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# Impact of *Chromolaena odorata* Ethanol Extract on Biofilm-Associated Corrosion of Mild steel in Enriched Artificial Seawater Medium

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## Abstract
In the present work, the effect of *Chromolaena odorata* ethanol extract (COEE) on biofilm-associated corrosion of mild steel was investigated. Mild steel coupons were immersed in enriched artificial seawater (EASW) medium inoculated with *Pseudomonas aeruginosa* for 7 days and 14 days, in the presence and absence of COEE. Three-dimensional surface analysis was carried out using Infinite Focus Microscopy (IFM) whilst phytochemical screening was conducted using gas chromatography-mass spectrometry (GC-MS). Results demonstrated that COEE inhibited pitting corrosion of mild steel in both 7-day and 14-day experiments. It was also found to contain major phytochemicals such as germacrene-D, caryophyllene and cadinene. The present work suggests the potential application of COEE as a biocorrosion inhibitor.

**Keywords**
Biocorrosion; *Chromolaena odorata*; Mild steel; *Pseudomonas aeruginosa*; Seawater medium

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## INTRODUCTION
Biocorrosion is deterioration of materials caused by the metabolic activities of microorganisms such as algae, fungi and bacteria [1]. It is characterized by localized corrosion such as pitting, crevice corrosion and erosion corrosion. The biocorrosion of steel has given rise to a wide range of problems associated with chemical processing, metal working, aviation, petrochemical and generation of nuclear power. There are several ways used by the microorganisms to initiate the corrosion process including production of corrosive metabolites, destruction of protective layers, stimulation of electrochemical reactions and colonization of microbial cells on the metal surface through the formation of biofilm [2]. In brief, biofilm is a microbial community that attaches to a hydrated

surface. It is protected from various extreme environmental conditions by self-produced extracellular polymeric substances (EPS) matrix which consists of lipids, proteins, polysaccharides, nucleic acids and glycolipids. One of the common biofilm-forming bacteria that causes biocorrosion of industrial pipeline system is *Pseudomonas aeruginosa*. It is abundant in aquatic environment and is capable of utilizing petroleum hydrocarbons [3]. Heterogeneity of *P. aeruginosa* biofilm on mild steel surface has previously been demonstrated [4].

There have been many studies reporting the use of plant-based materials to control the corrosion process. The plant-based materials have become the corrosion inhibitors of choice in the oil and gas industry because of its low production cost and environmentally friendly properties. Due to this current scenario, anticorrosion potential of Malaysian plant species deserves further attention. *Chromolaena odorata* is a widely distributed plant species in Malaysia which belongs to the plant family Asteraceae. It has been considered as a harmful weed due to its highly invasive and allelopathic nature. This plant species is known to possess a wide range of biological activities including antibacterial [5] and wound healing activities [6], however, its impact on biofilm-associated corrosion remains not well investigated. Thus, this work was conducted to evaluate the effect of *C. odorata* ethanol extract (COEE) on biofilm-associated corrosion of mild steel. Infinite Focus Microscopy (IFM) was used to analyze three-dimensional surface of mild steel while gas chromatography-mass spectrometry (GC-MS) was carried out to determine the phytochemicals in COEE.

## MATERIALS AND METHODS

### Plant extract and microorganism

Leaves of *Chromolaena odorata* were collected from a suburban area in Selangor state, Malaysia. The leaves were washed with tap water, ground and extracted using 100% ethanol solvent for three days at room temperature. The solvent was then filtered and removed using rotary evaporator. *Pseudomonas aeruginosa* strain ATCC 10145 was maintained on nutrient agar media. A batch culture of *P. aeruginosa* strain ATCC 10145 was grown at 37 °C in nutrient broth and its purity was assessed regularly by Gram-staining and colony morphology.

