Indian Journal of Chemical Technology
Vol. 26, July 2019, pp. 362-370

GCMS analysis of compounds extracted from actinomycetes AIA6 isolates and study of its antimicrobial efficacy

Nalinee Kumari*[,1], Ekta Menghani[2] & Rekha Mithal[3]

[1]Department of Microbiology, JECRC University, Jaipur, Rajasthan, India. [2]Department of Biotechnology, JECRC University, Jaipur, Rajasthan, India. [3]Department of Chemistry, JECRC College Jaipur, Rajasthan, India.
E-mail: nalineek9@gmail.com

Received 28 November 2018 ; accepted 26 March 2019

In this study the compounds have been analyzed by GCMS technique and checked for their antimicrobial potency on Muller Hinton agar media plates. Solvents for component extraction used were benzene, pet ether, ethyl acetate, chloroform and extraction is carried out by layer separation method. From Actinomycetes AIA6 isolate compounds are extracted and tested for antimicrobial activity against indicator pathogens. Cultures used are *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC 1688), *Klebsiella pneumonia* (MTCC-432), *Proteus vulgaris* (MTCC-7306), *Bacillus subtilis* (MTCC-441), *Aspergillus flavus* (MTCC-2206), *Aspergillus terreus* (MTCC-6324), *Aspergillus niger* (MTCC-961), *Saccharomyces cerevisiae* (MTCC-170), *Candida albicans* (MTCC-183). From four Solvents pet ether, benzene, chloroform and ethyl acetate major bioactive compounds, hexadecane, 2,6-di-Tert butyl phenol, 1-pentadecene, 1-hexadecene, heptadecane, 1-nonadecene, anthracene, heneicosane, pyrrolo[1,2-a] pyrazine-1,4-dione,hexahydro-3-(2-methylpro), 5,10-diethoxy-2,3,7,8-tetrahydro-1h,6h-dipyrrolo[1,2-a:1,2-d]pyrazine, n-hexadecanoic acid, hexadecanoic acid-butyl ester, n-acetyltriptamine, n-tetracosanol-1, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, octacosanol, eicosinoic acid 2,3 bis (acetyloxy) propyl ester, octadecanoic acid,2,3 dihydroxy propyl ester, geranyl linalool are present with antimicrobial activity. Compound identification was done with the help of NIST 14 library. Inhibition zones (IZ=mm) of activity test were observed against *Staphylococcus aureus* (IZ=Ben-11mm, E.A-11mm) *Pseudomonas aeruginosa* (IZ=Ben-18mm, E.A-21mm) *Bacillus subtilis* (IZ=Ben-19mm), *Klebsiella pneumonia* (IZ=E.A-12mm) *Proteus vulgaris* (IZ=P.E-21mm, E.A-15mm), *Aspergillus flavus* (IZ=Chl.-11mm), *Aspergillus niger* (IZ=Chl.-11mm), *Saccharomyces cerevisiae* (IZ=E.A-9mm). No antifungal activity was recorded against starins *Aspergillus terreus* and *Candida albicans.*

Keywords: Antimicrobial activity, Component extraction, Actinomycetes, Indicator pathogens

Antibacterial or anti-infection agents, compose a greater group of compounds. Earlier antibiotics used to imply as normally happening compounds that can be produced by a variety of microorganisms. Regardless, the term has not constrained but rather it is presently advanced to man-made engineered mixes as well. Antibiotics act diversely and can differentiate into four different classes in view of their mechanism of activity. Those four classes are (1) restraint of bacterial cell wall biosynthesis, (2) hindrance of protein biosynthesis, (3) restraint of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and (4) hindrance of folate synthesis[1,2]. Anti-microbial resistance has transformed into a universal well-being need worldwide and it's spreading at more rapid rate contrasted with the improvement of new compounds. A multidrug resistance expands patient treatment dis-satisfaction and mortality, and medical services expenditure. Utilization at extreme level and misleading of anti-infection agents exert the precise burden supporting the development, duplication and rapid spread of resistant strains. Moreover, the transmission of resistant organisms between individuals in relation to health security facilities and in community and among animal and their surrounding condition has lead to the spread of the anti-infection resistance[3]. The beta-lactam antibiotics, like penicillins, cephems, carbapenems, and monobactams were successful against gram positive organisms, prior after disclosure of penicillins. Cephems were made during the 1960s, and are categorized into ages on the basis of their antimicrobial potential. Cephems like cefazolin are powerful against gram-positive organisms and *E.coli*. Second-age cephems like cefotiam enclose a great action against both gram-positive and gram negative microorganisms. In addition third-age cephems are also very effective particularly for gram negative microbes and these cephems includes ceftazidime, cefotaxime.

