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Preprint · November 2018

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Bioactivity of natural compounds Produced by Marine *Alcaligenes faecalis* as Antimicrobial, antibiofilm formation and anti-biocorrosion effect against *Desulfovibrio* sp. Isolated from crude oil fluid

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ABSTRACT

Microorganisms extract have served are source of valuable diverse molecules in drug industries. This study successfully identified potentially new sources of antibacterial compounds against sulfate reducing bacterium, *Desulfovibrio* sp. , SRB)) which was isolated from crude oil field , PETRONAS Refinery , Melaka) Sdn. Bhd.) from Malaysia, and thus provide in protecting carbon steel coupons surface against biocorrosion caused by SRB present in crude oil .The marine , *Alcaligenes faecalis*) grown in marine broth extract , AF) were dissolved in chloroform , C).Chloroform extraction of *A. faecalis* , AFC) grown in marine broth was carried out to produce crude of *A. faecalis*. AFC was diluted at concentrations 0.2–12.8 mg/mL and was tested for antimicrobial activity by microdilution susceptibility tests in 96-wells plate. Using crystal violet assay method for confirm anti-biofilm activity. Inhibit the biocorrosion analyses were carried out by using weight loss. AFC was then analyzed by Gas Chromatography Mass Spectrometry, GC-MS). The results exhibited that, AFC achieved high significant effect for inhibiting growth in MIC 3.2 mg / ml against the concerned SRB. There were no biofilm formation or corrosion observed on carbon steel coupons after treatment with AFC were effective in controlling planktonic and sessile SRB growth. The GC-MS analysis showed the presence of 20 different chemical compounds in AFC, The major components in AFC can be related to antimicrobial, antifungal, antioxidant, pesticide, metabolism, toxicity, anticancer and corrosion inhibition activities. In conclusion, crude chloroform extract of *A. faecalis* has the ability to inhibit SRB growth and hindrance of biofilm formation and H_2S production which led to biocorrosion.

Key words: Antimicrobial activity; Antbiofilm, Anti-biocorrosion; *Alcaligenes faecalis* extracts, GC-MS, SRB.

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1. INTRODUCTION

Corrosion is the process of an electrochemical reaction occurring between environment and any substance such as metal that can be tank, pipeline miscellaneous[1]. Corrosion is an old industrial problem and causes a lot of dilemma in the industries because its leads to metallic structural failure. Sulfate-reducing bacteria , SRB) are the main bacterial group that cause metal biocorrosion and reservoir souring in petroleum factories. SRB utilized sulfate as a final electron acceptor and producing sulfide ions which are reaction with hydrogen and resulting height amount of H_2S in crude oil who is considered the major factor that in the corrosion process. This reaction result, the iron sulfide as a corrosive product [2]. Lowering oil recovery rates were due to deposition of SRB biomass, the iron sulfide and accumulation biofilm and accumulation biofilm [2, 3]. In order to mitigate threat corrosion, the major useful strategy is the addition appropriate biocides to inhibit microbial growth [4]. Antimicrobial resistance can often weaken the activity of biocides which can easily happen, biofilms with biocid [5] .It is now significant for the petroleum manufacturers to replace the utilization of biocides with other options for control SRB [6]. Environmental friendly methods of anticorrosion are sought by the petroleum manufacturers as substitutes to the use of manmade biocides which can cause harm in life forms by depositing in the environment. These days, Many innate products which contain medicinal value are derived from various substances such as marine organisms, terrestrial vertebrates and invertebrates, terrestrial plants and terrestrial microorganisms, many microbial extracts are contain a variety of important molecules in efforts to find out new compounds for inhibiting corrosion processes of carbon steel and other metals in acidic environments has been reported by several authors [7]. The current study highlights on the second metabolite extracted from marine bacterium which isolated from the marine , Malaysia) was identified as *Alcaligenes faecalis* which has the ability to produce active natural compounds can inhibit formation of biofilm [8]. Antagonistic activity is thought to be the Characteristic of genus Alcaligenes among the other species of bacteria which possessing the antagonistic ability [9]. *Alcaligenes denitrificans* has the ability to act as an algicidal against *Microcrocystis* sp. [10] and that is why *Alcaligenes xylosoxydans* act as a good antifungal biocontrol [11]. But no investigation has yet pointed towards the ability of *A. faecalis* which isolated from the marine environments against growth to *Desulfovibrio* sp. associated with MIC in Malaysian oil industries. The second metabolite effect of *A. faecalis* as an antibacterial ability is still not yet been clarified in Malaysian ecological zones, especially against sulfate-reducing bacteria.This will be the first time is proposing the use of *A. faecalis* to biocontrol and impeding SRB biofilm development and corrosion of metals such as tanks and pipes which induced by sulphides in the Malaysian petroleum industry.

