Plant Archives Volume 20 No. 2, 2020 pp. 3747-3753 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GCMS CHEMOPROFILING OF ANTIBACTERIAL PROFICIENT ACTINOMYCETES FROM MANGROVE SEDIMENTS OF COROMANDEL COASTLINE, PICHAVARAM, TAMIL NADU STATE, SOUTH INDIA

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Abstract

The sediment samples were collected from the Mangrove forest in Thandavarayan Sholagan Pet, Chidambaram Taluk, Cuddalore district, Tamilnadu, South India. Ethylacetate extracts were prepared from the actinomycete PMA2 and actinomycete PMA6, introduced in to GCMS column for GCMS analysis. The spectral studies showed that the actinomycete extracts were eluted in the Retention Time (RT) of 5.5 mins to 40 mins. After comparing with NIST (National Institute of Standard and Technology) library, 45 compounds were identified from the actinomycete PMA2 and 40 compounds were identified from the actinomycete PMA6.

Key words: Actinomycete, Ethylacetate extract, GCMS, Chromatogram

Introduction

Mangroves are one of the most important ecosystems of coastal and marine areas. They safeguard the ecology of the coastal areas and provide livelihood opportunities to the fishermen and pastoral families living in these areas. Mangroves also provide indirect benefits through its impact on up gradation of coastal and marine ecosystem. It is well known that coastal population succumbs to disasters of cyclones and Tsunamis, incurring heavy losses to their properties and live-stock. Bioactive natural compounds produced by microorganisms have been promising potential usefulness in safety and human health concerns, although there is still a significant demand of drug industry for synthetic products due to economic and time-consuming reasons. Recent review by Newman and Cragg, provides us a list of all approved agents from 1981 to 2006, from which a significant number of natural drugs are produced by microbes (Newman and Cragg, 2007).

According to World Health Organization (WHO) report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major issue to the WHO for the next millennium. Screening of plants for antimicrobial agents has gained much importance because WHO is encouraging and promoting in the development and utilization of medicinal plant resources in the traditional system of medicine. In addition, due to the fairly recent surge in the antibiotic resistance of pathogenic microorganisms and increasing consumer concerns regarding the negative health impact of synthetic preservative, there has been a dramatic increase in the application of natural antimicrobials of plant origin. The presence of natural products such as Terpenes, Alkaloids, Flavonoids, Coumarins and other secondary metabolites support the popular uses of medicines (Islam *et al.*, 2010).

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The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies. In recent years, the problem of multiple drug resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken not only to understand the genetic mechanisms of resistance but to develop new antimicrobial drugs especially from natural sources (Girish and Satish, 2008).

Bacterial resistance to currently used antibiotics is becoming a concern to public health (Monroe and Polk, 2000). The development of bacterial super resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents (Bhavnani and Ballow, 2000). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs (Santos *et al.*, 1995).

Antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin (Saxena *et al.*, 1994). Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. Many attempts have been made to eliminate *Streptococcus mutans* from the oral flora. Antibiotics such as Ampicillin, Chlorhexidine, Erythromycin, Penicillin, Tetracycline and Vancomycin have been very effective in preventing dental caries (Jarvinen *et al.*, 1993). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. The great biodiversity of plants found in Brazil might serve as an important source of new pharmacological agents (Basso *et al.*, 2005). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002). The aim of the present investigation is to identify the chemical constituents of ethylacetate extract from the actinomycete PMA2 and actinomycete PMA6 by Gas Chromatography and Mass Spectrometry.

Materials and Methods

Sample collection

The sediment samples were collected from the Mangrove forest in Thandavarayan Sholagan Pet, Chidambaram Taluk, Cuddalore district, Tamil Nadu, South India. The geological position is 11.41° N latitude, 79.79° E longitude and +5.25 M (MSL) altitude (Aarthi *et al.*, 2020).

Preparation of extract

Actinomycetes were isolated from the samples using Starch Casein Agar medium. Pure cultures were inoculated in Nutrient broth and incubated at 37°C for 10 days. Then the cultures were centrifuged at 2200 Xg for 10 mins. The supernatant was mixed with Ethyl acetate in 1:1 (v/v) ratio, extraction was done for 15 mins and then concentrated at 60°C up to complete dryness. The dried samples were exploited for Gas Chromatography and Mass Spectrometry studies (Kumari *et al.*, 2019).