Moreover carbapenem is another class of anti-microbial that incorporates panipenem, imipenem, and meropenem, which are dominant against gram-positive and also gram negative organisms[4]. Actinomycetes are unique group of aerobic, branched, unicellular and gram positive microscopic organisms found openly or saprophytically in various habitats, for example, soil, warm water, and marine deposit with high level of GC (70%) in their hereditary material. They incorporate essential genera, for example, micromonospora, nocardia and streptomyces whose extensive genomes allow them to deliver some different kinds of secondary metabolites, anti-microbial, industrially useful enzymes, antitumor materials, beneficial metabolites and pesticides. Actinomycetes especially streptomyces are well recognized source of secondary metabolites especially antibiotics. Commercially crucial molecules are significantly delivered by streptomyces, micromonospora, saccharopolyspora, amycolatopsis and actinoplanes. Almost, 80% of anti-infection agents have been delivered from streptomyces. Consequently the incredible significance was given to variety Streptomycetes as they are unique, has high metabolic generation rate and they have capability in recycling organic matter and to degrade chitin, lignocelluloses etc[5,6]. Actinomycetes exist as cohorts together with different microorganisms in diverse habitat. This association could initiate silent pathways that may result in the combination of novel secondary metabolites and eventually lead to chemical defense of the delivering microorganism[7]. Microbial diseases are expanding step by step and they are turning into a major danger to human health. There number of diseases is of 200 that are transmitted by microscopic organisms, parasites, infections, prions, rickettsia and different microorganisms to humans. Viruses or prions, among the distinctive microbial pathogens causes 37-44% of diseases, percentage of bacteria or rickettsia is 10-30%, for protozoa 10.7% diseases, fungi cause 6.3% of sicknesses and helminths cause 3.3% of diseases, prompting a huge number of death consistently[8]. The need for new antibiotics expanding every day because of the rise in drug resistant pathogens. Regardless of enormous potential sources of antimicrobial agents including therapeutic plants, soil is still the most essential store for novel antibiotics along with pharmaceutical and biological action[9]. The seriousness of the problem and its worldwide spread has encouraged the World Health Organization (WHO) and the European Union (EU) to initiate a few reconnaissance frameworks[4]. In 2015, a programme has been initiated, The National Action Plan for Combating Anti-infection Resistant Bacteria, which lay out to relieve anti-microbial resistance, jointly with requisition and improvement of ASPs[10]. Present study includes analysis of compounds from GCMS technique and their antimicrobial efficacy against some indicator pathogens causing various infectious diseases extracted from actinomycetes.

Experimental Section

Location of sample collection and isolation of Actinomycetes

Samples were collected from rhizospheric soil of different plants from different regions of Rajasthan. Samples were named as JPSN-I, UDSN-II, KOSN-III, ALSN-IV as per their locations like Jaipur, Udaipur, Kota and Alwar. To protect from other sources of contamination, soil was collected with hand gloves in clean, dry, sterile air tight bags from mentioned four locations and brought to laboratory for further processing of samples. Isolation of the actinomycetes was done by serial dilution[11], crowded plate technique and sprinkle method on actinomycetes isolation agar media (AIA). The plates were incubated inverted at 37°C for 7 days[12]. After incubation, individual colonies were maintained on actinomycetes isolation agar (AIA) media.

Media optimization, mass production and metabolite production

Actinomycetes were grown on actinomycetes isolation agar media (AIA) for the metabolite extraction. For extraction of compounds pure cultures were suspended in flasks filled with luria broth and kept at shaker incubator at 150 rpm at 30°C. Shaker incubation period was given for two weeks at same temperature and conditions given above. After two weeks cultures were centrifuged for separation of mycelium at 5000 rpm for 15-20 min. Filtration was done for extracts and kept safe at 4°C for further analysis of samples. Pet ether, benzene, ethyl acetate and chloroform solvents were used for compounds extraction from AIA6 culture broth. Solvent and broth of culture was blended in 1:1 (v/v) proportion and shaken well and kept undisturbed for 30 min till two isolate layers got completely separated from each other. Solvent beaker containing metabolites was kept at 60°C on water bath for complete dryness so that only compounds were left behind in the container[11,13-15]. Mixture of compounds was shifted to ependroff tube for Gas Chromatography Mass Spectrometry (GCMS) study. Compounds were studied from GCMS (Table 1-3) results.

