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Studies on Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Oil Contaminated Soil Samples

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Abstract: Biosurfactant are amphiphilic biomolecules, with hydrophobic and hydrophilic portions, the hydrophobic part of the molecule is based on saturated or unsaturated fatty acids, the hydrophilic portion can be either cationic and anionic amino acids or mono-and di-saccharides (rhamnose and trehalose respectively).To present study totally seven sampling stations were selected from Tirunelveli and its surrounding area in order to isolate biosurfactant producing organisms.Other more elaborate techniques including chromatography-mass spectroscopy (GC-MS). In the Gas Chromatography-Mass Spectroscopy (GC-MS) analysis, biosurfactant produced by Pseudomonas aeruginosa strain S1RU2 showed various compounds. The compounds such as, "Hexadecenoic acid, Z-11-", "9-Octadecenoic acid, (E)-", "Cyclotetradecane", "1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-","2-Ethylacridine","Pyrido[2,3-d]pyrimidine,4-phenyl","Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-","5-Methyl-2-trimethylsilyloxy-acetophenone", "5-Methyl-2-phenylindolizine", "n-Propyl 11-octadecenoate", "2,4-Cyclohexadien-1-one,3,5-bis(1,1-dimethylethyl)-4-hydroxy-","2-Ethylacridine", "Cyclobarbital", "Benzo[h]quinoline, 2,4-dimethyl-" were identified using Gas chromatography-mass spectroscopy (GC-MS) analysis.Together with the GC-MS research proves that the biosurfactant is a glycolipid.
Key-words: Biosurfactants, GC-MS,Glycolipid, *Pseudomonas aeruginosa*,Cyclotetradecane

Introduction :

The bioavailability of organic compounds, commonly is ruled by physical-chemical processes such as sorption and desorption, diffusion and dissolution. Some microorganisms improve bioavailability of biodegradable organic matter by production of biosurfactants (Abbasi et al., 2013). Biosurfactants (BS) are exopolymers mainly produced as secondary metabolites by bacteria, yeasts and fungi. By their molecular weight can be classified as biosurfactant of low molecular weight, such as glycolipids and lipopeptides; they are more effective at lowering the interfacial and surface tensions, and biosurfactant of high molecular weight, polysaccharides, lipoproteins and lipopolysaccharides; they are effective stabilizers of oil-in-water emulsions, also have emulsificant and dispersant properties (Shete et al., 2006).

Biochemically, biosurfactant are amphiphilic biomolecules, with hydrophobic and hydrophilic portions, the hydrophobic part of the molecule is based on saturated or unsaturated fatty acids,

the hydrophilic portion can be either cationic and anionic amino acids or mono-and di-saccharides (rhamnose and trehalose respectively). Both portions can have a wide range of chemical structural features ((Banat et al., 2000). The best-studied microbial surfactants are glycolipids, among these, the best-known compounds are rhamnolipids, trehalolipids, sophorolipids and mannosylerythritol lipids. The biosurfactant most studied and better known are rhamnolipids produced by Pseudomonas species, trehalolipids produced by Rhodococcus species, surfactin by Bacillus subtilis, sophorolipids by Candida bombicola (Ron and Rosenberg, 2001) and emulsan by Acinetobactercalcoaceticus (Karanth et al., 1999).

In general biosurfactant, and specifically rhamnolipids, have different physiological functions such as uptake of poorly accessible substrates like hydrophobic molecules due to their tensoactive properties. This is particulary useful, for competing with other microorganism due to their wide range of antimicrobial activity and the adhesion or contact to hydrophobic surfaces (Shete et al., 2006).In the past years, different range of techniques such as gas chromatography–mass spectrometry (GC–MS), are familiarized for characterizing and identifying the properties of biosurfactants compounds produced by a different micro-organism (Jimoh and Lin, 2019a)

