens. The potent isolate was chosen based on the good inhibitory activity and metabolite extraction was achieved using chloroform: methanol mixture. The metabolites were then checked for their antimicrobial activity by disk diffusion and also minimum inhibitory concentration was determined. Out of 20 bacterial strains, only one strain selected based on the good inhibitory activity against pathogens and the strain was identified as *Pseudomonas alcaliphila* based on the biochemical and16S rRNA sequencing. The results revealed that the metabolites exhibited high activity and it was found that *Klebsiella pneumoniae* was inhibited high with the diameter of 22 mm followed by *Salmonella* Typhi (15 mm), *E.coli* (12 mm), and *Bacillus subtilis* (15 mm). The MIC was observed at 31.25 µg/ml against all pathogens. Results of TLC exhibited the Rf value at 0.86 and the FTIR results revealed the presence of C=o, amide bond, amino acids and methoxy groups. In GC-MS results showed that the metabolites mostly contain fatty acids and alkenes compounds. Thus, this marine active compound was considered as a novel compound for biological applications and may be a potential drug for therapeutic use.

Key words: Marine sponge, Antibacterial activity, Minimum Inhibitory Concentration, GC-MS, Bioactive metabolites, *Pseudomonas alcaliphila.*

Introduction

In recent years, microorganisms associated with the surfaces of marine eukaryotes have been major targets for the discovery of new bioactive metabolites that can be used as an antibiotic agent (1-3). This is a primary notion of both microorganisms and host had developed their chemical defense strategies in the competitive environment for their survival (4-5). Around 2,500 bioactive metabolites produced by the microbes have been reported between 1985 - 2008 that are useful for human health (6). Many of the compounds having structural similarities which have shown the broad chemical diversity, and comprised of unusual nucleotides, terpenes, alkaloids, peptides and polyketides, they are extensively used as an antibiotic, anticancer drugs, anti infective, anti-inflammatory and neuro-protective agents (7). The marine derived drugs are used for several treatments, for example rifamycin (*Micromonospora* sp.); Salinosporamide-A (*Salinaspora* sp.,) and marinomycins (*Marinophilus* sp.,); are derived from the associated microbes and they are used as antimicrobial and anticancer drugs and they also increased the mortality rate among the immuno-compromised patients (8). To overcome the situation, there is a need to improve the drugs or discover the class of antibiotics with different mode of action. Many studies were reported antimicrobial activity of the volatile compounds, fatty acid, long chain fatty alcohols, esters and alkene. It has been reported that the activity increases with the length of the carbon chain. Several studies indicated the phytochemical compounds have the appropriate activities, shown the significant role of long chain fatty acid against pathogens (9). The present work focuses on the isolation of marine microbes from sponges and identification of the presence of natural compounds from sponge associated bacteria collected from the south

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east coast of Tamilnadu in the Rameshwaram region.

Materials and Methods

Pathogens used in this Study:

Clinical bacterial pathogens namely *Bacillus subtilis, Salmonella* Typhi, *E. coli* and *Klebsiella pneumoniae* were used in this study. All the bacterial pathogens were kindly provided by Dr.T.Ramamurthy, Dy. Director (Senior), National Institute of Cholera and Enteric Disease, Kolkata.

Sponge collection:

Sponges were collected from Mandapam (Tamilnadu, India) by the hand pricking method. The collected sponges were transferred to a sterile polythene bag and transported at 4° C to the laboratory for the isolation of sponge associated microorganisms. The sponges were identified and confirmed based on the skeleton and morphological characteristics.

Isolation of sponge associated bacteria:

The associated bacteria were isolated by standard method; sponges were rinsed with tap water to remove the debris present in the upper portion and again washed with sterile distilled water. Samples were cut into small pieces using sterile scissors, ground with mortar and pestle. Approximately 1g of the sample was taken and transferred into 250 ml Erlenmeyer flask containing 99 ml of sterile phosphate buffered saline (PBS) (or) sponge dissociation medium and shaken continuously for 30 min to separate the associated form of bacteria. Further tenfold dilution were made with PBS up to desired level and 0.1 ml was taken from the diluted sample and plated onto the ZoBell Marine Agar (ZMA) and Nutrient agar (NA) medium. Plates in duplicate were incubated at room temperature (25-28°C) for 3-5 days. At the end of the incubation period the different morphological colonies were selected and purified for the screening of bioactivity.