Table 1 — Compounds present in ethyl acetate extract sample of AIA6 isolate: Name, Retention time (RT) and Area % showing in GCMS analysis

S.No.	Compound	Retention. time (RT)	Sum of Area %
1	Benzenesulfonic Acid, 4-Hydroxy-	6.88	0.38
2	3-Octanol	11.206	0.34
3	Acetamide, N-(Aminoiminomethyl)-	13.395	6.09
4	Benzamide	13.82	0.52
5	Decanoic Acid	14.336	0.31
6	3-Hexadecene, (Z)-	14.82	1.01
7	Tetradecane	14.955	0.96
8	Phenol, 2,6-Dimethoxy-	15.387	0.6
9	Benzamide, 4-Methyl-	15.775	0.12
10	Hexadecane, 1-Chloro-	16.152	0.24
11	Hexadecane	16.38	2.68
12	10-Methyl-Octadec-1-Ene	16.445	0.18
13	Acetamide, N-(2-Phenylethyl)-	16.691	0.51
14	Dibenzofuran	16.986	0.33
15	N,N-Bis(2-Hydroxyethyl)Dodecanamide	17.485	0.41
16	Pentadecane, 3-Methyl-	17.644	0.26
17	1,2-Benzenedicarboxylic Acid, Diethyl Ester	17.903	0.13
18	1-Pentadecene	17.974	4.09
19	1,2-Epoxyundecane	18.4	0.19
20	Pyridine, 4,4'-(1,2-Ethenediyl)Bis-	18.764	0.31
21	8-Pentadecanone	19.109	0.15
22	Eicosane	19.518	0.38
23	3-Methyl-1,4-Diazabicyclo[4.3.0]Nonan-2,5-Dione, N-Acetyl-	19.711	2.07
24	3-Undecanone	19.983	0.1
25	Tetradecane, 5-Methyl-	20.212	0.23
26	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-	20.338	1.54
27	Heptadecane, 3-Methyl-	20.49	0.34
28	4-Pentenoic Acid, 2-Methyl-, Tetradecyl Ester	20.606	0.14
29	Formic Acid, Heptyl Ester	20.692	0.14
30	9-Eicosene, (E)-	20.789	2.57
31	Benzene, 1,1'-(1,2-Ethynediyl)Bis-	20.866	3.69
32	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-3-(2-Methylpropyl)-	21.126	4.17
33	Pentadecanoic Acid	21.184	0.62
34	Pyridine, 3-Ethyl-2,6-Dimethyl-	21.351	0.56
35	5h-Indeno[1,2-B]Pyridine	21.589	0.11
36	Diisobutyl Benzene-1,2-Dicarboxylate	21.663	0.24
37	9-Octadecanone	21.832	0.2
38	1-Hexadecanol	21.93	0.62
39	7,9-Di-Tert-Butyl-1-Oxaspiro(4,5)Deca-6,9-Diene-2,8-Dione	22.277	0.88
40	Palmitic Acid	22.478	0.67
41	2,5-Piperazinedione, 3,6-Bis(2-Methylpropyl)-	22.71	5.32
42	Dibutyl Phthalate	22.871	1.15
43	N-Hexadecanoic Acid	22.988	7.4
44	Eicosane, 2-Methyl-	23.07	0.21
45	9,10-Anthracenedione	23.271	0.41
46	1-Heneicosanol	23.34	1.5
47	Heptadecane	23.416	0.86
48	1-Hexanol, 2-Ethyl-	23.617	0.09
49	Eicosanoic Acid	23.727	2.76
50	9-Octadecenoic Acid (Z)-	23.814	0.37
51	Palmitic Acid, Tms Derivative	23.922	0.51

(*Contd.*)

Table 1 — Compounds present in ethyl acetate extract sample of AIA6 isolate: Name, Retention time (RT) and Area % showing in GCMS analysis (*Contd*.)