GC-MS analysis

Ethylacetate extracts of Actinomycetes were analyzed in Gas Chromatography and Mass Spectroscopy using Thermo GC – Trace Ultra Ver: 5.0, Thermo MS DSQ II equipment. DB 35 – MS Capillary standard non – polar column was used in this study. The dimension of Column: 30 M, ID: 0.25mm, Film: 0.25µm (Mokhtar and Osama, 2018). The Temperature of the equipment was set at 70°C and raised 6°C for every minute up to 260 °C. Helium gas acted as the Carrier gas at the flow rate of 1mL/min. The volume of the sample injected in the column was 1µL (Al-Marzogi *et al.*, 2015). The run time

was about 38 min (Al-Marzogi *et al.*, 2016). The scan range was set at 50-650 (m/z) in the mass spectrum (Kadhim *et al.*, 2016). Identification of the compounds has been done based on the peaks produced in mass spectra. The mass spectral peaks were compared with the known compounds available in NIST (National Institute of Standard and Technology) database (Rizwan *et al.*, 2017; Alghamdi *et al.*, 2018).

Results

GC-MS analysis of actinomycete PMA2

GCMS analysis of ethylacetate extract from actinmycete PMA2 has produced the spectrum in the range of 5.5 mins to 40 mins. The major peaks were appeared in the Retention time (RT) of 7.93, 18.34, 26.60 and 34.73. The spectrum was compared with the compounds deposited in NIST database. Fig. 1 shows the GCMS spectrum of actimycete PMA2.

The Retention time (RT) was compared with the known compounds deposited in the NIST database. Totally 45 compounds were identified from the actinomycete PMA2. Table 1 provides the information about the name of the compound, Molecular formula, Molecular weight and the peak area produced in percentage.

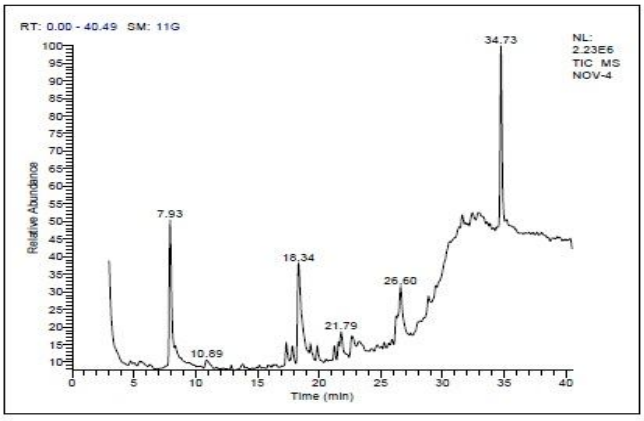

Fig. 1: Chromatogram of actinomycete PMA2

GCMS analysis of actinomycete PMA6

GCMS analysis of ethylacetate extract from actinmycete PMA6 has produced the spectrum in the range of 5.5 mins to 40 mins. The major peaks were appeared in the Retention time (RT) of 7.93, 18.30, 22.60, 26.60, 32.93 and 34.81. The spectrum was compared with the compounds deposited in NIST database. Fig. 2 shows the GCMS spectrum of actinomycete PMA6.

Based on the Retention time (RT), NIST database was compared for the similarity with the known compounds deposited. Totally 40 compounds were identified from the actinomycete PMA6. Table 2 provides the information about the name of the compound, Molecular formula, Molecular weight and the peak area produced in percentage.

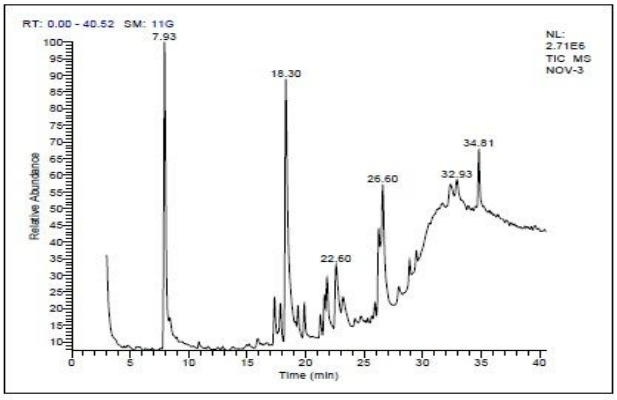

Fig. 2: Chromatogram of actinomycete PMA6.