Mass spectrometry is also used for determining the structure and chemical bonds present in the biosurfactant sample. In this method, the instrument is coupled with liquid or gas chromatography to identify its structure and gives the qualitative and quantitative analysis of each compound present in the biosurfactants sample (Sharma et al., 2014). The same result was also reported by Ibrahim et al. (2013), who showed that the hydrophilic and hydrophobic parts of the biosurfactants produced from bacteria could be analyzed by using LC–MS and GC–MS respectively. It has more advantages with accuracy than HPLC in the molecular determinants of the compound. In addition to the above methods, mass spectrometry coupled with electrospray ionization to identify the different biological molecules such as proteins, peptides from biosurfactants using high voltage separate fragments of macromolecules of ions produced gives better and precise accuracy (Monteiro et al., 2007). Jimoh and Lin (2019b) investigated that electrospray ionization coupled with liquid chromatography helps to identify the biomolecule with low concentration and secondary metabolites as in the biosurfactant compound. For instance, Yin et al. (2009) also used electrospray ionization coupled with liquid chromatography to identify the chemicals constituents from biosurfactants produced from *Paenibacillusdendritiformis CN5*. They identify eight amino acid constituents from biosurfactant biomolecule produced from *P. aeruginosa S6*, including four crucial amino acids, such as *RhaC12:1C10, RhaC8C10, RhaRhaC10C12:1, and RhaC10C10*.

Likewise, Guo et al. (2012) used matrix-assisted laser desorption/ionization-time of flight mass spectroscopy to identify the chemical constituent found in biosurfactants made from the genus *Paenibacillus.* They found that the biosurfactants have 13 amino acid residues with cyclic lipopeptide and finally noted that using this coupled technique can help identify the different complex compounds with less time with high-resolution data for the essential characterization in lass cost. However, it demands more energy for the formation of ions.

Materials and Methods :

For the present study, soil samples were collected from the zones contaminated with diesel (Automobile Service Station, Transport Depot, Diesel Mechanic Workshop etc) in and around Tirunelveli District. The samples were noted for any oil contamination and their nature of

texture was noted. The soils were collected in a sterile container in triplicate and were transported to the laboratory in aseptic condition. The samples were stored in the laboratory at ambient temperature until further use. Crude biosurfactant was extracted from cell-free supernatant collected. After removing the cell pellets, the pH of the supernatant was was adjusted to 2, using 1.0 M H_2SO_4. Then equal volume of chloroform: methanol (2: 1) was added. This mixture was shake well for mixing and kept for overnight for evaporation in a pre-weighed petri plate . Finally sediment was obtained.

A 1µl of biosurfactant solution was injected into the Gas Chromatography-Mass Spectroscopy (GC-MS) machine with a split detector and Mass Spectrometer Detector (MSD). Helium was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1µl, injector temperature 250˚C, and ion-source temperature 280˚C. Total GC running time was 90.67 min and the total length of time for running the analysis determined and programmed by the GC-MS analyst. Peaks in the chromatograms produced by these analyses were identified by a combination of references to their mass spectra and the NIST08 mass spectral database (Iowa State University (ISU) (2017). The National Institute of Standard and Technology (NIST) database, which contains more than 62,000 patterns, was used for mass-spectrum GC-MS interpretation. The spectrum of the unknown components was compared with the range of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained using CAS number. NIST and FAME software were used as MS library for FAME identification and analysis (Elumalai et al., 2014).

Result:

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis:

In the present study, biosurfactant produced by *Pseudomonas aeruginosa* strain S1RU2 showed various compounds. The compounds such as, “Hexadecenoic acid, Z-11-”, “9-Octadecenoic acid, (E)-”, “Cyclotetradecane”, “1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-”, “2-Ethylacridine”, “Pyrido[2,3-d]pyrimidine, 4-phenyl-”, “Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-”, “5-Methyl-2-trimethylsilyloxy-acetophenone”, “5-Methyl-2-phenylindolizine”, “n-Propyl 11-octadecenoate”, “2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-”, “2-Ethylacridine”, “Cyclobarbital”, “Benzo[h]quinoline, 2,4-dimethyl-” were identified using Gas chromatography-mass spectroscopy (GC-MS) analysis.