S.No.	Compound	Retention. time (RT)	Sum of Area %
52	3-Propionyloxytridecane	24.137	0.24
53	Carbonic Acid, Monoamide, N-Decyl-, 2-Ethylhexyl Ester	24.416	0.52
54	Octadecane	24.59	0.44
55	Heptadecyl Acetate	24.666	0.16
56	Dibenzothiophene 5,5-Dioxide	24.948	0.16
57	1-Docosanol	25.029	1.22
58	Octadecanoic Acid	25.328	2.24
59	Acetamide, N-[2-(1h-Indol-3-Yl)Ethyl]-	25.649	6.14
60	1-Decene, 3,3,4-Trimethyl-	25.719	1.09
61	1-Octanol, 2-Butyl-	25.814	0.28
62	Dodecane, 4-Cyclohexyl-	25.985	0.15
63	Octadecanoic Acid, Trimethylsilyl Ester	26.156	0.24
64	2,5-Piperazinedione, 3-Benzyl-6-Isopropyl-	26.468	0.16
65	Oxiraneoctanoic Acid, 3-Octyl-, Methyl Ester, Trans-	26.737	0.56
66	Oxalic Acid, 3,5-Difluorophenyl Tetradecyl Ester	27.199	0.23
67	Ergotaman-3',6',18-Trione, 9,10-Dihydro-12'-Hydroxy-2'-Methyl-5'-(Phenylmethyl)-, 76	27.297	0.86
68	Docosane	27.648	0.39
69	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-3-(Phenylmethyl)-	27.817	0.51
70	1-Nonadecene	27.921	0.68
71	Quinoline, 2-Propyl-	28.146	0.23
72	1,3,5-Trisilacyclohexane	28.967	0.25
73	D-Ribose, 2-Deoxy-Bis(Thioheptyl)-	29.232	0.28
74	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	29.559	2.8
75	1,2-Benzenedicarboxylic Acid	29.882	0.34
76	L-Prolinamide, 5-Oxo-L-Prolyl-L-Phenylalanyl-4-Hydroxy-	30.727	2.92
77	Hexadecanoic Acid, 2-(Acetyloxy)-1-[(Acetyloxy)Methyl]Ethyl Ester	32.401	0.22
78	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	33.155	1.6
79	2,2,20,20-Tetramethyl-3,7,11,15,19-Pentaoxa-2,20-Disilahenicosane	33.657	0.14
80	9-Octadecenamide	33.884	2.24
81	N-Tetracosanol-1	34.122	0.17
82	Squalene	34.323	0.18
83	Z-2-Acetoxy-12-Tetradecenitrile	34.6	0.18
84	9-Octadecenoic Acid (Z)-, 2,3-Bis(Acetyloxy)Propyl Ester	34.692	0.24
85	Eicosanoic Acid, 2,3-Bis(Acetyloxy)Propyl Ester	35.019	0.26
86	(3e,6e,10e)-3,7,11,15-Tetramethyl-1,3,6,10,14-Hexadecapentaene 99	45.06	0.31
87	1,6,10,14-Hexadecatetraen-3-Ol, 3,7,11,15-Tetramethyl-, (E,E)-	45.866	1.87
88	5,11,17,23-Tetratert-Butylpentacyclo[19.3.1.1~3,7~.1~9,13~.1~164166052	54.058	9.83

Microbial cultures and antimicrobial activity of ethyl acetate extract

For the antimicrobial activity test some microbial cultures were used For analysis of antimicrobial efficacy test. These were brought from IMTECH Chandigarh. Cultures were *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC 1688), *Klebsiella pneumonia* (MTCC-432), *Proteus vulgaris* (MTCC-7306), *Bacillus subtilis* (MTCC-441), *Aspergillus flavus* (MTCC-2206), *Aspergillus Terreus* (MTCC-6324), *Aspergillus Niger* (MTCC-961), *Saccharomyces cerevisiae* (MTCC-170), and *Candida albicans* (MTCC-183). The antibacterial, antifungal activity was measured utilizing the standard Kirby-Bauer disc diffusion strategy[13]. Petri plates were set up with 20 mL of sterilized Muller Hinton Agar (MHA) media and permitted to dry for 10 min. The crude extract were set on the surface of the medium and left for 20-30 min at room temperature for compound dispersion and then kept inverted for incubation at 37°C for 24-48 h[11].

GCMS analysis

Compound extracts of Actinomycetes AIA6 isolate were analyzed by GC-MS method. GC-MS analysis was executed using GC Shimadzu QP2010 ultra system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) and was operational with

Table 2 — Compounds present in chloroform extract sample of AIA6 isolate: Name, Retention time (RT) and Area % showing in GCMS analysis