Discussion

The breakthrough for new antimicrobial metabolites from medicinal plants arise an essential alternative to defeat the growing levels of drug resistance by human pathogens. Nowadays, lot of importance has been put in to the use of castor plant extracts as possible source of pest control agents and antimicrobial agents (Verma *et al.*, 2011).

GC–MS analysis plays an important role in the analysis of biocomponents. Normally, the plant materials are very difficult for identification, which made GC–MS well suited for their analysis. It is considered to be the gold standard in scientific analysis (Sajewicz *et al.*, 2009; Balamurugan *et al.*, 2012). Several phytochemical screening studies have been carried out in different parts of the world by using GC–MS (Dubey *et al.*, 2014; Doshi *et al.*, 2015).

The importance for the substitution of synthetic antimicrobial agents has emerged and promoted the research on vegetable sources. In order to discover the novel compounds phytoscreening of plant materials become an essential step (Ait-Ouazzou *et al.*, 2011; Lv *et al.*, 2011; Badawy and Abdelgaleil, 2014). Allicin is an antimicrobial substance due to its inhibitory action of sulfhydryl enzymes, it acts against a variety of organisms like bacteria, molds and viruses (Rico-Munoz and Davidson, 1983).

Among all actinobacteria, *Streptomyces* species are the most important producers of antimicrobial metabolites. *Streptomyces* sp. are known for the production of polyketides and nonribosomal polyketide compounds, which have important role in the Pharmaceutical community (Das *et al.*, 2018). 13 major compounds were identified from *Streptomyces* species, the presence of

Table 1: List of significant compounds identified from the actinomycete PMA2

S.No	Retention Time (RT)	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	7.93	4 – Methyl, 4 – hexen, 1 - ol	$C_7H_{14}O$	114	17.74
2.	7.93	4 – tert – Butyl – 3 – methyl, 2H Thiophen-5-one	$C_9H_{14}OS$	170	17.74
3.	10.87	Desoximetasone	$C_{22}H_{29}FO_4$	376	1.27
4.	15.88	4 – Thiouridine	$C_9H_{12}N_2O_5S$	260	0.55
5.	15.88	Cinchonine	$C_{19}H_{22}N_2O$	294	0.55
6.	15.88	Methyl 12-keto stearic acid	$C_{19}H_{36}O_3$	312	0.55
7.	17.35	1,10 – Cyclo eicosanedione	$C_{20}H_{36}O_2$	308	2.77
8.	17.35	12 – Hydroxy Stearic acid	$C_{18}H_{36}O_3$	300	2.77
9.	17.35	12 – Stearolactone	$C_{18}H_{34}O_2$	282	2.77
10.	17.35	Didemnin B	$C_{57}H_{89}N_7O_{15}$	1111	2.77
11.	17.83	2,4 – Hepta dienoic acid, 6-methyl, ethyl ester	$C_{10}H_{16}O_2$	168	2.02
12.	17.83	Imidazo[2,1-a] isoquinoline	$C_{11}H_8N_2$	168	2.02
13.	18.32	1-oxaspiro[4.5]decan-2-one	$C_9H_{14}O_2$	154	12.66
14.	19.30	α-L-Galactopyranose, 6-deoxy-, cyclic 1,2:3,4-bis(butylboronate)	$C_{14}H_{26}B_2O_5$	296	1.51
15.	21.26	Actinomycin C2	$C_{63}H_{88}N_{12}O_{16}$	1268	1.85
16.	21.26	Tetraneurin D	$C_{17}H_{24}O_6$	324	1.85
17.	21.79	Metconazole	$C_{17}H_{22}ClN_3O$	319	5.45
18.	22.64	9 – Octa Decenoic acid, 12 – hydroxy	$C_{18}H_{34}O_3$	298	3.05
19.	22.64	Bioepiderm	$C_{10}H_{16}N_2O_3S$	244	3.05
20.	22.64	Ricinoleic acid	$C_{18}H_{34}O_3$	298	3.05
21.	23.27	Stenophylline A	$C_{37}H_{55}NO_{10}$	673	1.96
22.	23.27	Veratroylzygadenine	$C_{36}H_{51}NO_{10}$	657	1.96
23.	24.70	5 – Chloro, 2 – nitro, Cinnamic acid	$C_9H_6ClNO_4$	227	0.68
24.	25.27	Endosulfan II	$C_9H_6Cl_6O_3S$	404	0.66
25.	25.27	Demecolcine	$C_{21}H_{25}NO_5$	371	0.66
26.	26.60	3,6 Diisobutyl 2,5 – Piperazinedione	$C_{12}H_{22}N_2O_2$	226	4.06
27.	28.00	2,5 – Dichloro 4 – nitro aniline	$C_6H_4Cl_2N_2O_2$	206	1.10
28.	28.00	Isopropyl 9Z – Tetradecenoate	$C_{17}H_{32}O_2$	268	1.10
29.	29.44	Beclomethasone	$C_{22}H_{29}ClO_5$	408	1.13
30.	29.44	Betamethasone acetate	$C_{24}H_{31}FO_6$	434	1.13
31.	29.44	Ingenol triacetate	$C_{26}H_{34}O_8$	474	1.13
32.	30.53	9,12 – Octadecadienoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester	$C_{27}H_{54}O_4Si_2$	498	3.42
33.	30.53	Fenretinide	$C_{26}H_{33}NO_2$	391	3.42
34.	31.26	3-Hydroxy Androsta-5,7,9(11)-trien-17-one	$C_{19}H_{24}O_2$	284	0.60
35.	31.59	9,12,15 – Octadecatrienoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester	$C_{27}H_{52}O_4Si_2$	496	1.46
36.	31.59	Curan, 16,17- didehydro-, (20.xi)	$C_{19}H_{24}N_2$	280	1.46
37.	31.59	Lucenin 2	$C_{27}H_{30}O_{16}$	610	1.46
38.	32.89	Rhodoxanthin	$C_{40}H_{50}O_2$	562	1.15
39.	32.89	Calix[4]arene	$C_{28}H_{24}O_4$	424	1.15
40.	34.73	9 – Octa decenamide	$C_{18}H_{35}NO$	281	22.81
41.	34.73	13 – Docosenamide	$C_{22}H_{43}NO$	337	22.81