2 MATERIALS AND METHODS

2.1 Microorganisms and Testing Medium

Marine bacterial isolates Aquatic bacterial samples utilized in this study were acquired from a biological laboratory, Faculty of Science and Technology, UKM culture collections. Stock cultures of *Alcaligenes faecalis* , STN17) and was grown on marine broth , MA, Difco, NJ, USA) at 30°C. [8]. This isolate was isolated from the marine environment, and recognized using PCR. Sulfate reducing bacteria used in this study was *Desulfovibrio* sp.KU892724 which isolated from a crude oil and was usually grown in VMNI medium [12].

2.2 Preparation Marine Bacterial for production second metabolites and Crude Extract Preparation

An inoculum of STN17 at OD $_{595}$ nm = 0.3 was cultivated under rotary agitation at 150 rpm at 30 ^{o} C for 96 h in 2 ml Erlenmeyer flask containing 1 L of marine broth , MB, Difco, NJ, USA). After incubation, the bacterial culture was centrifuged at 6000 rpm for 10 min at 4 $^{\circ}$C to obtain a clear supernatant for feather metabolite extraction. A liquid-liquid extrications were performed using chloroeorm , C). The supernatant was recuperated, cleansed and sterilized by filtration process and added to 500 ml C , GmbH, Germany, 99% of purity). Following the agitation for 1hr at room temperature and allowed to be left for a night in a separate container [13], this method of extrication was reiterated again until the supernatant assumed a colorless form. Vacuum evaporation at 37 degrees Celsius of the extricated samples was carried out to acquire the dry extracts; more dehydration took place under fume hood. The acquired solution was named as "crude extract" [14].

2.3 Determination of the minimum inhibitory concentration , MIC) and minimum bactericidal concentration , MBC):

The microdilution susceptible tests and growth under crude extraction exposure were carried out [15]. Working solutions of crude extracts , 1.0 mg /ml) were diluted in 96-well microtiter plates serially to make a group of dual concentrations slowly of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg /ml in sterile medium of VMNI separately to determine the lowest inhibitory and minimum bactericidal concentrations that effective. SRB which is the target isolate was grown for 7 days at 30°C in VMNI medium. The culture was then diluted to produce the final SRB inoculum size of 10^5 cells /ml. Microtiter plates which were put under incubation at 30 °C for 7 days. The SRB growth development was noticed by the blackish color of the medium which is because of iron sulfide precipitation in VMNI medium. The minimum inhibitory concentration , MIC) effect was ascertained when the minimum value of crude extraction was added which could not give the same black color of the medium. Supra-MIC , 2× MIC) and Sub-MIC , 0.5× MIC) of AFC was ascertained and additionally utilized for evaluation , antibiofilm assays and anti-corrosion). The minimum bactericidal concentration test was performed by utilizing 10 µl aliquots of the cell suspensions to inoculate fresh medium of VMNI , 90µl) and then put under incubation 30°C for 7 days. At the minimum of antimicrobial material concentration in which no growth of SRB indicator sample, minimum bacterial concentration , MBC) was ascertained. The methods of inoculation were all carried out in an anaerobic chamber , PlasLabs Inc., USA) within AnaeroGen , Oxoid, UK).Kill time procedure was also carried out using AFC macrodilutions in VNMI broth , 2 ml) using BD Vacutainer™ tubes,, SRB cells at 10^5 /ml were later inoculated. MIC levels of AFC was examined by incubating the tubes at 30 °C for 1, 2, 3, 6, 12 and 24 hours. Untreated specimen are used for the purposed of control for the whole incubation period. Recovery of biomass from the vessel tubes was performed after incubating by centrifugation and the cell pellets were washed by using sterile water , N2 purged sterile distilled) two times in order to remove the broth and AFC remains that may exist. Then, each cell pellet was isolated in fresh VMNI medium and put under incubation for 7 days at 30°C. All tests were performed in triplicate.