Screening and identification of the sponge associated bacteria:

All the pure isolates obtained from sponges with different morphological features were primarily screened for their bioactivity by cross streaking method against various bacterial pathogens such as *Bacillus subtilis, Salmonella* Typhi, *Pseudomonas aeruginosa, E.coli* and *Klebsiella pneumoniae*. The test strains were streaked perpendicularly across pathogens in Muller Hinton Agar plate (MHA) and the plates were incubated at 37°C for 24 h.

Identification of symbiotic bacteria:

All the isolates which exhibited antimicrobial activity was identified through microscopic and biochemical analysis described in the Bergeys Manual of determinative bacteriology (2nd Edition, 2001-2012).

Genomic DNA Extraction:

The Genomic DNA was isolated from bacteria by the following method. A single bacterial pure colony was inoculated into 15 ml of Luria Bertani (LB) broth and incubated for overnight at 37° C. The LB broth was taken and centrifuged at 10,000 rpm for 15 min. The supernatant was resuspended with 400 µl of TE buffer containing sucrose and lysozyme, the tubes were incubated at room temperature for 30 min. After incubation 100 µl of 0.5 M EDTA and 60 µl of 10% SDS was added to the tube and incubated for 30 min at room temperature, then the proteinase K was added to the tube and incubated for 55°C for 12 h. In addition phenol: chloroform: isoamyl alcohol (25:24:1) was added to the solution and gently mixed the tube carefully then centrifuged the tube at 10,000 rpm for 10 min. After that, the aqueous layer was collected and 1µl of sodium acetate was added into the tubes, then 1 ml of 100% ethanol was added and spins the tube at 12,000 rpm for 15 min. Then, the supernatant was discarded and dried the pellet; DNA was analyzed in 1% agarose gel electrophoresis. Finally, the DNA was stored at -20°C for further use.

Amplification of Genomic DNA:

The potent strain was characterized by 16S rRNA gene sequencing. The isolated DNA was amplified using the Universal primers 27F-5' AGAGTTTGATCMTGGCTCAG 3', 1492R-5' TACGGYTACCTTGTTACGACTT 3'. A PCR was performed with DNA 5 µl, forward primer 1.5µl, reverse primer1.5 µl, Taq master mix 12 µl and the total volume was 25 µl. The amplification conditions were as follows initial 94°C for 3min (denaturation), 94°C for 30sec (denaturation), 60°C for 30sec (annealing), 72°C for 1 min (extension) and 72°C for 10 min (final

extension). Expected PCR products were electrophoresed on a 1% agarose gel electrophoresis and illuminated by UV transluminator. The PCR product was purified by Montage PCR Clean up kit (Millipore) to remove the incorporated dNTPs. One microlitre of purified product was sequenced by automated sequence analyzer (Applied Biosystem-3500,capillary sequencer).Further analysis of sequence was performed by similarly searching tool, NCBI BLAST server (http://www.ncbi.nlm.nih.gov/BLAST) and the phylogeny also constructed by the use of Phyml tool.

Production and extraction of the bioactive compounds:

The potent isolate was cultivated in nutrient broth with $CaCl_2$ 0.015 g, $MgSo_4$ 0.02 g, KCl 0.01g, (NH4)2SO4 0.05g, NaCl 0.01g, and glucose 1% and sterilized at 121° C for 15 min. A single colony from the nutrient agar plate was inoculated into the 10 ml of nutrient broth and incubated at room temperature for 24 h. After incubation, the bacterial culture was transferred into the 1000 ml Erlenmeyer flask containing 500 ml of sterile nutrient broth and incubated for 48 h at room temperature. After incubation, the culture was filtered through Whattman filter paper no: 1, then the cells was harvested at 10000 rpm for 20 min and the cell free supernatant was collected. The metabolite was extracted by solvent extraction method by adding an equal volume of chloroform and methanol. Then metabolites were separated in separating funnel and concentrated under vacuum evaporator at 40°C and the extract was maintained at 4°C for further analysis.