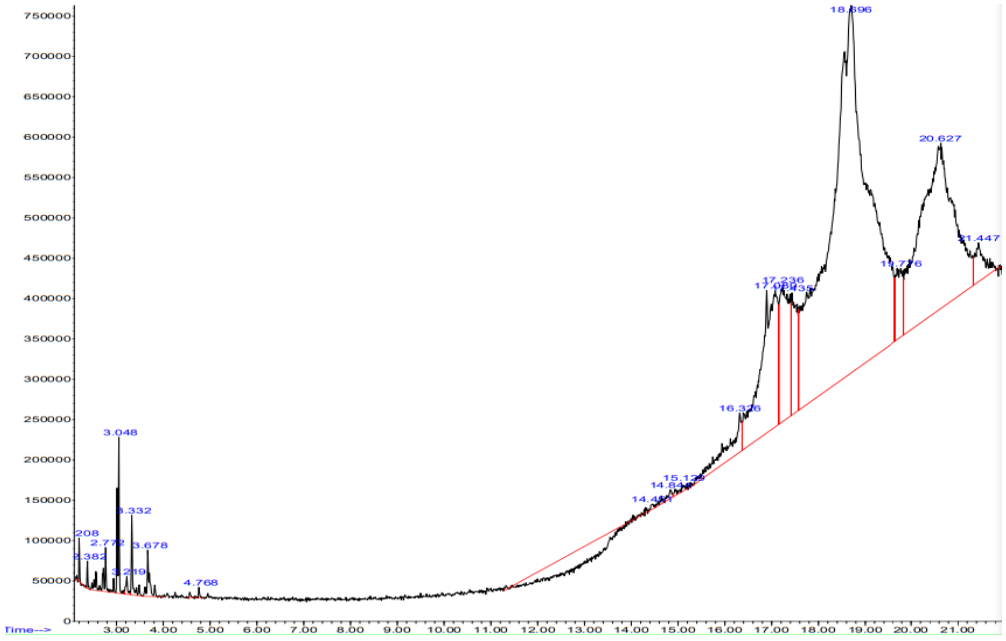

Figure 1- Analysis of biosurfactant produced by *Pseudomonas aeruginosa* strain S1RU2 using Gas chromatography-mass spectroscopy (GC-MS) analysis

GC – MS spectrum of Hexadecenoic acid, Z-11- produced by *Pseudomonas aeruginosa* strain S1RU2 :

The GC-MS spectrum of Hexadecenoic acid, Z-11- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 5 and the details of Hexadecenoic acid, Z-11- was given in table 1.

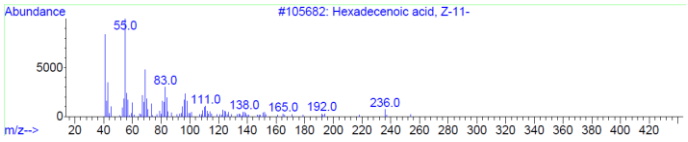

Figure 2-GC – MS spectrum of Hexadecenoic acid, Z-11-

Table 1- Details of Hexadecenoic acid, Z-11-

Hexadecenoic acid, Z-11-	
Formula	$C_{16}H_{30}O_2$
Molecular weight	254.4082
IUPAC Standard InChI	InChI=1S/C16H30O2/c1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16(17)18/h5-6H,2-4,7-15H2,1H3,(H,17,18)/b6-5-
IUPAC Standard	JGMYDQCXGIMHLL-WAYWQWQTSA-N

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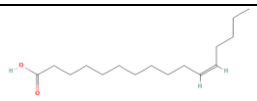
InChIKey	
CAS Registry Number	2416-20-8
Chemical structure	
Other names	Z-11-Hexadecenoic acid

GC – MS spectrum of 9-Octadecenoic acid, (E)- produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 9-Octadecenoic acid, (E)- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 6 and the details of 9-Octadecenoic acid, (E)- was given in table 2.