S.No.	Compound	Retention. time (RT)	Sum of Area %
1	2-Pyrrolidinone	8.771	0.12
2	Cyclohexane, Eicosyl-	11.236	0.36
3	Acetamide, N-(Aminoiminomethyl)-	13.657	6.81
4	Ethyl Cyclopropanecarboxylate	14.027	1.88
5	Methacrylic Acid, Tms Derivative	14.711	0.19
6	3-Hexadecene, (Z)-	14.833	0.88
7	Tetradecane	14.963	0.08
8	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	16.754	8.36
9	4-Undecene, 3-Methyl-, (Z)-	17.911	0.09
10	1-Hexadecene	18.022	8.94
11	Hexadecane	18.099	0.21
12	Tridecane, 3-Methylene-	18.164	0.23
13	3-Methyl-1,4-Diazabicyclo[4.3.0]Nonan-2,5-Dione, N-Acetyl-	19.834	2.6
14	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-	20.56	1.8
15	1-Nonadecene	20.846	19.03
16	Octadecane	20.902	0.15
17	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-3-(2-Methylpropyl)-	21.214	1.88
18	2-Piperidinone, 1-(3,4,5,6-Tetrahydro-2-Pyridinyl)-	21.988	0.09
19	7,9-Di-Tert-Butyl-1-Oxaspiro(4,5)Deca-6,9-Diene-2,8-Dione	22.295	0.15
20	3-Isobutylhexahydropyrrolo[1,2-A]Pyrazine-1,4-Dione #	22.509	0.09
21	5,10-Diethoxy-2,3,7,8-Tetrahydro-1h,6h-Dipyrrolo[1,2-A:1,2-30	22.805	3.39
22	N-Hexadecanoic Acid	22.976	0.54
23	3,5-Di-Tert-Butyl-4-Hydroxyphenylpropionic Acid	23.269	0.35
24	Hexadecanoic Acid, Trimethylsilyl Ester	23.918	0.09
25	Tricyclo[20.8.0.0e7,16]Triacontan, 1(22),7(16)-Diepoxy-	25.043	0.05
26	1-Decanethiol	25.091	0.07
27	Octadecanoic Acid	25.322	0.32
28	2,5-Piperazinedione, 3,6-Bis(2-Methylpropyl)-	25.621	0.37
29	N-Tetracosanol-1	25.714	11.64
30	2-Ethylhexyl Acrylate	25.834	1.11
31	Tetradecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	26.868	0.06
32	Ergotaman-3',6',18-Trione, 9,10-Dihydro-12'-Hydroxy-2'-Methyl-5'-(Phenylmethyl)-, 42	27.358	0.33
33	Octadecanoic Acid, 3-Oxo-, Ethyl Ester	28.985	0.74
34	1h-Indene, 1-Hexadecyl-2,3-Dihydro-	29.288	0.18
35	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	29.7	8.68
36	1,2-Benzenedicarboxylic Acid, Dioctyl Ester	29.923	0.06
37	3-Hydroxypropyl Palmitate, Tms Derivative	30.367	0.28
38	1-Methyl-2,3-Cis-Dimethylaziridine	30.957	0.03
39	Octacosanol	31.09	7.23
40	3-Benzyl-6-Isobutyl-2,5-Dioxo-Piperazine	31.805	0.53
41	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	33.273	7.85
42	6-Ethyl-3-Decanol, Tms Derivative	33.571	0.07
43	2-Hydroxyethyl Palmitate, Tms Derivative	33.696	0.53
44	9-Octadecenamide	33.911	0.14
45	Squalene	34.343	0.09
46	Hexacosyl Heptafluorobutyrate	46.932	0.41
47	5,11,17,23-Tetratert-Butylpentacyclo[19.3.1.1~3,7~.1~9,13~.1~	54.136	0.93

Elite-1 fused silica capillary column. Helium gas (99.99%) was the carrier gas with a constant flow rate of 1.21 mL/min and with split ratio: 10. Temperature of Injector was 260°C; Ion-source temperature 200°C. The oven temperature was intended from 60°C (constant for 3 min.) with an increment as of 280°C for 22 min. Mass spectra were taken at 70 eV with a scan interval of 0.5 sec[13].

Table 3 — Compounds present in benzene extract sample of AIA6 isolate: Name, Retention time (RT) and Area % showing in GCMS analysis

S.No.	Compound	Retention. time (RT)	Sum of Area %
1	Tridecane	13.249	0.21
2	Pentadecane	14.981	3.03
3	5-Decyne-4,7-Diol, 2,4,7,9-Tetramethyl-	15.115	0.61
4	2,6,10-Trimethyltridecane	15.943	0.29
5	Octadecanoic Acid	17.541	0.75
6	3-Octadecene, (E)-	17.985	0.23
7	Heptadecane	18.129	5.01
8	Pentadecane, 2,6,10-Trimethyl-	18.773	0.71
9	Nonadecane	19.538	1.88
10	1,1'-Biphenyl, 2,2',5,5'-Tetramethyl-	19.694	0.33
11	10-Methoxy-Nb-.Alpha.-Methylcorynantheol	19.835	0.19
12	2-Bromotetradecane	20.12	0.33
13	Tetradecane, 5-Methyl-	20.225	0.27
14	Undecanoic Acid, 10-Bromo-	20.353	0.4
15	1-Docosene	20.805	0.36
16	Heneicosane	20.931	13.41
17	Pentadecanoic Acid	21.283	1.75
18	1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester	21.688	0.68
19	Hexadecanoic Acid	22.523	0.38
20	Piperazine, 2,5-Dimethyl-3-(2-Methylpropyl)-	22.76	1.14
21	N-Hexadecanoic Acid	23.033	1.93
22	1-Hexadecanol	23.356	0.33
23	Heptadecanoic Acid	23.759	0.48
24	Eicosane, 2,4-Dimethyl-	25.177	0.85
25	Hexadecanoic Acid, Butyl Ester	25.607	5.23
26	Decane, 4-Cyclohexyl-	25.999	0.23
27	Pyrrolo[1,2-A]Pyrazine-1,4-Dione, Hexahydro-3-(Phenylmethyl)-	27.836	0.22
28	4-Cyclohexylnonadecane	28.358	0.19
29	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	29.615	1.48
30	Di-N-Octyl Phthalate	29.909	0.25
31	Eicosanoic Acid, 2-(Acetyloxy)-1-[(Acetyloxy)Methyl]Ethyl Ester	32.349	2.12
32	Eicosanoic Acid, 2,3-Bis(Acetyloxy)Propyl Ester	32.499	8.87
33	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	33.204	1.08
34	2-Methylhexacosane	34.196	0.93
35	2,3,4-Trifluorobenzoic Acid, Undec-10-Enyl Ester	34.981	41.55
36	Tetratetracontane	37.043	0.45
37	5,11,17,23-Tetratert-Butylpentacyclo[19.3.1.1~3,7~.1~9,13~.1	54.203	1.88