The antimicrobial activity:

The bioactivity of the extract was analyzed by disk diffusion method with MHA plates. Overnight grown test pathogens were plated as lawn culture and left it for 60 sec. Then the disks were impregnated with extracts in different concentration ranged from 10-50 µg/ml and placed the disks onto the plates and incubated the plates at 37°C for 48 h. After incubation the inhibitory activity was observed and recorded.

The determination of minimum inhibitory concentration (MIC):

The minimum inhibitory concentration of metabolites was tested against pathogenic microbes in 96-well microtitre plate method. The pathogenic bacterial cultures were grown on nutrient broth for overnight which was adjusted with 0.5 McFarland tubes. The 0.3 mg of metabolites were dissolved in 2ml of chloroform and methanol solutions and filter through the whatmann filter no: 1. Then it was diluted into 500 µg, 250 µg, 125 µg, 62.5 µg and 31.25 µg/ml in PBS. Aliquots of 100 µl of media and 100 µl of pathogens were seeded into the appropriate well. The test pathogens were used as negative control and the amoxicillin used as positive control. The MIC was determined by the lowest concentration of inhibition after incubation at 37°C for 24 h and the plate was read at 593 nm.

Characterization with thin layer chromatography (TLC):

The extract was collected and the purity was characterized by thin layer chromatography. The sample was spotted by using capillary tubes on to the silica coated plate, allowed to dry and then the plates were developed with the solvent mobile phase by using chloroform: methanol solvent system in 1:1 ratio for 30 min. The developed plates were observed through UV- transilluminator and the retention factor was calculated by the formula:

RF value = Distance travelled by the solute / Distance travelled by the solvent

FTIR:

The FTIR spectrum of the extract was conducted via the IR-470 model. The functional group of IR spectra was determined with the help of FTIR correlation charts. The FTIR % transmittance was recorded in various numbers (cm^{-1}). The wave number region was 3500-500 (cm^{-1}). The metabolite was prepared in one compression drop between two KBr disks.

GC-MS analysis:

The solvent extract was subjected to GC-MS analysis using the model instrument Quadrapole spectrometer with a capillary column of 0.25 µm film thickness × 0.25 mm Id × 30 mm length. Analysis was performed by injecting 1µl of the sample with a split

ratio of 20:1 Helium gas (99.9%) as the carrier gas at a flow rate of 1 ml/min. The reaction was performed with 70 eV of ionization energy and the injector column temperature was maintained at 25°C (held 3 min) and raised at 10°C per min to 280°C (3 min) and finaly it was held at 300°C for 10 min. The compounds were identified after comparing the spectral configuration with available database (NIST library).

Results

Isolation, screening and identification of sponge associated bacteria:

A total of 20 isolates were obtained from the sponge *Haliclona* sp., the isolates were recovered and maintained on Zobell marine agar plates. The isolation based on the cultural characteristics mostly they are round, smooth, translucent, some of the colonies were pigment producers, round and smooth surfaces and they are fast growers on the nutrient's rich plates. All the twenty isolates were screened for antibacterial activity against *Bacillus subtilis, Salmonella* Typhi, *E.coli,* and *Klebsiella pneumoniae* by cross streaking method and the results revealed that only five strains exhibited the good inhibition against the *Bacillus subtilis, E.coli* and *Salmonella* Typhi (table 1 and fig 1). From these five isolates, only one isolates showed very high activity against the pathogens tested and was tentatively identified as *Pseudomonas* sp., through phenotypic characterization (table 2). This was reconfirmed through 16Sr RNA sequencing analysis it showed the potent bacterial isolate was 99% similarity with the species of *Pseudomonas alcaliphila* (fig 2).

