Indian Journal of Geo Marine Sciences
Vol. 50 (01), January 2021, pp. 29-36

Evaluation of actinomycetes isolated antimicrobial metabolites as potent inhibitor of multidrug resistant organisms

N Kumari*[,a], S Pandey[b] & E Menghani[c]

[a]Department of Microbiology, JECRC University, Jaipur, Rajasthan – 303 905, India
[b]Department of Zoology, Dr. Bhimrao Amberdkar University, Agra, Uttar Pradesh – 282 002, India
[c]Department of Biotechnology, JECRC University, Jaipur, Rajasthan – 303 905, India
*[E-mail: nalineek9@gmail.com]

Received 09 February 2020; revised 24 February 2020

With the increased metabolic diversity and adaptability, microorganisms are capable of living in diverse environmental conditions. Various microorganisms present in natural environment are resistant to some of the current antibiotics that has lead to discovery of new antibiotic compounds. Soil organisms have been paid a great attention and are recognized as a good source of antibiotic compounds. Actinomycetes are well known for their importance as bioactive metabolites in different sectors. Present study was carried out via isolation of actinomycetes from rhizospheric soil from four different locations of Rajasthan and the isolation was performed on Actinomycetes Isolation Agar (AIA) medium. In this study AIA10 isolate was screened for their antimicrobial potential against five selected microbial pathogens *viz*. *Staphyloccocus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Bacillus subtilis*. After Solvent extraction, crude extracts of benzene and ethyl acetate were tested for antibacterial activity. GC-MS technique identified the presence of major components like Heptadecane at retention time (RT) - 18.131 (5.07 %), Heneicosane at RT - 20.935 (14.63 %), Hexadecanoic acid, Butyl ester at RT - 25.611 (4.92 %), Eicosanoic acid, 2,3-Bis(Acetyloxy)Propyl ester at RT - 32.509 (8.15 %), 9-Octadecenoic acid (Z)-, 2,3-Bis(Acetyloxy)Propyl ester at RT - 34.978 (34.32 %) in benzene crude extract. In ethyl acetate extract major compounds identified were, Pentadecanoic acid at RT - 6.25 (21.251 %), N-Hexadecanoic acid at RT - 22.527 (12.79 %), Eicosanoic acid at RT - 23.788 (14.18 %), Hexadecanoic acid, 2-Hydroxy-1-(Hydroxymethyl) Ethyl ester at RT - 29.593 (5.59 %), Octadecanoic acid, 2,3-Dihydroxypropyl ester at RT - 33.182 (3.50 %) and 5,11,17, 23-Tetratert Butyl pentacyclo [19.3.1.1~3,7~.1~9,13~.1~15,19~]Octacosa1(25),3(28),4,6,9(27),10,12,15(26),16,18,21, 23-Dodecaene-25,26,27,28 Tetrol at RT - 54.152 (9.92 %). Primary screening was tested and the activity was observed against *S. aureus* and *P. vulgaris*. In secondary screening, benzene and ethyl acetate extract was found active against *S. aureus*, at inhibition zone IZ = 10 and 20 mm, respectively. The study conducted proposes these actinomycetes as a potent source for antimicrobial metabolites.

[**Keywords**: Actinomycetes, Actinomycetes isolation agar (AIA) media, Antimicrobial metabolites, Mueller Hinton Agar (MHA) media, Solvent extraction]

Introduction

As a result of the treatment of multiple infectious diseases, the use of new antibiotics has faced increased resistance from multidrug resistant microorganisms. Therefore, many antibiotics are ineffective or very less effective to gradually increasing resistant strains of pathogenic bacteria. Antibiotics which are old gradually declining and the resistant method adapted by bacterial cells for which, many antibiotics were not at all applied. Drug resistance in bacterial cells gained the potential of surviving, growing and multiplying under antibiotic-pressure conditions. Some of the current antibiotics from microbial cells are successful upon in vivo testing. Any bacterial cell that produces an antibiotic compound has the characteristics of self-protective method because of the genes, which govern the methods of resistance for example, efflux mechanism for alteration in the cell membrane or external enzymes production such as, b-Lactamases[1]. *Staphyloccocus aureus* is the most repeatedly found bacterial pathogenic cell in hospital acquired infections in addition to the common cause of community-acquired infections including endocarditis, osteomyelitis, pneumonia and abscesses, and septic arthritis. It is also an important pathogen in economically important species[2]. Due to resistance from drugs such as synthetic penicillins, the treatment of methicillin resistant *S. aureus* (MRSA) causes significant problems and the variants of which are increasingly growing. The appearance of strains of MRSA that are much less susceptible to vancomycin, called last