Compounds identification

Identification of active components interpretation of GC-MS samples were finished using the database of Willey library and National Institute of Standards and Technology (NIST), which is having over 62,000 patterns for assessment of diverse compounds. The results recorded were compared with the library of the known components stored in the NIST 14.lib and Wiley 8.lib. Compounds with the name, mass, molecular formula, molecular weight etc. of the components were compared from above mentioned library.

Results and Discussion

Natural products have been considered the largely significant source of potential drugs since ancient era. On the other hand, due to the emergence of different human diseases with the changing environment, continuous screening along with validation of secondary metabolites in the form of effective drug needs to be updated[16]. This work is directed to the extraction of bioactive components from the soil actinomycetes isolated on actinomycetes isolation agar (AIA) media from Rajasthan and was tested for antimicrobial activity against selected pathogens

brought from IMTECH Chandigarh. Pet ether, benzene, ethyl acetate and chloroform solvent were used for the extraction of compounds present in AIA6 isolate. Extraction process was held by layer separation technique and compounds were applied on prepared Muller Hinton Agar (MHA) media that occupied with pathogens growth. The isolate was characterized by means of GCMS. A relative concentration of compounds was shown by chromatogram of GCMS. Height of every peak is proportional to its present concentration of compounds. Mass spectrometer identifies the structure and nature of the compound structure, molecular weight, molecular formula, retention time and area percentage of components was identified from NIST 14.lib and Wiley 8.lib. Fatty acids (saturated, unsaturated), phenols, terpenes, alcohols etc. were present in good to moderate amount. Actinomycete produces different compounds showing a good range activity against variety of test pathogen. Actinomycetes have capability to deliver excellent range of antimicrobial compounds[17]. Bioactive compounds found (Table 4) in GCMS results have been reported with antimicrobial activities (antimicrobial[20], antibacterial[19], antifungal[18], antioxidant[20], anti-inflammatory[25], hypocholesterolemic[28], antieczemic properties[33], antihistaminic[33]. Compounds were hexadecane with RT 16.380, 2,6-di-tert butyl phenol with RT 16.754, 1-pentadecene with RT 17.974, 1-hexadecene with RT 18.022, heptadecane with RT 18.129, 1-nonadecene with RT 20.846, anthracene with RT 20.866, heneicosane with RT 20.931, pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpro) with RT 21.126, 5,10-diethoxy-2,3,7,8-tetrahydro-1h, 6h-dipyrrolo[1,2-a:1,2-d] pyrazine with RT 22.805, n-hexadecanoic acid with RT 22.988, hexadecanoic acid-butyl ester with RT 25.607, N-acetyltriptamine with RT 25.649, n-tetracosanol-1 with RT 25.714, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with RT 29.700, octacosanol with RT 31.090, eicosinoic acid 2,3 bis acetyloxy) propyl ester with RT 32.499, octadecanoic acid,2,3 dihydroxy propyl ester with RT 33.273, geranyl linalool with RT 45.866. Inhibition zones (IZ) of antibacterial and antifungal activity was observed.

Antibacterial activity was observed against *Pseudomonas aeruginosa, Staphylococcus aureus,*

Table 4 — Major Compounds present in all three samples of AIA6 isolate: Name, Retention time (RT), Molecular formula, Molecular weight, Activity present in samples of GCMS analysis