Table 1 continued .....

Table 1 continued .....

42.	34.73	Trans – 13 – Docosenamide	$C_{22}H_{43}NO$	337	22.81
43.	39.44	Bergenin	$C_{14}H_{16}O_9$	328	0.75
44.	40.34	Methyl (3alpha)-3-(acetyloxy) -12-oxocholan-24-oate	$C_{27}H_{42}O_5$	446	1.48
45.	40.34	Methyl betulate	$C_{31}H_{50}O_3$	470	1.48

Table 2: List of significant compounds identified from the actinomycete PMA6

S.No	Retention Time (RT)	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	5.57	Quercetin 7,3',4' – Trimethyl ether	$C_{18}H_{16}O_7$	344	0.48
2.	5.57	Cis –Isohumulone	$C_{21}H_{30}O_5$	362	0.48
3.	5.57	Dotriacontane	$C_{32}H_{66}$	450	0.48
4.	7.93	(3R) – 3 – (2 – Propan – 2 – yloxyethyl) Piperidine	$C_{10}H_{21}NO$	171	23.55
5.	7.93	4 – Methyl, 4 – hexen, 1 - ol	$C_7H_{14}O$	114	23.55
6.	7.93	4 – tert – Butyl – 3 – methyl, 2H Thiophen-5-one	$C_9H_{14}OS$	170	23.55
7.	8.42	Debenzoylaralionine	$C_{27}H_{34}N_4O_4$	478	0.57
8.	8.42	Veratramine	$C_{27}H_{39}NO_2$	409	0.57
9.	10.87	2 – Bromo, Tetradecanoic acid	$C_{14}H_{27}BrO_2$	306	0.52
10.	15.17	Cinchonine	$C_{19}H_{22}N_2O$	294	1.08
11.	15.17	Octyloxy, diphenyl Silane	$C_{20}H_{27}OSi$	312	1.08
12.	15.88	Proceroside	$C_{29}H_{40}O_{10}$	548	0.73
13.	17.35	12 – Stearolactone	$C_{18}H_{34}O_2$	282	3.47
14.	17.35	6-Thioxanthine	$C_5H_4N_4OS$	168	3.47
15.	17.35	Cyclohexyl Carbamoyl Azide	$C_7H_{12}N_4O$	168	3.47
16.	17.85	Imidazo (5,1-a) isoquinoline	$C_{11}H_8N_2$	168	2.42
17.	18.30	Hexahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_7H_{10}N_2O_2$	154	19.46
18.	19.34	Sylvopinol	$C_8H_{10}O_3$	154	1.53
19.	19.89	2 – Ethyl, 5 – propyl, Thiophene	$C_9H_{14}S$	154	2.37
20.	19.89	3,5 – Dimethoxy Phenol	$C_8H_{10}O_3$	154	2.37
21.	21.26	Actinomycin C2	$C_{63}H_{88}N_{12}O_{16}$	1268	1.74
22.	21.83	Boroxine, Diethyl Methyl	$C_5H_{13}B_3O_3$	154	6.94
23.	22.58	Barbital	$C_8H_{12}N_2O_3$	184	6.02
24.	22.58	Spinulosin	$C_8H_8O_5$	184	6.02
25.	22.58	Trimethyl Aconitate	$C_9H_{12}O_6$	216	6.02
26.	24.21	methyl 8-ketostearate	$C_{19}H_{36}O_3$	312	0.67
27.	24.72	Metconazole	$C_{17}H_{22}ClN_3O$	319	0.48
28.	26.60	3,6 Diisobutyl 2,5 – Piperazinedione	$C_{12}H_{22}N_2O_2$	226	5.92
29.	28.88	Aspartame	$C_{14}H_{18}N_2O_5$	294	1.71
30.	28.88	Ergotamine	$C_{33}H_{35}N_5O_5$	581	1.71
31.	29.45	Chloro Anthraquinone	$C_{14}H_7ClO_2$	242	1.21
32.	30.51	2,5 – Dichloro 4 – nitro aniline	$C_6H_4Cl_2N_2O_2$	206	1.17