resort antibiotics, is another major concern. Vancomycin-intermediate *S. aureus* (VISA) variant have emerged and elevated the staphylococcal infections[3]. Marine organisms are rapidly becoming a significant basis in the search for scientifically important molecules. Due to the unusual biologically dynamic metabolites of marine microbes, both academic and industrial interest in marine microorganisms is now at the top of the list. Micro-organisms of actinomycetes are abundant in such environmental habitats such as soil, fresh water, marine water, compost, etc[4].

Marine actinomycetes have been reported to be involved in various tasks like organic material transformation and mineralization process[5]. Actinomycetes are a diverse group of bacteria comprising of several genera. They are gram-positive microorganisms, similar to fungi, and are structurally filamentous. A wide variety of secondary metabolites of actinomycetes are well established in manufacturing many biotechnologically important compounds. Actinomycetes, especially those strains belonging to the genus *Streptomyces* have been considered a great reservoir of antimicrobial and antibiotic components[6]. They are considered as extremely valuable as they produce a broad range of antibiotics and other antimicrobials which are therapeutically active compounds with diverse biological actions. The huge bulk of these significant metabolites have been isolated from actinomycetes that comprises a total of 70 % compounds, in which 20 % are from fungi , 7 % compounds from *Bacillus* sp. and about 1 – 2 % from *Pseudomonas* species. Consequently, actinomycetes are those most significant group of organisms considered widely for the continuous detection of novel drugs and other active metabolites[7]. Studies have been suggested that a large number of antimicrobial compounds can be obtained from terrestrial microbes like fungi, actinomycetes and from parts of medicinal plants[8].

Materials and Methods

Chemical and reagents

Actinomycetes isolation agar (AIA) medium (Hi media, India) was used for the isolation of actinomycetes pure colony. Petroleum ether, benzene, ethyl acetate and chloroform solvents were provided from laboratory of Jaipur Engineering College and Research Centre (JECRC) University, Jaipur, Rajasthan. Double distilled and autoclaved water were used for the experiments conducted.

Compilation of rhizospheric samples and isolation of pure actinomycetes strains

Samples of rhizospheric soil were assembled from four separate rhizospheric locations in the state of Rajasthan. The samples collected were from places like Jaipur, Udaipur, Kota and Alwar. To avoid any contamination, rhizospheric samples were assembled with hygienic, fresh gloves and spatula in sterile bags from these locations. 12-15 cm deep soil was digged and the samples were collected. After collection, samples were processed and stored at 4 °C in research laboratory for further tests of samples[7]. After isolation of actinomycetes, on AIA medium, 10 gm each soil sample was mixed with 90 ml of distilled & sterilized water at vortex shaker for even mixing of samples and was subjected to dilutions up to 10^{-5} and 100 µl of sample was spread onto sterile plates containing AIA medium. Plates were held in reverse side at 37 °C in incubator for 7-10 days for development of isolated colonies. Morphological characteristics, gram staining and different biochemical tests were performed for the identification of actinomycetes[9].

Primary screening

Primary screening was executed for AIA10 isolated pure colony which was performed to reveal antimicrobial actions against five indicator organisms. Agar well and Disc diffusion method was applied for primary screening of isolates[6,10].

Cultivation of Actinomycetes

Luria broth (LB) was the base for the bulk production of metabolites from actinomycetes. 500 ml of LB were prepared and divided to two parts and autoclaved. Each flask was inoculated with culture aseptically in laminar air flow hood and was subjected to 30 °C in shaker incubation at 150 rpm around 23-30 days. Immediately when incubation time is completed, the LB was centrifuged at 5000 rpm for about 15-20 min for separation of supernatant and the biomass. They were taken out separately in different beakers and kept for further tests[11].