Retention time (RT)	Compound	Molecular formula	Molecular weight	Activity
16.380	Hexadecane	$C_{16}H_{34}$	226	Antibacterial, Antifungal, Antioxidant [18]
16.754	2,6-Di-Tert butyl phenol	$C_{14}H_{22}O$	206	Antibacterial, Anti-inflammatory[19]
17.974	1-Pentadecene	$C_{15}H_{30}$	210	Antimicrobial, Antioxidant[20]
18.022	1-Hexadecene	$C_{16}H_{32}$	224	Antimicrobial [21]
18.129	Heptadecane	$C_{17}H_{36}$	240	Antifungal[22]
20.846	1-Nonadecene	$C_{19}H_{38}$	266	Antifungal[23]
20.866	Anthracene	$C_{14}H_{10}$	178	Antimicrobial[24]
20.931	Heneicosane	$C_{21}H_{44}$	296	Antibacterial, Anti-inflammatory, Anti-larvalsettle[25]
21.126	Pyrrolo[1,2-a] pyrazine-1,4-dione,hexahydro-3-(2-methylpro)	$C_{11}H_{18}N_2O_2$	210	Antifungal[26]
22.805	5,10-diethoxy-2,3,7,8-tetrahydro-1h,6h-dipyrrolo[1,2-a:1,2-d]pyrazine	$C_{14}H_{22}N_2O_2$	250	Antimicrobial [27]
22.988	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, Pesticide, Hypocholesterolemic, Nematicide, Lubricant, Antiandrogenic,Hemolytic 5 alpha reductase inhibitor,Antipsychotic[28]
25.607	Hexadecanoic acid- butyl ester	$C_{20}H_{40}O_2$	312	Antimicrobial, Antioxidant[29]
25.649	N-acetyltriptamine	$C_{12}H_{14}N_2O$	202	Antibacterial[30]
25.714	n- Tetracosanol-1	$C_{24}H_{50}O$	354	Antibacterial[23]
29.700	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	Antimicrobial[28]
31.090	Octacosanol	$C_{28}H_{58}O$	410	Anti-inflammatory, Antinociceptive[31]
32.499	Eicosinoic acid 2,3 bis(acetyloxy) propyl ester	$C_{27}H_{50}O_6$	470	Antifungal[32]
33.273	Octadecanoic acid,2,3 dihydroxy propyl ester	$C_{21}H_{42}O_4$	358	Antioxidant, Hepatoprotective, Antihistaminic, Hypocholesterolemic and Antieczemic activities[33]
45.866	Geranyl linalool	$C_{20}H_{34}O$	290	Antimicrobial[34]

Table 5 — Inhibition zones (IZ=mm) of antibacterial activity of AIA6 Isolate against different test pathogens

Solvents	Test pathogens				
	Staphylococcus aureus (MTCC-3160)	*Pseudomonas aeruginosa* (MTCC 1688).	*Bacillus subtilis* (MTCC 441)	*Klebsiella pneumonia* (MTCC 432)	*Proteus vulgaris* (MTCC 7306)
Benzene	11mm	18mm	19mm	-	-
Pet ether	-	-	-	-	21mm
Chloroform	-	-	-	-	-
Ethyl acetate	11mm	21mm	-	12mm	15mm

Table 6 — Inhibition zones (IZ=mm) of antifungal activity of AIA6 Isolate against different test pathogens

Solvents	Test pathogens				
	Aspergillus flavus (MTCC-2206)	*Aspergillus terreus* (MTCC-6324).	*Aspergillus niger* (MTCC-961)	*Saccharomyces cerevisiae* (MTCC-170)	*Candida albicans* (MTCC-183)
Benzene	-	-	-	-	-
Pet ether	-	-	-	-	-
Chloroform	11mm	-	11mm	-	-
Ethyl acetate	-	-	-	9mm	-

Bacillus subtilis, Klebsiella pneumonia and *Proteus vulgaris* indicator pathogens. Although antibacterial activity was observed against all test pathogens but the isolate was found to be more sensitive against two gram negative test pathogens- *Pseudomonas aeruginosa* and *Proteus vulgaris*. A good range activity was observed against two test pathogens such as *Pseudomonas aeruginosa* (IZ=Ben-18mm, E.A-21mm) and *Proteus vulgaris* (IZ=P.E-21mm, E.A-15mm). Further results showed antibacterial activity against gram positive bacteria *Staphylococcus aureus* (IZ=Ben-11mm, E.A-11mm) and *Bacillus subtilis* (IZ=Ben-19mm). The Isolate showed moderate activity against gram negative strain *Klebsiella pneumonia* (IZ=E.A-12mm) (Table-5). Antifungal activity was checked against five fungal strains such as *Aspergillus flavus, Aspergillus niger, Saccharomyces cerevisiae, Aspergillus terreus* and *Candida albicans*. Results showed that the isolate was having moderate antifungal activity against three fungal strains, *Aspergillus flavus* (IZ=Chl.-11mm), *Aspergillus niger* (IZ=Chl.-11mm) and *Saccharomyces cerevisiae* (IZ=E.A-9mm). No antifungal activity was recorded against strains *Aspergillus terreus* and *Candida albicans* (Table-6).

Conclusion

On the whole, examination of actinomycetes acquired from rhizospheric soil demonstrates their antibacterial, antifungal, antioxidant and antitoxins etc activity in analysis of GCMS. Under a scope of various development conditions, actinomycetes delivers such compounds which is beneficial against multidrug resistance pathogens (MDR).In this manner numerous screening events center around discovering novel antimicrobial components that focus on these pathogens. Various untapped rhizospheric regions need to be targeted continuously in search of novel antimicrobial agents which are very significant to the clinic and pharmaceutical industries. So this research is continued in such area to get higher quality effective antimicrobial compounds.