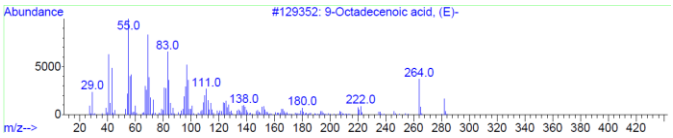


Figure 3- GC – MS spectrum of 9-Octadecenoic acid, (E)-

Table 2- Details of 9-Octadecenoic acid, (E)-

9-Octadecenoic acid, (E)-	
Formula	$C_{18}H_{34}O_2$
Molecular weight	282.4614
IUPAC Standard InChI	InChI=1S/C18H34O2/c1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18(19)20/h9-10H,2-8,11-17H2,1H3,(H,19,20)/b10-9+
IUPAC Standard InChIKey	ZQPPMHVWECSIRJ-MDZDMXLPSA-N
CAS Registry Number	112-79-8
Chemical structure	
Other names	trans-δ^9-Octadecenoic acid; trans-Octadec-9-enoic acid; trans-Oleic acid; trans-9-Octadecenoic acid; Elaidic acid; (E)-9-Octadecenoic acid; (E)-Palmitoleic acid; trans-Elaidic acid; (E)-Palmitelaidic acid; trans-.delta.(sup 9)-Octadecenoic acid

GC – MS spectrum of Cyclotetradecane produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of Cyclotetradecane produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 7 and the details of Cyclotetradecane was given in table 3.

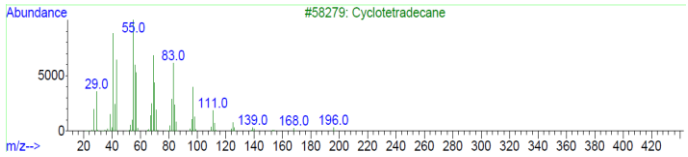


Figure 4-GC – MS spectrum of Cyclotetradecane

Table 3 Details of Cyclotetradecane

Cyclotetradecane	
Formula	$C_{14}H_{28}$
Molecular weight	196.3721
IUPAC Standard InChI	InChI=1S/C14H28/c1-2-4-6-8-10-12-14-13-11-9-7-5-3-1/h1-14H2
IUPAC Standard InChIKey	KATXJJSCAPBIOB-UHFFFAOYSA-N
CAS Registry Number	295-17-0
Chemical structure	

GC – MS spectrum of 1, 2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 1, 2-Benzenediol, 3,5-bis(1,1-dimethylethyl)- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 8 and the details of 1, 2-Benzenediol, 3,5-bis(1,1-dimethylethyl)- was given in table 4.

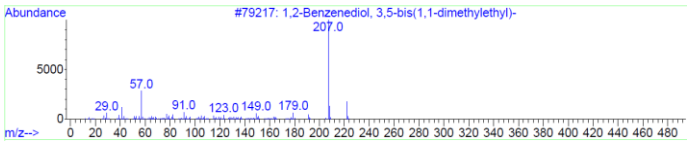
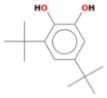


Figure 5-GC – MS spectrum of 1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)

Table 4-Details of 1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-

1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	
Formula	$C_{14}H_{22}O_2$
Molecular weight	222.3233
IUPAC Standard InChI	InChI=1S/C14H22O2/c1-13(2,3)9-7-10(14(4,5)6)12(16)11(15)8-9/h7-8,15-16H,1-6H3
IUPAC Standard InChIKey	PJZLSMMERMMQBJ-UHFFFAOYSA-N
CAS Registry Number	1020-31-1
Chemical structure	
Other names	1,2-Benzenediol, 3,5-di(1,1-dimethylethyl)-; 3,5-di-tert-Butylcatechol; 3,5-di-t-Butylcatechol; Pyrocatechol, 3,5-di-tert-butyl-; 3,5-Di-tert-butylpyrocatechol; 4,6-Di-tert-butylpyrocatechol; 3,5-Di-tert-butyl-1,2-benzenediol; 3,5-Bis(1,1-dimethylethyl)catechol; NSC 59767

GC – MS spectrum of 2-Ethylacridine produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 2-Ethylacridine produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 9 and the details of 2-Ethylacridine was given in table 5.