2.4 Antibiofilm action of AFC on SRB biofilm formation and stability on carbon steel coupon surfaces

Biofilm formation was tested according to [16]with same modification. The device used to biofilm formation was a 24 well-plate with a carbon steel coupon , Chemical composition , wt %) of this steel was C 0.12, Mn 0.5, S 0.045, P 0.04 and Fe is balance) each. Assay was utilized to assess the effectiveness of the AFC against SRB biofilm formation. In this assay,

concentrating of AFC as described above in determination MIC, these concentrations was added at the exact same time with SRB cells and VMNI medium were placed in the biofilm device.

A control without AFC was performed in this assay. 0.5ml of the cell suspended fluid was added to each well, covering the carbon steel surfaces by added sterile VMNI medium , and put under incubation at 30°C for 7 days [17]. At the conclusion of the biofilm formation incubation timeframe on the carbon steel coupons surface, the carbon steel coupons were separated aseptically from the fluid culture for biofilm computation utilizing the crystal violet merging assay explained by [16]. The assessment was performed in triplicate. The method was reiterated three times for each concentration and the acquired results were laid out as the mean value and standard deviation as required.

2.5 Antibiocorrosion action of AFC

In this research, a quantitative analysis method is used to ascertain the corrosion rate on the basis of the weight loss method. It requires revealing the specimen coupons by total submersion into the simulated solution of distinct concentration and computing the loss of weight of the material as a function of time. The computations of the samples are picked while the exposure and after extraction according to ASTM G162-99 , American Standard of Material and Testing, 2000). Observational materials utilized are carbon steel coupons , 15 mm×10 mm×1.5 mm) by submerging coupons in a 24-well-plate having VMNI medium inoculated with 0.5 ml , 10%) of 10^5 cells / ml of SRB with crude extracts at MIC, sub-MIC and supra-MIC solutions and incubated for 7 days at 30°C. Total surface region and starting weight were determined. The positive control , without crude extracts) and negative controls , without cells) were carried out using carbon steel coupons. Treatment and control of coupons was carried out in triplicate. Weight was measured in grams, and the corrosion rate , CR) of carbon steel coupons was calculated and expressed in mm/yr .The corrosion rate was calculated using the formula below:

$$\textbf{Corrosion rate , mm/y)} = \frac{(\mathbf{K}\times\mathbf{W})}{(\mathbf{T}\times\mathbf{A}\times\mathbf{D})}$$

Where, K is a factor accounting , 8.76×10^4), W is the difference between initial weight‖ and secondary sample weight after cleaning in gram, T is time of exposure of coupon in hours, and A is total area of the coupon in cm². While the surface area of each coupon was calculated using: Surface area , A) = 2 [, L×B) + , B×T) + , L×T)] Where, L = , 15 mm) Length of the coupon. B = , 10 mm) Width of the coupon. T = , 1.5 mm) Thickness of the coupon.

2.6 Screening of Bacterial metabolite extracted with GC-MS:

The ethyl acetate extract from *A. faecalis* were analyzed by Gas Chromatography and Mass Spectrophotometer, utilizing this technique the main compound having the antimicrobial activity can be detected. Ten , 10) µl of the crude extraction from *A. faecalis* by ethyl acetate was directly injected into the port of injection of gas chromatograph , Agilent Technologies 7890A GC system) straightly combined with a mass spectrometer system , MS) , Agilent Technologies 5975C inert MSD with Triple-Axis Detector).The GC was worked on an Agilent DB-5MS UI GC column , 30 m x 0.25 mm, id. with 0.25µm film thickness of 5%-phenyl-methylpolysiloxane) and helium was utilized as the transportation gas. The temperature command was started with a starting temperature of 50 degrees Celsius and held for 2 minutes at this exact temperature, then 6 °C/min to 280 °C for 10 min a flow rate of 1 mL/min and Run Time 50.333 minutes. The MSD Chemstation was utilized to ascertain all the crests in raw GC chromatogram. Library investigation was performed for all the crests using the using the National Institute of Standards and Technology NIST/EPA/NIH version 2.0, all outcomes was adjoined in a single crest table.

2.7 Statistical analysis

The Excel data analysis package was used to calculate mean, standard deviation of the mean. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.20. Significant differences , P<0.05). All the results were calculated from the mean of three replicate samples for each data point.