References

1 Atul P S, Singh R B & Mishra S, *The Open Nutraceuticals J*, 5 (2012) 107.

2 Kapoor G, Saigal S & Elongavan A, *J Anaesthesiol Clin Pharmacol*, 33 (2017) 300.

3 Prigitano A, Romanò L, Auxilia F, Castaldi S & Tortorano A M, *J Infect Public Health* (2017).

4 Saga T & Yamaguchi K, *Japan Med Assoc J*, 52 (2009) 103.

5 Mary S P, Guntukua G S, Muthyalaa M K K, Pingalia S & Sahu P K, *J Basic Appl Sci*, 7 (2018) 250.

6 Farshid K & Dehdari F, *Egypt J Aquat Res*, 41 (2015) 197.

7 Usama R A, Grkovic T, Balasubramanian S, Kamel M S, Quinn R J & Hentschel U, *Biotechnol Adv*, 33 (2015) 798.

8 Pathalam G, Reegan A D, David R H A, Gandhi M R, Paulraj M G, Al-Dhabi N A & Ignacimuthu S, *India Alexandria Journal of Medicine*, 53 (2017) 101.

9 Manal M A, Awaad A S, AL-Mudhayyif H A, Alothman M R, Alqasoumi S I & Zain S M, *Saudi Pharm* J, 27 (2019) 401.

10 Shashi N Kapadia, Erika L Abramson, Eileen J Carter, RN, Angela S Loo, Rainu Kaushal, David P Calfee & Matthew S Simon, *J Comm J Qual Patient Safety*, (2017).

11 Kumar P S, Duraipandiyan V & Ignacimuthu S, *Kaohsiung J Med Sci*, 30 (2014) 435.

12 Gobalakrishnana R & Sivakumar K**,** *Biotechnol Rep,* 13 (2017) 30–36.

13 Zothanpuia, Passari A K, Leo V V, Chandra P, Kumar B, Nayak C, Hashem A, Elsayed Fathi Abd Allah, Abdulaziz A Alqarawi & Singh B P, *Microb Cell Fact*, 17 (2018) 68.

14 Balasubramaniam V, Ganesh S, Karunanithi V & Perumal P, *Int J Pharm Ind Res*, 1 (2011).

15 Kandasamy S, Muthusamy G, Thangaswamy S & Kumar S, *World res J Antimicr Agents*, 1 (2012) 07.

16 Amit K S, Singha S K, Singha P P, Srivastavaa A K, Pandeya K D, Kumara P & Yadav H, *Biotechnol Rep*, 18 (2018).

17 Jeffrey L S H, Sarawak *African J Biotechnol*, 7 (2008).

18 Balamurugan M, Selvam G G, Thinakaran T & Sivakumar K, *American-Eurasian J Sci Res*, 8 (2013) 117.

19 Milaeva E R, Filimonova S I, Meleshonkova N N, Dubova L G, Shevtsova E F, Bachurin S O & Zefirov N S, *Bioinorg Chem Appl*, (2010).

20 Renukadevi K P, Saravana P S & Angayarkanni J, *IJPSR*, 2 (2011) 1467.

21 Naragani K, Mangamuri U, Muvva V, Poda S & Munaganti R K, *Int J Pharm Pharm Sci*, 8 (2016).

22 Abubackerand M N, Devi P K, *British Biomedical Bulletin* (2015)

23 Kuppuswamy K M, Jonnalagadda B & Arockiasamy S, *Int J Pharm Bio Sci*, 4 (2013) 613.

24 Kim M G, Jeong E Y & Lee H S, *J Korean Soc Appl Biol Chem*, 52 (2009) 327.

25 Kamiyama M, Horiuchi M, Umano K, Kondo K, Otsuka Y & Shibamoto T, *Int J Nutr Food Sci*, 2 (2013) 85.

26 Dash S, Jin C, Lee O, Xu Y & Qian P Y, *J Ind Microbiol Biotechnol*, 36 (2009) 1047.

27 Khattab A, Babiker E H & Saeed H A, *Int Current Pharm J*, 5 (2016) 27.

28 Tyagia T & Agarwala M, *Int J Current Pharm Res*, 9 (2017).

29 Belakhdar G, Benjouad A & Abdennebi, E H, *J Mater Environ Sci*, 6 (2015) 2778.

30 Okudoh V I & Wallis F M, *South Africa Trop J Pharm Res*, 11 (2012) 729.

31 Oliveira A M D, Conserva L M, Ferro J N D S , Brito F D A, Lemos R P L & Barreto E, *Int J Mol Sci*, 13 (2012) 1598.

32 Abdallah H & Ibrahim H, *Egypt J Aquatic Res*, 38 (2012) 185.

33 Fadeyi B O, Olatunji GA & Ogundele VA, *Nat Prod Chem Res*, 3 (2015).

34 Ramanujam P K, Sivasankaran C, Elumalai R S, Xavier V A J, Sekar K & Balasubramanian B, *Int J Chem Tech Res*, 6 (2014) 5071.