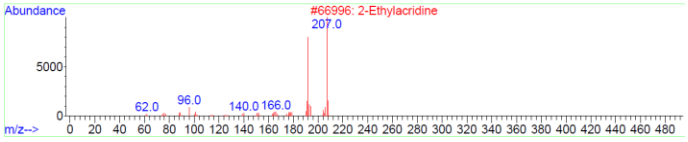
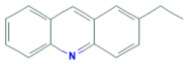


Figure 6-GC – MS spectrum of 2-Ethylacridine

Table 5- Details of 2-Ethylacridine

2-Ethylacridine	
Formula	$C_{15}H_{13}N$
Molecular weight	207.27
IUPAC Standard InChI	InChI=1S/C15H13N/c1-2-11-7-8-15-13(9-11)10-12-5-3-4-6-14(12)16-15/h3-10H,2H2,1H3

IUPAC Standard InChIKey	LMTKWIQCTSKTDN-UHFFFAOYSA-N
CAS Registry Number	55751-83-2
Chemical structure	

GC – MS spectrum of Pyrido[2,3-d]pyrimidine, 4-phenyl- produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of Pyrido[2,3-d]pyrimidine, 4-phenyl- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 10 and the details of Pyrido[2,3-d]pyrimidine, 4-phenyl- was given in table 6.

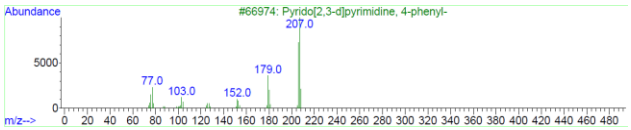
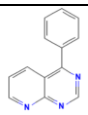


Figure 7-GC – MS spectrum of Pyrido[2,3-d]pyrimidine, 4-phenyl-

Table 6- Details of Pyrido[2,3-d]pyrimidine, 4-phenyl-

Pyrido[2,3-d]pyrimidine, 4-phenyl-	
Formula	$C_{13}H_9N_3$
Molecular weight	207.23
IUPAC Standard InChI	InChI=1S/C13H9N3/c1-2-5-10(6-3-1)12-11-7-4-8-14-13(11)16-9-15-12/h1-9H
IUPAC Standard InChIKey	IXBMZXJFPBMPDB-UHFFFAOYSA-N
CAS Registry Number	28732-75-4
Chemical structure	
Other names	4-Phenylpyrido[2,3-d]pyrimidine

GC – MS spectrum of Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 11

and the details of Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- was given in table 7.

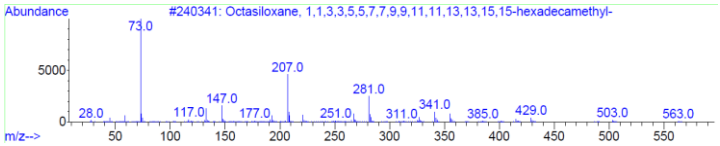


Figure 8-GC – MS spectrum of Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-

Table 7- Details of Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-

Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	
Formula	$C_{16}H_{48}O_7Si_8$
Molecular weight	577.2
IUPAC Standard InChI	InChI=1S/C16H48O7Si8/c1-24(2)17-26(5,6)19-28(9,10)21-30(13,14)23-31(15,16)22-29(11,12)20-27(7,8)18-25(3)4/h1-16H3
IUPAC Standard InChIKey	JETFAOWLNCGULZ-UHFFFAOYSA-N
CAS Registry Number	19095-24-0
Chemical structure	
Other names	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane,Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-2,4,4,6,6,8,8,10,10,12,12,14,14,16-tetradecamethyl-3,5,7,9,11,13,15-heptaoxa-2,4,6,8,10,12,14,16-octasilaheptadecane

GC – MS spectrum of Octasiloxane, 5-Methyl-2-trimethylsilyloxy-acetophenone produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 5-Methyl-2-trimethylsilyloxy-acetophenone produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 12 and the details of 5-Methyl-2-trimethylsilyloxy-acetophenone was given in table 8.