Extraction process for bioactive compounds isolation

Mycelium was extracted from the fermented broth after fermentation and then apparent filtrate was taken to expose antimicrobial activity. Thereafter, antibiotic compounds isolation from AIA10 isolate was performed by the process of solvent extraction. The method of solvent extraction was followed by a similar ratio of mixing of both filtrate and solvent (1:1) and energetically shaken for some time in order to complete metabolite extraction. It was held back

uninterrupted until two distinct layers were clearly and completely removed. After separation of layers, both were collected in different beakers. Solvent present in beakers was evaporated by using water bath on constant temperature of 50-60 °C. Crude extracts were weighted and sterilized discs were kept inside beakers after evaporation of solvents followed by activity tests[12].

Antimicrobial activity screening

Microbial pathogens

Five standard microbial cultures such as *S. aureus* (MTCC 3160), *P. aeruginosa* (MTCC 1688), *K. pneumonia* (MTCC 432), *P. vulgaris* (MTCC 7306) and *B. subtilis* (MTCC 441) were used for activity test taken from the laboratory of IMTECH Chandigarh.

Testing of crude extracts on MHA media

Activity test of pure AIA10 isolate was carried out using both disc diffusion method and agar well diffusion method. For this, selected microbial test pathogens were spread onto prepared Mueller Hinton Agar (MHA) media plates. After solvent extraction from petroleum ether, benzene, chloroform and ethyl acetate solvent system, discs filled with crude of AIA10 isolate was set on plates after test pathogens spread on the MHA media plates. By using two methods, antibacterial activity was checked against above mentioned cultures. Plates were kept at 30 °C temperature for around 1-2 days. After obtaining the results, complete inhibition zones (IZ) were measured in millimeter (mm)[13].

Metabolites identification by GC-MS analysis

The chemical composition of AIA10 benzene and ethyl acetate extract were detected by GC Shimadzu QP2010 ultra system. The GC-MS instrument was operational by means of Elite-1 fused silica capillary column-Rtx-Ms (30 m × 0.25 mm i.d. × 0.25 µm film thickness) applied for uncovering of sample mixture. Sample with respective solvent with little quantity was loaded in injector and processed. Chromatograms obtained for each sample with information present in (NIST14. LIB) library of compounds. Helium gas (99.99 %) was used as the carrier gas. Flow rate was set as 1.21 ml/min and split ratio: 10. Temperature of injector was set on 260 °C; Ion-source temperature was 200 °C. Temperature of oven was proposed from 60 °C (constant for 3 min) with an raise of 280 °C for about 22 min. Mass spectra were taken at 70 eV with a scan interval of 0.5 sec[14,15].

Results and Discussion

Collected soil samples from four different locations of Rajasthan were assembled in research laboratory. Isolation of pure strains was executed on AIA medium. Identification of actinomycetes was performed on the basis of morphological, gram staining and biochemical tests of AIA10 isolate. Primary screening of isolate was positive for activity test against *S. aureus* and *P. vulgaris*. Inhibition zones were measured against *S. aureus* (IZ = 9 mm) & *P. vulgaris* (IZ = 15 mm; Fig. 1a). For extraction of metabolites AIA10 isolate was kept for fermentation process onto shaker incubator for antimicrobial production followed by extraction of bioactive metabolites. After extraction from four solvents, crude extracts were tested against selected microbial pathogens such as *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *P. vulgaris* and *B. subtilis*. Extraction of metabolites from benzene and ethyl acetate extract was applied onto petri-plates and zones of inhibitory actions were measured. Activity of benzene and ethyl acetate crude extract isolate AIA10 was observed against test organisms *i.e. S. aureus*. In secondary antimicrobial screening of AIA10, isolate was found positive against *S. aureus*, so inhibition zone (IZ) of crude extract of benzene and ethyl acetate was measured against *S. aureus* as inhibition zone, IZ = 10 mm & 20 mm, respectively (Fig. 1b). For that reason isolate AIA10, was subjected to GC-MS analysis for