3. Result and discution

3.1 Determination of the minimum inhibitory concentration , MIC) and minimum bactericidal concentration , MBC):

The MIC of AFC was determined at 3.2 , mg/ ml) according to the result which obtained by using spectrophotometric assay , Fig. 1), Compared with the control negative culture , sterile VMNI without SRB) and control positive culture , sterile VMNI with SRB).Thus, AFC was evinced to be responsible for the antimicrobial effect. In order to proceed with the following tests with a AFC threshold levels, MIC, sub-MIC and supra-MIC levels were considered as 3.2, 1.6 and 6.4 , mg / ml), respectively. The minimum bactericidal concentration , MBC) of AFC was determined as the same value as the MIC, as no cell growth was recovered from any of the three replicate wells. The optical density , OD) reading on 630 nm wavelength of untreated SRB is around 1.750 , ± 0. .0520) , Fig. 1). According to the OD reading results any wells that give OD reading less this range can be considered to have effect with the AFC .Therefore, AFC showed a bactericidal effect against the SRB. The time kill test at MIC level showed an immediate bactericidal effect, that is, no viable cells were recovered after SRB growth in VMNI medium.

The antibacterial assay showed the antimicrobial effect of the MIC is 3.2 mg /ml. This result indicates that *A. faecalis* acts by substance , s) secreted in the medium and which are soluble in chloroform , AFC).considering the large number of different groups of chemical compounds present in AFC, it is likely that their antibacterial activity is not attributable to one specific mechanism but to several targets in the cell. An important characteristic of AF is their hydrophobicity, which allows the accumulation and partition of the lipids in bacterial cell membranes modifying their structure, distorting the lipid/protein interactions and disturbing their function [18]

Antibacterial activity which was evaluated by microdilution methods resulted in clear data [19], broth microdilution was faster, easier and more reproducible than diffusion method. The result of microdilution assay showed that antibacterial activity of AFC depended on the extracting concentration. Most of the extracts showed that the higher the extract's concentration, the lower the optical density , OD) reading. It then meant that the extract inhibited SRB growth. However, the extracts caused a turbid phase when mixed with the VMNI media without addition of the optical density , OD) reading , >0.2) and the optical density , OD) reading in MIC , ≤ 1.5).

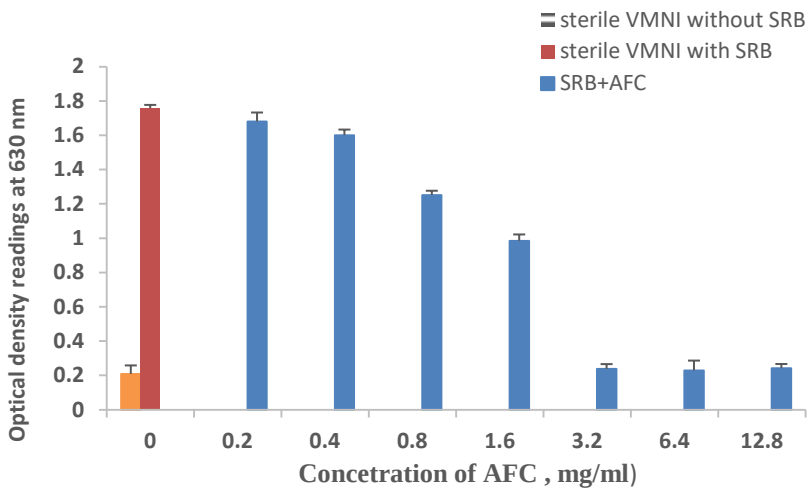

Fig 1: Determination of the MIC of AF using optical densities readings , OD) of SRB cell cultures treated with different concentrations.

Mulyono et al , 2013) stated that the spectrophotometer will not give accurate results if the optical density , OD) reading is either too high , >1.5) or too low , <0.02).

Fig.. 1 indicated that chloroform extract of *A. faecalis* , AFC) could inhibit SRB growth in concentration of 3.2 mg/ml and the optical density , OD) reading was 0.3313 , ± 0.057). The AFC seemed to be more effective to inhibit SRB growth. Fig..1 Therefore, ethyl acetate extract was suggested to be the good solvent for extract to obtain antimicrobial agent from *A. faecalis*.

3.2 Antibiofilm action of AF on *Desulfovibrio* SP biofilm formation on the surfaces of carbon steel metal.

Desulfovibrio SP. , SRB) biofilm removal by applied AFC was observed when testing biofilm formation on the surfaces of carbon steel metal coupons , Fig. 2). The compounds present in the AFC were a highly effective biofilm inhibitor. AFC exterminated completely at MIC and supra- MIC. Sub-MIC levels showed the OD reading on 630 nm wavelength of SRB becomes less than the OD reading for untreated assay by the AFC, Thus, AFC were evinced to be responsible for the antibiofilm effect. Control positive , VMNI medium with SRB only) the SRB cells formed biofilms on carbon steel coupons surfaces in all assays showed the OD reading in 2.14 , ±0.0744) , Fig. 2).