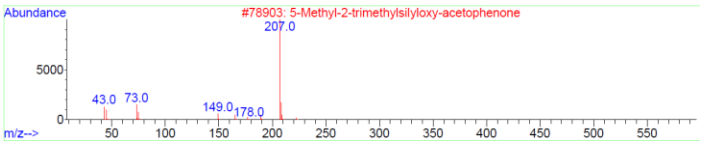
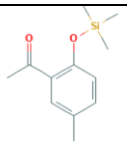


Figure 9- GC – MS spectrum of 5-Methyl-2-trimethylsilyloxy-acetophenone

Table 8- Details of 5-Methyl-2-trimethylsilyloxy-acetophenone

5-Methyl-2-trimethylsilyloxy-acetophenone	
Formula	$C_{12}H_{18}O_2Si$
Molecular weight	222.35
IUPAC Standard InChI	InChI=1S/C12H18O2Si/c1-9-6-7-12(14-15(3,4)5)11(8-9)10(2)13/h6-8H,1-5H3
IUPAC Standard InChIKey	LZFQRXBEWMGXCV-UHFFFAOYSA-N
CAS Registry Number	97389-69-0
Chemical structure	
Other names	2'-Hydroxy-5'-methylacetophenone, TMS derivative,1-(5-Methyl-2-((trimethylsilyl)oxy)phenyl)ethanone,Ethanone, 1-[5-methyl-2-[(trimethylsilyl)oxy]phenyl]-5-Methyl-2-trimethylsilyloxy-acetophenone

GC – MS spectrum of 5-Methyl-2-phenylindolizine produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 5-Methyl-2-phenylindolizine produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 13 and the details of 5-Methyl-2-phenylindolizine was given in table 9.

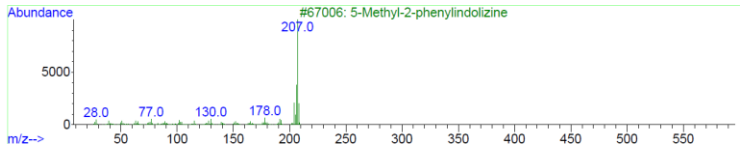
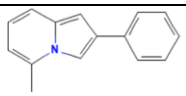


Figure 10-GC – MS spectrum of 5-Methyl-2-phenylindolizine

Table 9- Details of 5-Methyl-2-phenylindolizine

5-Methyl-2-phenylindolizine	
Formula	$C_{15}H_{13}N$
Molecular weight	207.27
IUPAC Standard InChI	InChI=1S/C15H13N/c1-12-6-5-9-15-10-14(11-16(12)15)13-7-3-2-4-8-13/h2-11H,1H3
IUPAC Standard InChIKey	INOVZESTRNGOLS-UHFFFAOYSA-N
CAS Registry Number	36944-99-7
Chemical structure	
Other names	5-Methyl-2-phenylindolizine,2-Phenyl-5-methylindolizine,5-methyl-2-phenyl-indolizine

GC – MS spectrum of n-Propyl 11-octadecenoate produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of n-Propyl 11-octadecenoate produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 14 and the details of n-Propyl 11-octadecenoate was given in table 10.

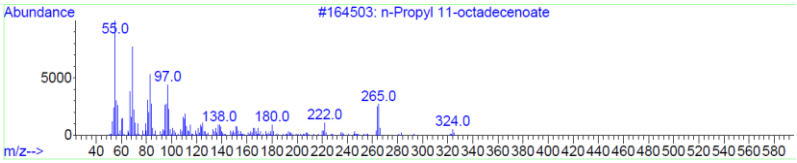
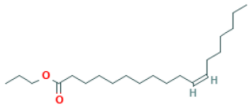


Figure 11-GC – MS spectrum of n-Propyl 11-octadecenoate

Table 10- Details of n-Propyl 11-octadecenoate

n-Propyl 11-octadecenoate	
Formula	$C_{21}H_{40}O_2$
Molecular weight	324.5
IUPAC Standard InChI	InChI=1S/C21H40O2/c1-3-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-21(22)23-20-4-2/h9-10H,3-8,11-20H2,1-2H3/b10-9-
IUPAC Standard InChIKey	XFPIVTOWUUZNNP-KTKRTIGZSA-N

Chemical structure	
Other names	propyl (*Z*)-octadec-11-enoate

GC – MS spectrum of 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 15 and the details of 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- was given in table 11.