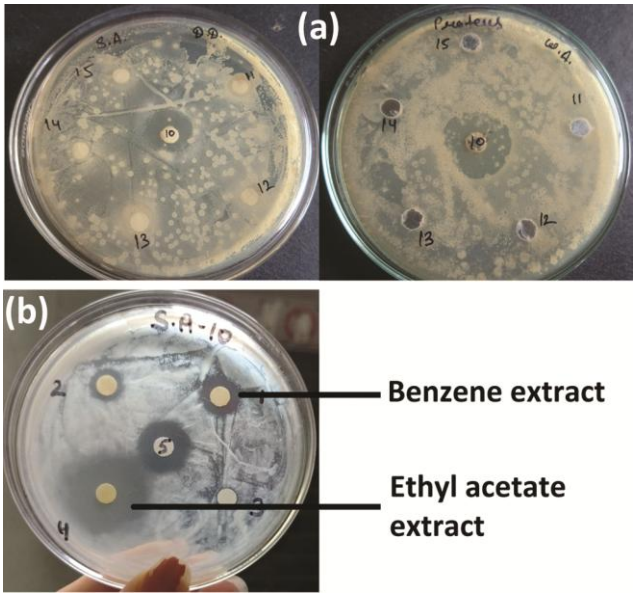

Fig. 1 — Measurement of inhibition activity: a) Primary screening of AIA10 strain against *S. aureus* and *P. vulgaris*; and b) Secondary screening of benzene and ethyl acetate crude extract of AIA10 isolate against *S. aureus*

the detection of bioactive compounds from mixture present in sample. GC-MS analysis revealed the occurrence of a number of components in AIA10 isolate. The Retention Time (RT) of every peak indicates relevant compound in the chromatogram (Figs. 2a, b). Every peak showed, is proportional to the percent quantity of compound there in the solvent. Mixture of compounds were analyzed with WILLEY 8 and NIST 14 LIB and identified with the list of compounds obtained from the library. GC-MS analysis detected some of the major compounds from the benzene and ethyl acetate extract (Tables 1 & 2). In Benzene crude extract major compounds detected were, Heptadecane at RT 18.131 (5.07 %), Heneicosane at –RT 20.935 (14.63 %), Hexadecanoic acid, and Butyl ester at RT 25.611 (4.92 %), Eicosanoic acid, 2,3-Bis (Acetyloxy) Propyl ester at RT 32.509 (8.15 %), 9-Octadecenoic acid (Z)-, 2,3-Bis (Acetyloxy) Propyl ester at RT 34.978 (34.32 %). In ethyl acetate extract major compounds detected were Pentadecanoic acid at –RT 6.25 (21.251 %), N-Hexadecanoic acid at –RT 22.527 (12.79 %),