In this experiment, the inhibit attachment of SRB cell on the surface of carbon steel and the capability to separation pre-established biofilms were also observed when sub- MIC, MIC and supra-MIC levels of AFC was applied , Fig. 2).Even though the effect of bioactive compounds extracted from bacteria against biofilms formation was proved to be medically significant bacteria [17], in This study will be first demonstrating the antimicrobial activity of other second metabolites of marine , *A. faecalis*) against the anaerobic bacteria which isolated from the petroleum manufacturers.

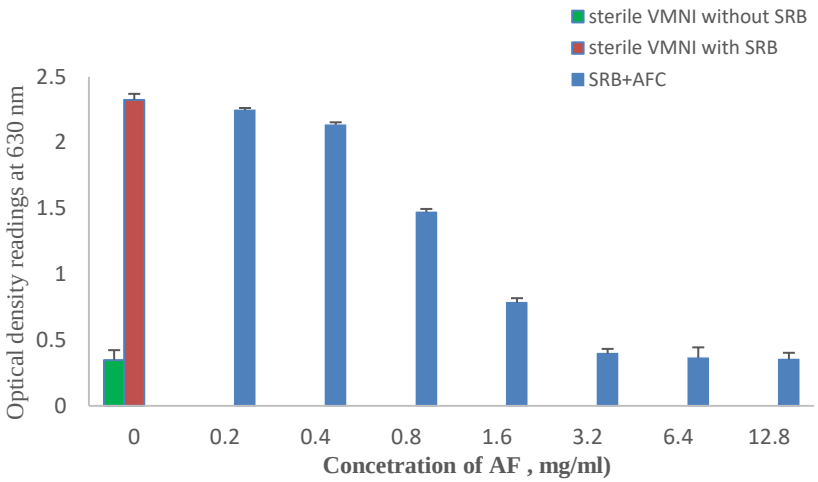

Fig 2: Determination of the inhibited biofilm formation on carbon steel surfaces of AF using optical densities readings , OD) of SRB cell cultures treated with different concentrations

7.4 Conditioning carbon steel coupons with AFC:

The carbon steel coupons which used as a positive control was showed black corrosion precipitates, while in contrast the coupons which were treated with AFC were kept safe from production of biofilm and also from SRB-induced biocorrosion mechanism. The conditioned coupons suffered a bit from corrosion which looked the same as that noticed on coupons which were left blank and was termed as chemical corrosion. The highest amount of loss of mass was noticed on untreated coupons instead of conditioned coupons, after subtracting the rate of corrosion of blank coupons. Conditioned coupons were not affected by bio corrosion, and on the other hand it was observed on coupons which were not treated , 0.099390 , ± 0.01583) mm/year, $p<0.001$). It was made evident that biocorrosion which was calculated by used weight loss method could be impeded by AFC , Fig. 3) if sulfate reducing bacteria growth was inhibit that mean gotten rid of which produced biofilm on carbon steel coupons. Biofilms are accumulations of microorganisms cells which adhere to the carbon steel coupon surface and are made up of a structural component built by the microbes known as a polymeric matrix The physiological state is affected as a result of this structure when compared to their planktonic compliment, with the sessile cells having a preferable fitness in their normal surroundings [23].

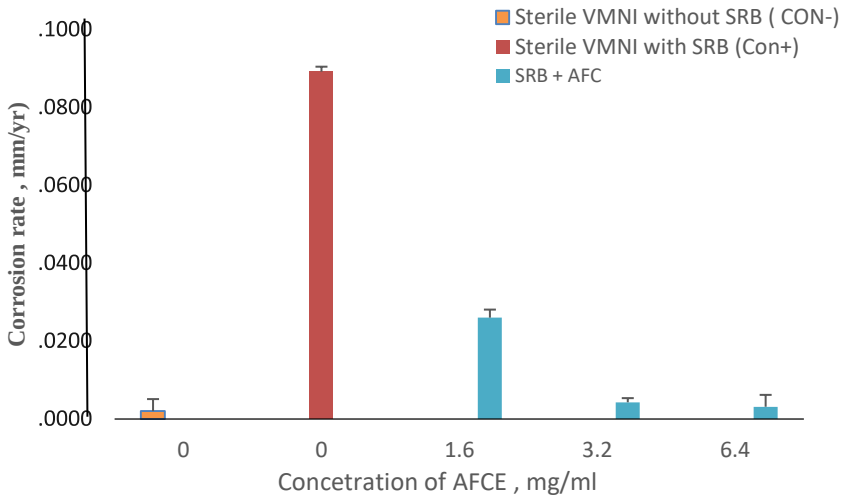

Fig .3: Corrosion rate on control coupon and inhibition of corrosion on coupons conditioned with AFC at MIC, sub-MIC and supra-MIC.