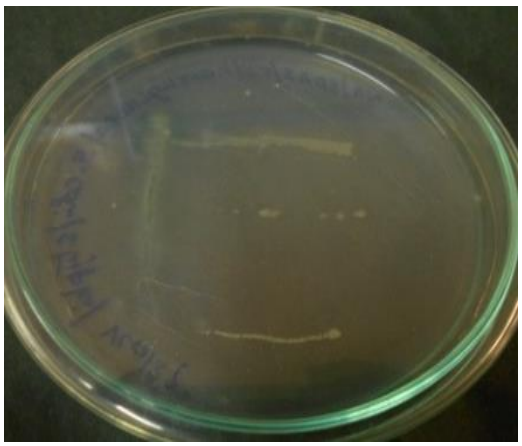


Figure 1: Showing the primary screening on Muller Hinton agar plate

Table 1: Primary Screening of bioactivity of marine bacterial isolates against bacterial pathogens by the cross-streaking method

S.No.	Isolated strains	*Bacillus subtilis*	*E. coli*	*Salmonella* Typhi	*Klebsiella pneumoniae*
1	SgM1	+++	+++	++	+++
2	SgM2	++	+	+	+
3	SgM3	++	+	++	++
4	SgM4	+++	+	++	+
5	SgM5	+	+	++	+
6	SgM6	-	-	-	-
7	SgM7	+	-	++	++
8	SgM8	+	+	-	+
9	SgM9	+	+	++	+
10	SgM10	+	+	++	+
11	SgM11	+	+	++	+
12	SgM12	+	+	++	+
13	SgM13	-	+	-	+
14	SgM14	++	-	-	-
15	SgM15	-	+	-	-
16	SgM16	-	-	-	-
17	SgM17	+	-	-	+
18	SgM18	-	-	+	-
19	SgM19	-	-	-	-
20	SgM20	-	+	-	+

(+ mild inhibition, ++ good inhibition, +++ high inhibition, - no inhibition)

Antimicrobial activity:

The extract was subjected to antibacterial activity against human bacterial pathogens in the disk diffusion assay. It was found that the high activity was observed against *Klebsiella pneumoniae* and the zone of inhibition was 22mm in diameter. The moderate activity was found against *Bacillus subtilis* (15 mm), *Salmonella* Typhi (15 mm) and *E.coli* (12 mm) with 30 µg/ml of concentration (table 3).

Table 2: The biochemical identification of marine bacteria SGM1

Biochemical Tests	SgM1
Cultural characters	Small Creamy, Translucent
Isolation medium	ZMA
Simple staining	rod
Gram staining	-
Catalase	+
Oxidase	+
TSI	K
Citrate utilization	+
Carbohydrate fermentation	+
Indole	+
MR-VP	-
Gelatin hydrolysis	+
Motility with H_2S	+

(+ Positive, - negative, k-alkali)

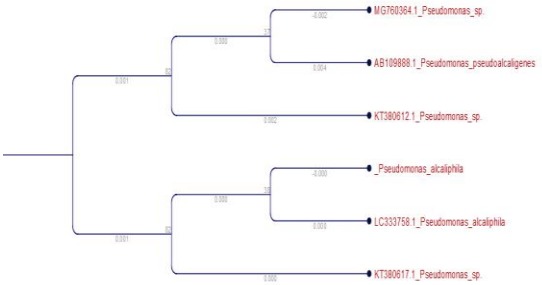


Figure 2: Showing the Phylogenetic tree of the potent SGM1 strain.