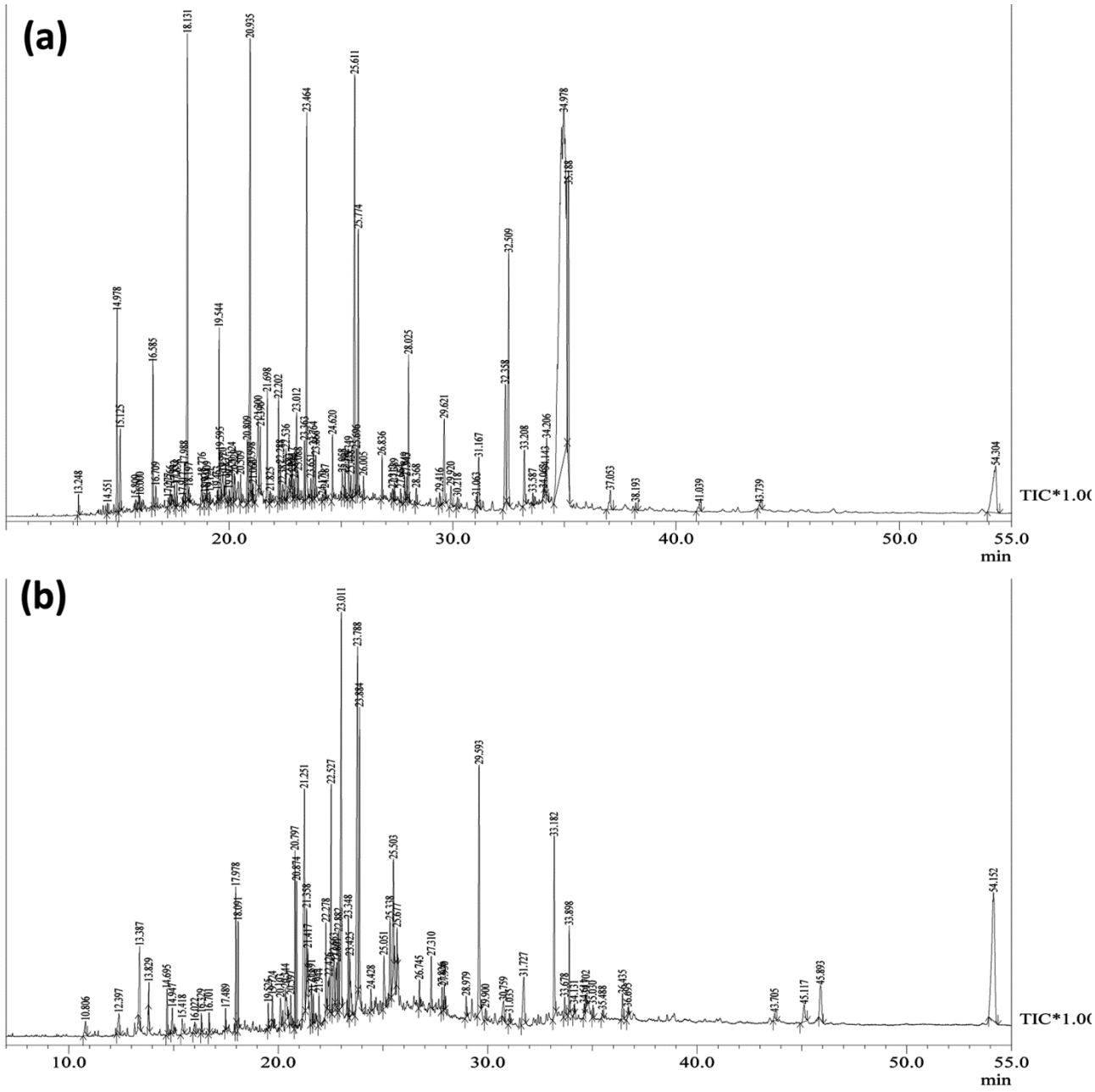

Fig. 2 — Chromatogram of AIA10 strain obtained from GC-MS analysis: a) for benzene extract; and b) for ethyl acetate extract

Table 1 — Compounds of benzene crude extract of AIA10 strain obtained from GC-MS analysis