33.	31.70	9,12,15 – Octadecatrienoic acid, 2,3-bis [(trimethylsilyl) oxy] propyl ester	$C_{27}H_{52}O_4Si_2$	496	0.69
34.	31.70	Fenretinide	$C_{26}H_{33}NO_2$	391	0.69
35.	31.70	Lucenin 2	$C_{27}H_{30}O_{16}$	610	0.69
36.	32.35	3-Hydroxy Androsta-5,7,9(11)-trien-17-one	$C_{19}H_{24}O_2$	284	2.29
37.	32.35	Curan, 16,17- didehydro-, (20.xi)	$C_{19}H_{24}N_2$	280	2.29

Table 2 continued .....

Table 2 continued .....

38.	32.35	Endosulfan II	$C_9H_6Cl_6O_3S$	404	2.29
39.	32.35	Rhodoxanthin	$C_{40}H_{50}O_2$	562	2.29
40.	34.81	13 – Docosenamide	$C_{22}H_{43}NO$	337	4.30

further tiny peaks in the spectrum indicated that the extract might contain other unidentified compounds. The major component alone or in combination with minor constituents might be responsible for the antibacterial activity. It has exposed the potentials impregnated in marine *Streptomyces* as a source of newer and more valuable antibiotics (Faja *et al.*, 2017). The crude extract of Actinobacteria contains different types of compounds like fatty acid methyl ester, long chain alkanes, fatty alcohol, piperazinedione and pyrrolizidine. Among seven vital compounds, four compounds exhibited antimicrobial activity (Singh and Wahla, 2018).

Cinnamic acid proved as a powerful antibacterial and antifungal effect with Minimum Inhibitory Values ranging from 50.4 and 449 μM. Fascinatingly, it has displayed higher inhibitory effect against fungal species compared to bacteria, and Gram-negative and Gram-positive bacteria were equally inhibited by the compound. The derivative 3,4-methylenedioxycinnamic acid has been reported to inhibit *Mycobacterium tuberculosis*. The effect of the position of the nitro group showed 4-nitrocinnamic acid exhibited more powerful antimicrobial effect (Guzman, 2014).

Conclusion

In the present study, 85 compounds have been identified from two actinomycetes through Gas Chromatography and Mass Spectrometry. Several compounds have medicinal value. Though, the compound isolation might lead to successful result. Hence, these actinomycetes are recommended for Biopharmaceutical importance.

Acknowledgement

The authors are thankful to the Head, Department of Biotechnology, Principal and Management of K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode for providing necessary laboratory facilities during the study period.

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