The SRB biofilms are related to biocorrosion of metal exterior of petroleum development line, tanks and vessels in petroleum environments [24]. Therefore, the utilize of AFC, at MIC and supra- MIC levels in controlling sulfate reducing bacteria biofilms is a golden standard which needs to be applied in industrial systems as described by , Videla. 2002), which maintains the organization clean and prevents biocorrosion. The evidence gained by this research exhibited that the ATAE had antimicrobial activities as opposed to SRB and antibiocorrosion impact on carbon steel metal exterior.

7.5 Gas Chromatography Mass Spectrometry Analysis , GC-MS) of crude extraction from *A. faecalis*

The GC-MS profile analysis was performed to investigation chemical compounds presence in crude extract from the second metabolite of *A.faecalis*, extracts by used chloroform, which demonstrated that around 52 different chemical compounds , Fig. 4). The major chemical compounds were identified with similarity index ≥90% showed the presence of 20 different chemical compounds extracts from ***A. faecalis*** by used chloroform. It was also known this particular technique could not be investigated all chemical compounds which presence in crude extraction. The retention time, name of identified compound, molecular weight, percentage peak area, and their biological activities of the major constituent's compounds are given in , Tables). Also, the existence of the various small peaks in the spectrum points towards the fact, the extract may contain many other unidentified chemical compounds. More importantly, the GC/MS data also has the ability to give investigative proof that biomass extracts which had different organic species might possess growth, biofilm and corrosion inhibiting potential.

The presence of many groups of chemical compounds in AFC was demonstrated by the GC-MAS analysis , Table 1) as revealed by the present study directed to focus towards the characterization of potential compound from this variety that have antimicrobial activity characters. The major components in AFC were antimicrobial, antifungal, antioxidant, pesticide, metabolism, toxicity, anticancer and inhibit corrosion activities , Table 1). The characteristic in AFC which was in majority due to peak area was antimicrobial their composition in AFC was 30.905 % , Table 4) and most were fatty acids. This recognition was the same as Sujarwo et al. , 2010) who utilized GC/MS to identify and state that *apus* bamboo consisted of fatty acids and

few aromatic substances. [39]. Desbois and Smith , 2010) stated that free fatty acids possessed antibacterial activity [40]. Electron transport chain and oxidative phosphorylation was impeded by Free fatty acids, and had affected cellular energy development, enzymatic ability and nutrient uptake, toxic peroxidation production, direct lysis of bacterial cells and stopping bacterial adhesion which led to biofilm production. Different organisms can utilize those natural substances to protect itself against pathogenic or parasitic bacteria.

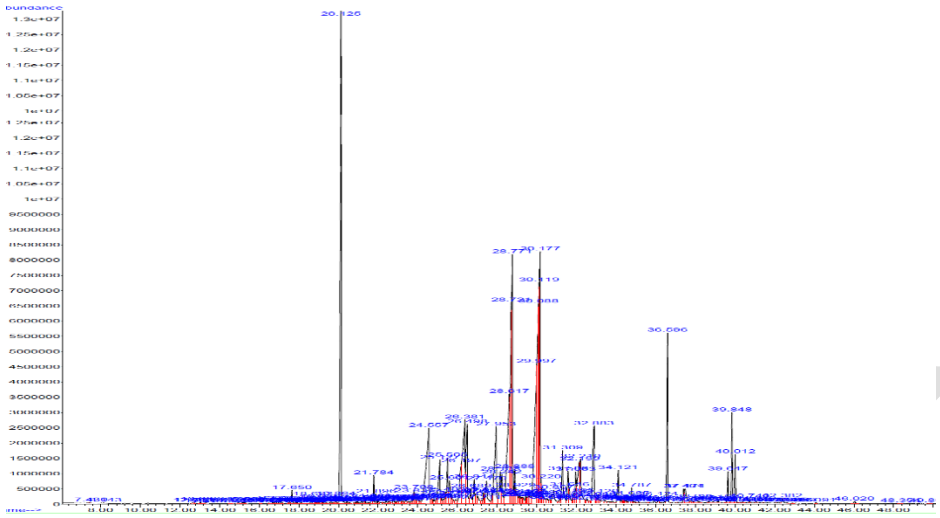

Fig 4: *GC-MS chromatogram of the* Chloroform bacterial crude extracts from *Alcaligenes faecalis*

Table 1: GC-MS analysis showed biochemical compounds identified from Chloroform extraction bacterial extracts from Alcaligenes faecalis.