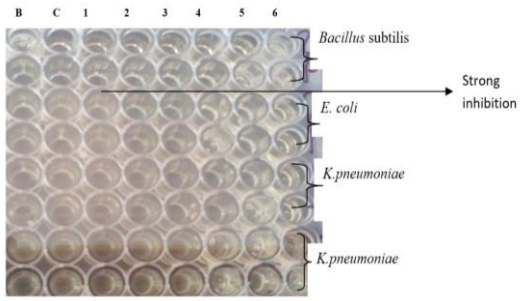
Table 3: Antimicrobial activity of the metabolite SGM1 against human bacterial pathogens

Metabolite Concentration (µg/ml)	Human bacterial pathogens			
	Bacillus subtilis	*E. coli*	*Salmonella typhi*	*Klebsiella pneumoniae*
10	5 mm	12mm	15mm	19 mm
20	14 mm	8 mm	16 mm	15 mm
30	15 mm	12mm	15 mm	22mm

The MIC of the secondary metabolite:

The MIC of the extracts was determined by microtitre plate assay using different concentrations ranged from 31.25-500 µg/ml. The compounds were tested against *Bacillus subtils, E. coli, Salmonella* Typhi and *Klebsiella pneumoniae* pathogens (fig 3). Among the various concentration 31.25 µg/ml concentration showed the good inhibitory on the microtitre plates and the remaining were shown the moderate inhibition when compared with the 31.25 µg/ml.

Figure 3: Determination of minimum inhibitory concentration of metabolite.



(B-negative control, C-positive control, 1-31.25, 2-31.25, 3-61.25, 4-125, 5-250, 6-500 µg/ml).

Thin Layer Chromatography:

The solvent system of chloroform: methanol (30:70) selected based on the polarity to separate the active compound.

The metabolites were spotted on the analytical TLC plate which was examined in UV and iodine chamber and the results showed the single spot on the plate, the Rf value was found as 0.86 (fig.4). The spot showed blue color on UV illumination and iodine chamber. The active compound was characterized for further analysis.

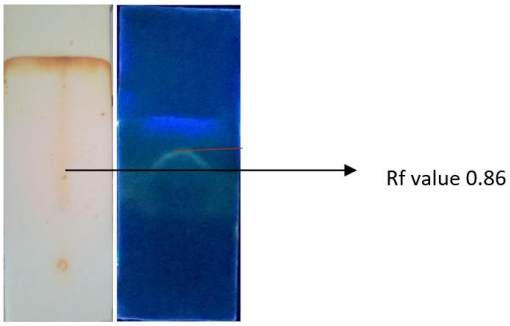


Figure 4: The active metabolites on TLC plates

FTIR:

A total of nine functional groups were observed and the wave range was 3361.00, 2928.84, 2161.79, 1637.12, 1557.12, 1405.07, 1271.01, 1069.07, 799.13, 648.65(cm-1) (fig 5 &table 4). However, in addition, one unknown wave range of 2161.79 was detected and this functional group was classified as fatty acids, alkene, amino groups, volatile oil, hydrocarbons, fatty acid esters.

Table 4: The functional groups of active compound

FTIR	Functional groups
648.85	Fatty acids
799.13	Fatty acids C-H-CH2
1069.07	C=N stretches aliphatic amines
1271.01	C-O stretch, ester, carboxylic acids
1405.07	C=0 symmetric stretch COO-amino acids, fatty acids
1557.12	Aromatic C-C ring
1637.12	Amide I of β-pleated sheet stretch
2161.79	Unknown compound
2928.84	C-H stretch of CH2
3361.00	N-H, O-H vibration

GC-MS:

The extract was partially purified by TLC analysis. The partially purified extract was subjected to the GC-MS analysis, chemical profile was also obtained with NIST library. The molecular weight also identified based on the spectral values of the mass spectrometry. The GC-MS analysis of the chloroform, methanol extract was carried out and the fractions showed the presence of fatty acids, esters, alkenes and acidic compounds. 23 peaks indicated the presence of active compounds. Most widely, dodecene (1.6%), eicoscene (0.2%), tetradecene (0.9%), cyclodecasiloxane eicosamethyl (1.37%), and heptasiloxasane hexadecamethyl (0.351%) showed the antimicrobial and anticancer activity and exihibitted antioxidant activity (fig 6 & table 5).