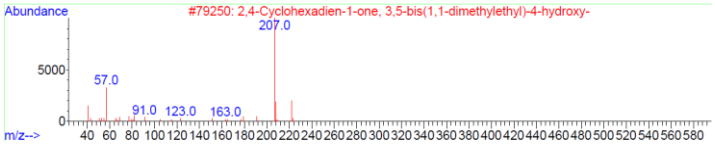
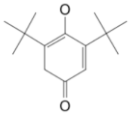


Figure 12-GC – MS spectrum of 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-

Table 11-Details of 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-

2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	
Formula	C14H22O2
Molecular weight	222.33
IUPAC Standard InChI	InChI=1S/C14H22O2/c1-13(2,3)10-7-9(15)8-11(12(10)16)14(4,5)6/h7,16H,8H2,1-6H3
IUPAC Standard InChIKey	VMIBWUBKVGANRX-UHFFFAOYSA-N
Chemical structure	

GC – MS spectrum of 2-Ethylacridine produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of 2-Ethylacridine produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 16 and the details of 2-Ethylacridine was given in table 12.

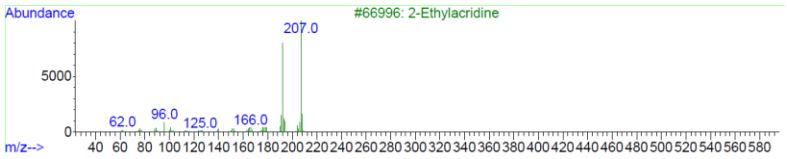
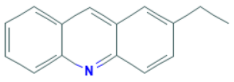


Figure 13- GC – MS spectrum of 2-Ethylacridine

Table 12- Details of 2-Ethylacridine

2-Ethylacridine	
Formula	$C_{15}H_{13}N$
Molecular weight	207.27
IUPAC Standard InChI	InChI=1S/C15H13N/c1-2-11-7-8-15-13(9-11)10-12-5-3-4-6-14(12)16-15/h3-10H,2H2,1H3
IUPAC Standard InChIKey	LMTKWIQCTSKTDN-UHFFFAOYSA-N
CAS Registry Number	55751-83-2
Chemical structure	

GC – MS spectrum of Cyclobarbital produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of Cyclobarbital produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 17, and the details of Cyclobarbital was given in table 13.

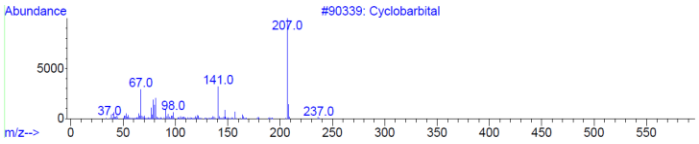
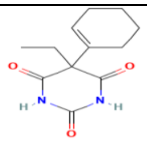


Figure 14-GC – MS spectrum of Cyclobarbital

Table 13- Details of Cyclobarbital

Cyclobarbital	
Formula	$C_{12}H_{16}N_2O_3$
Molecular weight	236.27
IUPAC Standard InChI	InChI=1S/C12H16N2O3/c1-2-12(8-6-4-3-5-7-8)9(15)13-

	11(17)14-10(12)16/h6H,2-5,7H2,1H3,(H2,13,14,15,16,17)
IUPAC Standard InChIKey	WTYGAUXICFETTC-UHFFFAOYSA-N
CAS Registry Number	52-31-3
Chemical structure	
Other names	5-(cyclohexen-1-yl)-5-ethyl-1,3-diazinane-2,4,6-trione

GC – MS spectrum of Benzo[h]quinoline, 2,4-dimethyl- produced by *Pseudomonas aeruginosa* strain S1RU2:

The GC-MS spectrum of Benzo[h]quinoline, 2,4-dimethyl- produced by *Pseudomonas aeruginosa* strain S1RU2 was given in figure 18, and the details of Benzo[h]quinoline, 2,4-dimethyl- was given in table 14.