R. T.	Compound Name	M. W.	Formulae	Area %	Co. Na.	Activity	Ref
28.72	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O2$	5.567	Fattt acid	Cytotoxic	[25]
39.85	Decanedioic acid, bis, 2-ethylhexyl) ester	426.67	$C_{26}H_{50}O4$	1.663	organic compound	Antimicrobial and antioxidant	[26]
17.65	5-Octadecene, , E)-	252	$C_{16}H_{32}$	0.28	long-chain fatty acid	Stronger sexual characters	[27]
19.08	Hexacosane	226.45	$C_{26}H_{54}$	0.11	Alkane	Antimicrobial	[28]
20.12	Phenol, 2,4-bis, 1,1-dimethylethyl)-	206.323	$C_{14}H_{22}O$	8.65	organic compound	Antimicrobial	[29]
24.55	Tetradecanoic acid	228.38	$C_{14}H_{28}O_2$	4.88	Fatty acid	Cancer reventive	[30]
25.50	7-Hexadecene, , Z)-	224	$C_{16}H_{32}$	1.0105	long-chain fatty acid	Antibacterial	[31]
25.50	9-Eicosene, , E)-	280	$C_{20}H_{40}$	1.01	Chain fatty acid	Antimicrobial	[32]
27.74	hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	0.21	Fatty acid methylester	Antioxidant	[33]
30.27	Palmitoleic acid	254.41	$C_{16}H_{30}O_2$	6.58	Fatty acid	Antibiotic and Pesticide	[34]
28.72	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	5.57	Fatty acid	Antimicrobial	[33]

28.88	1-Nonadecene	266	$C_{19}H_{38}$	0.68	long-chain fatty acid	Anti-fungal activity	[27]
29.60	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	0.29	Fatty acid	Antimicrobial	[33]
31.47	cis-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	0.14	Faty acid	Antibacterial	[33]
31.58	Octadecanoic acid	284	$C_{18}H_{36}O_2$	0.88	Fatty acid	Antimicrobial	[35]
32.88	9-Hexadecenoic acid	254	$C_{16}H_{30}O_2$	2.3704	Organic compound	Pesticide and antibiotic	[34]
25.17	1-Dodecanol, 2-octyl-	298.54	$C_{20}H_{42}O$	0.13	long-chain fattylcohol	Anti-bacterial	[33]
36.59	Bis, 2-ethylhexyl) phthalate	390.56	$C_{24}H_{38}O_4$	2.91	organic compound	Antitoxicity and intioxide.	[36]
39.64	9-Octadecenamide	281.48	$C_{18}H_{35}NO$	0.60	fatty acid	Treatment of sleep disorders and pain	[37]
40.01	Squalene	410	$C_{30}H_5$	0.81	organic compound	Antibacterial and antioxidant	[38]

Note :, R. T.): Retention Time, , M. W.): Molecular Weight, , Co.Na.)Compound Nature , Ref.): Reference