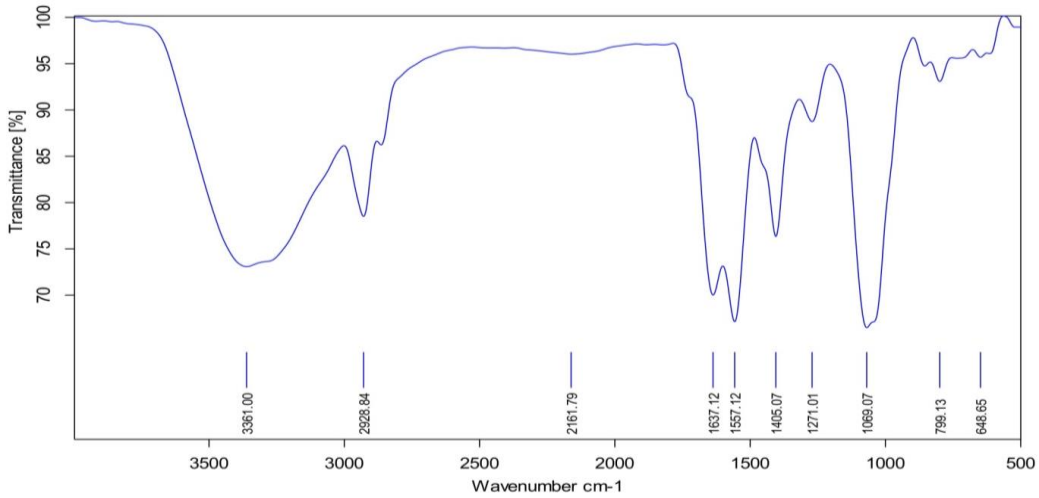


Figure 5: The active molecules functional groups of isolated compounds

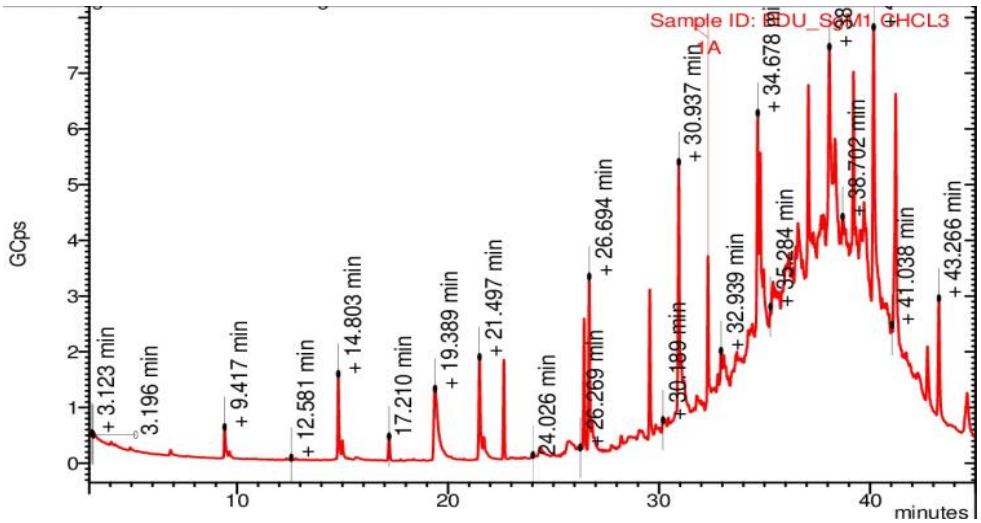


Figure 6: Partially purified molecule of SGM1 analyzed by Gas chromatography (NIST library).