Sr. No	Compounds	Sum of Area (%)	Retention Time
1	Tridecane	0.16	13.248
2	Dodecane, 2,6,10-Trimethyl-	0.09	14.551
3	Tetradecane	1.79	14.978
4	5-Decyne-4,7-Diol, 2,4,7,9-Tetramethyl-	1.00	15.125
5	1-Hexacosanol	0.37	15.809
6	Eicosane, 2-Methyl-	0.28	16.000
7	Pentadecane	1.20	16.585
8	Di-T-Butyl-Phenol	0.24	16.709
9	Decane, 5-Propyl-	0.13	17.277
10	Tetradecane, 5-Methyl-	0.51	17.366
11	Pentadecane, 3-Methyl-	0.19	17.658
12	1,2-Benzenedicarboxylic Acid, Diethyl Ester	0.07	17.917
13	3-Octadecene, (E)-	0.30	17.988
14	Heptadecane	5.07	18.131
15	Pentafluoropropionic Acid, Tetradecyl Ester	0.11	18.197
16	Pentadecane, 2,6,10-Trimethyl-	0.34	18.776
17	Benzene, (2,3-Dimethyldecyl)-	0.09	18.923
18	Pentadecane, 8-Hexyl-	0.10	19.009
19	8-Heptylpentadecane	0.11	19.121
20	Phenol, Nonyl-	0.18	19.463
21	Nonadecane	1.43	19.544
22	Hexadecane, 1-Bromo-	0.52	19.595
23	4-Nonylphenol	0.39	19.720
24	Phenol, 4-Dodecyl-	0.29	19.837
25	1,2-Benzenedicarboxylic Acid, Dinonyl Ester	0.49	20.124
26	Heptadecane, 3-Methyl-	0.24	20.509
27	1-Heptadecene	0.53	20.809
28	Heneicosane	14.63	20.935
29	Hexadecane, 2,6,10,14-Tetramethyl-	0.24	20.998
30	Tridecane, 4-Cyclohexyl-	0.15	21.066
31	Pentadecanoic Acid	1.63	21.300
32	9-Octadecenoic Acid (Z)-	1.07	21.396
33	Octadecane, 3-Methyl-	0.12	21.825
34	Pentadecanoic Acid, Tms Ester	0.63	22.288
35	Eicosane, 2,4-Dimethyl	0.37	22.817
36	Dibutyl Phthalate	0.16	22.894
37	Nonadecane, 3-Methyl-	0.21	23.088
38	1-Nonadecene	0.83	23.363
39	Cyclodecane, Octyl-	0.20	23.651
40	Eicosanoic Acid	0.64	23.764
41	Heptadecanoic Acid	0.06	24.170
42	2-Methyltetracosane	0.09	24.287
43	1-Decanol, 2-Hexyl-	0.40	25.058
44	Eicosane, 2,4-Dimethyl-	0.50	25.182
45	Octadecanoic Acid	0.43	25.349
46	3-Methylhenicosane	0.19	25.438
47	Hexadecanoic Acid, Butyl Ester	4.92	25.611
48	Decane, 4-Cyclohexyl-	0.23	26.005

(*Contd*.)

Table 1 — Compounds of benzene crude extract of AIA10 strain obtained from GC-MS analysis

Sr. No	Compounds	Sum of Area (%)	Retention Time
49	1-Hexadecanol, 3,7,11,15-Tetramethyl-	0.13	27.312
50	2-Methyloctacosane	0.13	27.664
51	Hexanedioic Acid, Bis(2-Ethylhexyl) Ester	0.28	27.849
52	Behenic Alcohol	0.19	27.945
53	4-Cyclohexylnonadecane	0.18	28.368
54	Octacosane	0.16	29.416
55	Di-N-Octyl Phthalate	0.33	29.920
56	N-Tetracosanol-1	0.13	31.063
57	Tetratetracontane	1.37	31.167
58	Eicosanoic Acid, 2,3-Bis(Acetyloxy)Propyl Ester	8.15	32.509
59	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	0.78	33.208
60	Tetracosane, 11-Decyl-	0.10	33.587
61	2-Methylhexacosane	0.68	34.206
62	9-Octadecenoic Acid (Z)-, 2,3-Bis(Acetyloxy)Propyl Ester	34.32	34.978
63	Gamma.-Sitosterol	0.14	43.739
64	5,11,17,23-Tetratert-Butylpentacyclo[19.3.1.1~3,7~.1~9,13~.1~15,19~]Octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-Dodecaene-25,26,27,28-Tetrol	2.72	54.304

Table 2 — Compounds of ethyl acetate crude extract of AIA10 strain obtained from GC-MS analysis

Sr. No.	Compounds	Sum of Area (%)	Retention Time
1	Benzoic Acid	0.47	10.806
2	Benzeneacetic Acid	0.63	12.397
3	Acetamide, N-(Aminoiminomethyl)-	1.90	13.387
4	Ethyl Cyclopropanecarboxylate	0.84	13.829
5	Methacrylic Acid, Tms Derivative	0.75	14.695
6	Benzoic Acid, 2-Amino-	0.57	14.947
7	Benzeneethanol, 4-Hydroxy-	0.44	15.418
8	Anthranilic Acid, Tms Derivative	0.27	16.329
9	Acetamide, N-(2-Phenylethyl)-	0.29	16.701
10	Dodecanoic Acid	0.28	17.489
11	1-Pentadecene	1.65	17.978
12	Nonadecane	1.31	18.091
13	Eicosane	0.30	19.525
14	1-Decene, 3,3,4-Trimethyl-	0.80	20.103
15	Tetradecanoic Acid	1.04	20.344
16	1-Heptadecene	2.09	20.797
17	Benzene, 1,1'-(1,2-Ethynediyl)Bis-	2.93	20.874
18	Pentadecanoic Acid	6.25	21.251
19	9-Octadecenoic Acid (Z)-	1.32	21.358
20	Tyrosol, Acetate	0.21	21.417
21	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester	0.38	21.678
22	1-Hexadecanol	0.54	21.944
23	Pentadecanoic Acid, Tms Derivative	1.31	22.278
24	N-Hexadecanoic Acid	12.79	22.527
25	Dibutyl Phthalate	1.28	22.882
26	9-Tricosene, (Z)-	1.21	23.348
27	Heptadecane	1.58	23.425
28	Eicosanoic Acid	14.18	23.788
29	Pyrene	0.39	24.428
30	9-Hexadecenoic Acid	1.38	25.051