Also, ester compounds possessed antibacterial ability due to the fact that fatty acids were produced by metabolizing and with the help of lipase enzyme. The majorly present fatty acid in AFC were n-Hexadecanoic acid, Palmitoleic acid , 9-Hexadecenoic), Pentadecanoic acid, cis-13-Octadecenoic acid, Octadecanoic acid and Heptadecanoic acid , Tadel 1). Fatty acids are numerous in natural fats and dietary oils, they playing the important roles of metabolites and nutritious compounds in living organisms [41]. Russell , 1991) reported the role of fatty acids as antifungal and antibacterial properties [42]. Manila *et al* , 2009) reported that extraction from red algae *Laurencia brandenii* in southwest coast of India, have cytotoxic activity. The extract had a main constituent which known as Fatty Acid, and revealed that the main acids were n-n-Hexadecanoic acid , 14.24 %) 9, 12-Octadecadienoic acid , Z,Z)- , 49.75%). The crude extraction from root of *Guiera senegalensis* J.F. Gmel was investigated using , GC-MS) analysis showed, n-Hexadecanoic acid , 46.6%) was the main constituent after which came 9-Hexadecenoic acid , 20.93%), which had strong activities as a antimicrobial, antioxidant and antitumour [34]. Pentadecanoic acid , 2.65%) and more such Fatty Acid Methyl Esters derivations of *Excoecaria agallocha* were observed with the help of GC and exhibited antifungal and antibacterial abilities in opposition to 11 microorganisms , 4 yeast and 7 bacteria) [43].Oleic acid and cis Octadecanoic acid inhibited the primary adhesion of *Staphylococcus aureus*, the bacterial population which had adherent ability reduced in relation to higher concentration levels of oleic acid while a contrasting phenomenon was observed on the planktonic population [44]. Mujeeb et al , 2014) demonstrated that the medicinal worth of *Aegle marmelos* Correa , Rutaceae), usually termed as "Bael," has been recognized as conventional medication of many human illnesses. According to Orishadipe, Ibekwe et al. , 2012) reported the methanol Seed oil extraction from *Entandrophragma angolense* was analysed by GC-MS produced their main constituents as fatty acid, and also had cis-vaccenic acid, then antimicrobial investigation showed seed oil had positive activity in opposition to *Salmonella gallinarum* and *Klebseilla pneumonia* [45].

The GC-MS Analysis were found many bioactive substances along with fatty alcohol such as 1-Dodecanol, 2-octyl- in AFC. Fatty acid was produced by oxidation of the alcohol which possessed antibacterial activity. Essential oil of air-dried *Minuartia meyeri* , Boiss.) which was an organic substance and the mentioned alcohol was mixed together, which has antibacterial ability again Gram-positive and Gram-negative bacteria [46, 47]. A long-chain fatty alcohol was reported with a very high antibacterial activity against *Staphylococcus aureus*, there were

numerous studies associated with the antibacterial ability of long-chain fatty alcohols [48]. According to Kabelit, Santos et al. , 2003) reported increased of effective with the carbon chain length [49]. C10-OH and C11-OH possessed antibacterial ability which were revealed by a destruction of cell membranes due to which K+ ions effluxes, and lead to exacerbation of leakage [50].

AFC contained the following organic compounds: Phenol, 2,4-bis, 1,1-dimethylethyl), Squalene, Bis, 2-ethylhexyl) phthalate, Decanedioic acid, bis, 2-ethylhexyl) ester and 9-Hexadecenoic acid. According to Govindappa, Prathap et al. , 2014) Organic substances in AFC were report that, the bioactive compounds in endophytic fungus, *Alternaria sp* methanol extract were identified as phenol, 2,4-bis, 1,1-dimethylethyl) and other compounds. These compounds exhibited strong antibacterial activity in combination with other compounds [51]. The leaf extract derived from *Nicotiana tabacum* , NT) had a corrosion impeding effect on mild steel which showed that NT stops the mechanism of corrosion and potential of stunting becomes higher with higher concentration levels of NT.GC/MS. Analysis showed precedent Octacosyl trifluoroacetate of a percentage similar to the obtained in this study [52]. *Pseudomonas putida* segregated from root endosphere bacterium can inhibit the growth of a vast number of pathogens. Chemical profiling done with the help of GC/MS showed that some main compounds existed such as Octadecyl trifluoroacetate [53].

4. CONCLUSION

It was demonstrated by the evidence gained in this study that the second metabolites taken from marine *A. faecalis* consist of compounds that can cause bioactivity. These compounds have been identified and recognized as an antimicrobial activity that acts against the growth of SRB and can stop biocorrosion effects on the surface of carbon steel metal. The inhibiting mechanism of biocorrosion associated with activity of SRB by applied 3.2 mg/ml of AFC gave us promising results. AFC derivations lowered the part played by SRB in the corrosion mechanism by inhibiting growth of SRB cells, which also cut down on production of sulphide, mass of sessile cells and formation of biofilm in petroleum factories.

5. ACKNOWLEDGMENT

We would like to appreciate Universiti of Kebangsaan Malaysia , UKM)) for their support through research grants 04-01-02-SF014 from Ministry of Science, Technology and Innovation , MOSTI) of Malaysia and UKM , GUP BTK 07-75-198). We also acknowledge Makmal Pencirian Struktur Molekul , MPSM), Centre for Research and Instrumentation Management , CRIM), Universiti Kebangsaan Malaysia , UKM). The authors also acknowledge the Ministry of Higher Education and Scientific Research of Iraq to providing first author Ph.D scholarship.

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