Table 5: The bioactive property of compounds on GC-MS analysis

Compound name	Formula	% Area	RT	Activity	Co.Na
9-Eicoscene	$C_{20}H_{40}$	1.01	26.694	Antimicrobial	Chain fatty acid
Nonadecene	$C_{19}H_{38}$	0.68	28.88	Antifungal	Long chain fatty acid
Cyclohexasiloxane dodecamethyl	$C_{12}H_{36}O_6Si_6$	1.37	9.46	Antifungal activity	Long chain fatty acid
Cyclodecasiloxane, eicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	2.01	11.3	Antimicrobial	Esters
Hexacosene	$C_{26}H_{54}$	0.11	19.08	Antimicrobial	Alkenes
9-Eicosene	$C_{20}H_{40}$	1.01	25.50	Antimicrobial	Chain fatty acid
Dodecane	$C_{12}H_6$	170.34	0.339	Antioxidant	Chain chain alkene
Eicosane	$C_{20}H_{42}$	282.54	0.250	Antibacterial	Alkene
1-Dodecanol	$C_{20}H_{42}O$	186.33	0.676	Antioxidant Antibacterial	Long chain fatty acid alcohol
Phenol,2,4-bis (1,1-dimethyl ethyl)	$C_{14}H_{22}O$	206.32	1.115	Antibacterial	Aromatic hydrocarbons
Nonadecanol-1	$C_{19}H_{40}O$	284.52	7.956	Antimicrobial, cytotoxic	Long chain fatty acids
Hexadene	$C_{16}H_{34}$	226.44	0.806	Antimicrobial Antioxidant	Long chain hydrocarbons
Octodecanoic acid, 9,10-dichloro-methyl ester	$C_{19}H_{36}Cl_2O_2$	367.40	4.166	Antibacterial	Unsaturated fatty acids
Cyclo-decasiloxane eicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	740	1.65	Antimicrobial	Cyclic methyl siloxane
Octasiloxane1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15 hexadecamethyl	$C_{16}H_{50}O_7Si_8$	578	1.90	Antimicrobial and Pharmacological	Fatty acid ester
Cyclohexasiloxane dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	2.61	Pharmacological	Volatile organic
Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$		1.75	Pharmacological	Cyclic methyl siloxane

Discussion

The search of novel antimicrobials has gained the urgency because of increased development of resistance by pathogens against one or more antibiotics. Marine bacteria have been recognized as an important source for novel bioactive compounds. The coastal region found to be excellent to collection of sponges (9). They are the part of benthic fauna and live in all area of the marine world. During the last decade, several bioactive compounds have been isolated from marine bacteria which are new sources for development of medically useful compounds (10). Recent studies indicated that the symbiotic bacteria showed the specificity against pathogenic bacteria, they may also contain several bioactive metabolites and potentially able to inhibit the target organism (11). Approximately, 99% of sponge associated bacterial endosymbionts are uncultivable under laboratory condition using available media. So, investigations based associated bacterial study was expanded. In this study, the bacteria were cultivated from the sponge *Haliclona* sp., a total of 20 culturable bacteria was obtained and screened against bacterial pathogens.

Among 20 strains, only five showed the antibacterial activity in cross streaking method, rest of the bacteria showed the mild inhibition against all tested pathogens. Among five, only one strain was chosen based on the good inhibitory activity against pathogens. In this study, the marine bacteria was named as SGM1 and this isolate was identified through biochemical and molecular level, it was catalase positive, oxidase positive, gram negative rod shaped motile organism. It was also identified through 16S rRNA sequencing as *Pseudomonas alcaliphila.* Many of the studies have reported the gram-negative bacteria was isolated from the sediments, algae, water and sponges from marine environment, it contains a large diversity of gram-negative bacteria (12). The genus *Pseudomonas* is known for the good candidate for the production of secondary metabolites with good antimicrobial, antifungal, and cytotoxic property (13-14). The microbial fermentation will provide the tremendous amount of metabolites that are useful for as antibiotic and therapeutic agents. Marine sponge derived bacterial metabolites were extracted by fermentation sequentially with chloroform and methanol extraction with the production