(*Contd*.)

Table 2 — Compounds of ethyl acetate crude extract of AIA10 strain obtained from GC-MS analysis

Sr. No.	Compounds	Sum of Area (%)	Retention Time
31	Octadecanoic Acid	1.62	25.338
32	Octacosanol	0.53	25.677
33	1-Nonadecene	0.33	27.930
34	Octadecanoic Acid, 3-Oxo-, Ethyl Ester	0.37	28.979
35	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	5.59	29.593
36	1,2-Benzenedicarboxylic Acid	0.27	29.900
37	2,5-Piperazinedione, 3-(2-Methylpropyl)-6-(Phenylmethyl)-,	0.63	30.759
38	N-Tetracosanol-1	0.22	31.035
39	3-Benzyl-6-Isobutyl-2,5-Dioxo-Piperazine	1.89	31.727
40	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	3.50	33.182
41	2-Hydroxyethyl Palmitate, Tms Derivative	0.43	33.678
42	9-Octadecenamide	1.91	33.898
43	Behenic Alcohol	0.13	34.131
44	Z-2-Acetoxy-12-Tetradecenitrile	0.17	34.611
45	9-Octadecenoic Acid (Z)-, 2,3-Bis(Acetyloxy)Propyl Ester	0.16	34.702
46	Eicosanoic Acid, 2,3-Bis(Acetyloxy) Propyl Ester	0.21	35.030
47	Tetratetracontane	0.18	35.488
48	Cis-9,10-Epoxyoctadecanamide	0.20	36.695
49	Stigmast-5-En-3-Ol, (3.Beta.)-	0.28	43.705
50	1,6,10,14,18,22-Tetracosahexaen-3-Ol, 2,6,10,15,19,23-Hexamethyl-, (All-E)-(.+/-.)-	1.69	45.893
51	5,11,17,23-Tetratert-Butylpentacyclo[19.3.1.1~3,7~.1~9,13~.1~15,19~]Octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18 ,21,23-Dodecaene-25,26,27,28-Tetrol	9.92	54.152

Eicosanoic acid at RT 23.788 (14.18 %), Hexadecanoic acid, 2-Hydroxy-1-(Hydroxymethyl) Ethyl ester at –RT 29.593 (5.59 %), Octadecanoic acid, 2, 3-Dihydroxypropyl ester at RT 33.182 (3.50 %) and 5,11,17, 23-Tetratert Butylpentacyclo [19.3.1.1~3,7~.1~9,13~.1~15,19~]Octacosa1(25),3(28), 4,6,9(27),10,12,15(26),16,18,21,23-Dodecaene 25,26, 27,28-Tetrol at RT 54.152 (9.92 %). These are the compounds having antimicrobial activity against multidrug resistant pathogens which are useful in industries and in treatment of various diseases caused due to these pathogenic bacteria.

Conclusion

The present study indicates that the rhizospheric soil of Rajasthan exhibited a great source for antimicrobial compounds and showed a good antimicrobial activity against the tested multidrug resistant pathogen like *Staphylococcus aureus*. The bacterial cell possesses compounds produced by the AIA10 strain were recovered by four solvents. Benzene & ethyl acetate crude extracts displayed an antibacterial activity against *Staphylococcus aureus* in secondary screening. GC-MS technique was successful in identifying bioactive metabolites of the both the extracts. On the whole, the study was successful in the discovery of antimicrobial compounds from the rhizospheric soil of Rajasthan and suggests a continuous research for the discovery of newer antibiotics or antimicrobial compounds from these unexplored regions of Rajasthan.