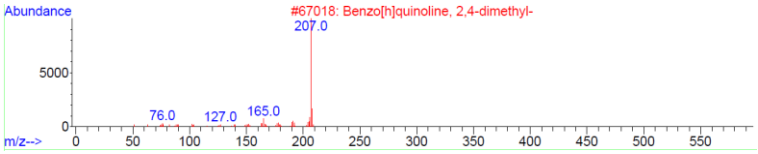
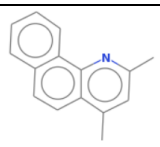


Figure 15-GC – MS spectrum of Benzo[h]quinoline, 2,4-dimethyl-

Table 14-Details of Benzo[h]quinoline, 2,4-dimethyl-

Benzo[h]quinoline, 2,4-dimethyl-	
Formula	$C_{15}H_{13}N$
Molecular weight	207.2704
IUPAC Standard InChI	InChI=1S/C15H13N/c1-10-9-11(2)16-15-13(10)8-7-12-5-3-4-6-14(12)15/h3-9H,1-2H3
IUPAC Standard InChIKey	VPSXZUORXQYBAT-UHFFFAOYSA-N
CAS Registry Number	605-67-4
Chemical structure	

Other names	2,4Dimethylbenzo[h]quinoline,Benzo(h)quinoline,2,4-dimethyl2,4Dimethylbenzo(h)quinoline,Benzo[h]quinoline, 2,4-dimethyl-

Our GC-MS research proves that the biosurfactant is a glycolipid.

Discusion :

In the present study, biosurfactant produced by *Pseudomonas aeruginosa* strain S1RU2 showed various compounds. The compounds such as, "Hexadecenoic acid, Z-11-", "9-Octadecenoic acid, (E)-", "Cyclotetradecane", "1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-", "2-Ethylacridine", "Pyrido[2,3-d]pyrimidine, 4-phenyl-", "Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-", "5-Methyl-2-trimethylsilyloxy-acetophenone", "5-Methyl-2-phenylindolizine", "n-Propyl 11-octadecenoate", "2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-", "2-Ethylacridine", "Cyclobarbital", "Benzo[h]quinoline, 2,4-dimethyl-" were identified using Gas chromatography-mass spectroscopy (GC-MS) analysis.

The GC-MS analysis on biosurfactant by Anaukwu et al. (2020), revealed the presence fatty acid components of the biosurfactant produced by CGA1 which include cyclotetrasiloxane, methyl stearate, octadecanoic acid, cyclododecanol, and tert-butyl isopropyl disulphide. In our present study, Hexadecenoic acid, Octadecenoic acid, Cyclotetradecane, Octasiloxane were present with octadecanoic acid occurring most. Octadecanoic acid commonly called stearic acid is a surface-active agent derived from natural fatty acids, which has excellent surfactant properties and is easily biodegraded . Cyclotetrasiloxane is used as a hair conditioner, skin conditioner, and in other cosmetics as a foaming agent . Recovery of these components as the functional components of the biosurfactant produced by *Pseudomonas aeruginosa* strain CGA1isin line with the reports of other researchers (Parthipan et al., 2017; Deepansh et al., 2014; Lobna and Ahmed, 2013).

Conclusion:

Many useful bioactive compounds are found out from the oil contaminated soil extract. These compounds are play important role as well as several antimicrobial molecules were detected for further studies.Biosurfactants are natural, greener, and more environmentally friendly alternatives to chemical or synthetic surfactants. They can be made from bioresources at a cheaper cost by using low-cost materials and biotechnological techniques. However, due to its low insolubility and bioavailability, significant adsorption to soil particles, and pollutant hydrophobicity, its biotechnological and environmental applications processes may be hampered. This problem can be solved by optimising growth/production conditions, employing economically viable renewable substrates, and implementing efficient multi-step downstream processing. This would aid in the development of a more profitable biosurfactant. Furthermore, given the social and economic benefits of these minerals, the best circumstances for their preparation must be researched further.

Conflict of Interest: The authors declare that there are no conflicts of interest regarding publication of this paper.

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