media contained carbon and nitrogen sources and the desired growth was determined up to 48 h. In addition to that the MIC also determined the active compound using different concentration, the IC_{50} value showed 31.25 µg/ml against the pathogens and the antimicrobial activity was also determined in disk diffusion method and the zone of inhibition was at the concentration of 30 µg/ml against bacterial pathogens which showed the good inhibition against *Bacillus subtilis, Salmonella* Typhi, *E.coli* and *Klebsiella*. Mukherjee (15) and Al-abd (16) reported that the long chain fatty acids had the antibacterial activity against pathogens and another study (Naoko *et al.,* 2007) also reported the same type of fatty acids had the antibacterial activity against *Klebsiella pneumoniae.* Most of the studies revealed that the fatty acids and alcohols had the good inhibitory activity against both gram - positive and gram- negative bacteria (*Bacillus subtilis, Enterobacter fecalis, E. coli, S. aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) (17). The compound extraction is one of the most effective and useful for pharmacological applications from marine microbes. In this study chloroform and methanol was used based on the polar and non-polarities. The metabolites possesd the good inhibitory activity against the human pathogens so, the compound was characterized by chromatographical methods like TLC, FTIR and GC-MS analysis. In TLC result, it showed the Rf value was found at 0.86, it was closely matched with the lipopeptide compounds reported by Walter *et al.* (2010) (18). So, it was confirmed that the compound had both fatty acid and some kinds of active molecules. In yet another report, *Streptomyces* isolates obtained from marine sponges produced antimicrobial compounds that showed the Rf value at 0.78 in TLC plates which was also closely related one and confirmes the presence of lipids and polyene compounds (19). From that the functional group was determined by FTIR analysis which showed active molecules like carboxyl, hydroxyl and amino acid, alkenes, hydrocarbons profile at 3361, 2928, 2161, 1637, 1557, 1405, 1271, 799, 648 (cm-1) at various retention time the comparison. This was endorsed by several reports that showed the lipopetide functional characterization (20). In GC-MS also it was found 23 active metabolomic peaks at various retention time and the result revealed that tetracosamethyl-cyclododecasiloxane is one of the biologically active compound possessed hepato protective activity, cyclohexasiloxane, dodecamethyl- is widely used as a conditioning agent, emollient, in personal care products, lubricant and de-foaming agent. This was confirmed with the previous studies of Ghoibi *et al.,* (2011) (21-22). Also, in this study the same type of molecules was screened and had the potent antimicrobial activity, silicone oil is a polymerized siloxane with organic side chains. Consumer products to control flatus (anti- flatulents) often contain silicone oil. Silicone oils have been used as a vitreous fluid substitute to treat difficult cases of retinal detachment (23). The fatty acid ester group contains effective anti arthritic, anti-acne and anti-androgenic property. The alkene and hydrocarbons were essential for oil production have good antimicrobial activity (24-27). Maheswari and Saraswathy reported that ethyl acetate extract of *Bacillus* derived alkene compounds possessed the pharmacological activity and also various *Streptomycete* and *Actinoycete* derived compounds showed the biological activity when compared to the plant derived phytochemicals (28). Thus, this study concluded that all active compounds contained the biological activity and beneficial for human health, hence, the bacteria derived compounds are considered as a novel therapeutic drug.

Conclusion

Based on this finding we suggested that the symbiotic associated bacteria derived metabolites are used as an antibiotic for future purpose and they are used as a good drug for therapeutical applications.

Acknowledgement

Financial support as University Research Fellow by Bharathidasan University, Tiruchy, and Tamilnadu, India is gratefully acknowledged

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Cite this article as:

Bavithra H. and Sathiyamurthy K. Bioactive potential of *Pseudomonas alcaliphila* isolated from a marine sponge against human pathogens. *International Journal of Bio-Pharma Research*, Volume 8, Issue 3 (2019) pp. 2494-2503.

http://dx.doi.org/10.21746/ijbpr.2019.8.3.2

Source of support: Nil; **Conflict of interest:** Nil.