Conflict of Interest

Authors declare no conflict of interest.

Author Contributions

NK: Investigation, original content preparation, review & editing of manuscript; SP: review & editing; and EM: Resources, supervision, and review & editing.

References

1 Rout S, Dubey D, Panigrahy R & Padhy R N, Surveillance of extended-spectrum b-lactamase producing bacteria in an Indian teaching hospital, *J Taibah Univ Med Sci*, 9 (2014) 274-281.

2 Tenhagen B A, Hansen I, Reinecke A & Heuwieser W, Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition, *J Dairy Res*, 76 (2) (2009) 179-187.

3 Datta S, Pal N K & Nandy A K, Inhibition of the emergence of multi drug resistant *Staphylococcus aureus* by *Withania somnifera* root extracts, *Asian Pac J Trop Med*, (2011) 917-920.

4 Subramani R & Mathivanan N, Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes, *World J Microbiol Biotechnol*, 25 (2009) 2103–2111. DOI 10.1007/s11274-009-0113-4.

5 Gobalakrishnan R, Sivasankar P & Sivakumar K, Marine actinobacteria: A concise account for young researchers, *Indian J Geo-Mar Sci*, 47 (3) (2018) 541-547.

6 Adeyemo O M, Onilude A A & Babatola L J, Effect of production parameters and inhibitory activity of antimicrobial compounds produced by co-cultured strains of *Streptomyces xinghaiensis* - OY62 and *S. rimosus* - OG95, *J King Saud University – Sci*, 32 (2020) 294-301.

7 Prabavathy V R, Mathivanan N & Murugesan K, Control of blast and sheath blight diseases of rice using antifungal metabolites produced by *Streptomyces* sp. PM5, *Biol Control*, 39 (2006) 313-319.

8 Dogan E, Demir O, Sertdemir M, Ali Saracli M & Konuklugil B, Screening of the selected marine sponges from the coasts of Turkey for antimicrobial activity, *Indian J Geo-Mar Sci*, 47 (6) (2018) 1193-1198.

9 Gobalakrishnana R & Sivakumar K, Systematic characterization of potential cellulolytic marine actinobacteria *Actinoalloteichus* sp. MHA15, *Biotechnol Rep*, 13 (2017) 30-36.

10 Tendencia E A, Disk diffusion method, In: *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment*, (Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines), 2004, pp. 13-29.

11 Al-Dhabi N A, Mohammed Ghilan A K, Ali Esmail G, Valan Arasu M, Duraipandiyan V, *et al.*, Bioactivity assessment of the Saudi Arabian Marine *Streptomyces* sp. Al-Dhabi-90, metabolic profiling and it's in vitro inhibitory property against multidrug resistant and extended-spectrum beta-lactamase clinical bacterial pathogens, *J Infect Pub Health*, 12 (4) (2019) 549-556.

12 Kumari N, Menghani E & Mithal R, Bioactive compounds characterization and Antibacterial potentials of Actinomycetes isolated from Rhizospheric soil, *J Sci Ind Res*, 78 (11) (2019) 793-798.

13 Sanghvi G V, Ghevariya D, Gosai S, Langa R, Dhaduk N, *et al.*, Isolation and partial purification of erythromycin from alkaliphilic *Streptomyces werraensis* isolated from Rajkot, India, *Biotechnol Rep*, 1-2 (2014) 2-7.

14 Kumari N, Menghani E & Mithal R, GCMS analysis of compounds extracted from actinomycetes AIA6 isolates and study of its antimicrobial efficacy, *Indian J Chem Technol*, 26 (2019) 362-370.

15 Kumari N, Menghani E & Mithal R, GCMS analysis and Assessment of Antimicrobial Potential of Rhizospheric Actinomycetes of AIA3 isolate, *Indian J Tradit Know*, 19 (1) (2020) 111-119.