### Enriched artificial seawater medium

Each litre of the nutrient rich medium consists of 23.476 g NaCl; 3.917 g $Na_2SO_4$; 0.192 g $NaHCO_3$; 0.664 g KCl; 0.096 g KBr; 10.61 g $MgCl_2.H_2O$; 1.469 g $CaCl_2.2H_2O$; 0.026g $H_3BO_3$; 3 g of bacterial peptone and 1.5 g of yeast extract. The pH was adjusted to 7.2 ± 0.1 using 5 M NaOH solution and sterilized by autoclaving for 20 minutes at 120 °C at 15 psi.

### Mild steel coupons

Mild steel with composition of 0.04% C, 0.022% P, 0.35% Mn, 0.036% S, 99.552% Fe was cut into 15 mm x 15 mm x 2.05 mm coupons and were sequentially ground with a series of grit SiC papers (320, 600, 800, 1000 and 1200) to a smooth surface. The coupons were then polished to a mirror finish using suspended alumina powder, rinsed thrice with deionized (DI) water, dried by storing in airtight desiccator, fully immersed in 70% ethanol and dried by flaming the coupons over Bunsen burner. The newly prepared mild steel coupons were immediately exposed to the test medium for the biocorrosion assay.

### Biocorrosion inhibition assay

Biocorrosion inhibition assay was conducted at room temperature under stagnant condition for 7 days and 14 days. A number of 24 mild steel coupons were segregated into eight groups [four groups (A-D) for 7-day and 14-day assays respectively, n=3]: A[+EASW–plant extract–biofilm cells]; B[+EASW–plant extract+biofilm cells]; C [+EASW+plant extract+biofilm cells] and D [+EASW+plant extract–biofilm cells]. A volume of 5 ml of the *P. aeruginosa* culture was introduced into 100 ml of the EASW medium in sterile conical flask. When the optical density of bacterial suspension in EASW medium from group B and C reached ~ 0.4, all the mild steel coupons were immersed in the respective EASW medium simultaneously with the surface of interest facing upward. For group C and D, an amount of 0.1 g of *C. odorata* ethanol extract was dissolved in 1 ml of DMSO solution making 100 mg/ml concentration of plant extract and was added into the EASW medium.

### Infinite focus microscopy

After 7 and 14 days of experiments, the biofilm layer was removed from the mild steel surface by wiping the surface with alcohol and then subjected to ultrasonication for 90 seconds with a frequency of 30 kHz. The mild steel coupons were then rinsed with deionized water, purged with nitrogen and stored in airtight desiccator. Analysis of corrosion images were carried out using Alicona Infinite Focus Microscope G4 according to [4].

### Gas chromatography-mass spectrometry

Chemical constituents of COEE were analysed using a Clarus 600 GC-MS system. The constituents were separated on 30 m x 0.25 mm x 0.25 µm Elite-5MS column and the column temperature was set from 40-220° C at a rate of 4°C $min^{-1}$; injector temperature,

250° C; injection volume, 1 µL; transfer temperature, 280° C. The peaks in chromatogram were identified based on library search using NIST and Wiley Registry 8 Edition.

## RESULTS AND DISCUSSION

### Two- and three-dimensional surface profiles

Figure 1 and 2 show the surface profile of mild steel coupons from 7-day and 14-day experiments. Uniform corrosion occurred on mild steel coupons immersed in EASW without biofilm cells whilst biocorrosion occurred on mild steel coupons immersed in EASW with biofilm cells. The biocorrosion of mild steel coupons was characterized by pitting formation. Furthermore, treatment with COEE was found to inhibit the biocorrosion. *P. aeruginosa* is a common biofilm-forming bacterial species that causes corrosion of metal and alloy surfaces. Numerous investigations have been conducted to understand how this anaerobic microorganism accelerates the corrosion rate. For example, Li et al. [7] reported that the biofilm accelerates the corrosion rate by accepting electrons from the metal surface leading to creation of a pathway of electron transfer from the anodic site to cathodic site on the metal surface. The role of biofilm in deterioration of various materials has also been reported by Sand [8]. Inorganic acids excreted by biofilms often reduces pH of water and interrupts passivation of steels by strong alkali. Passivation refers to a process of reducing chemical reactivity of metal surface in order to prevent corrosion. In general, inhibitory action of COEE against biocorrosion of mild steel observed herein is in agreement with [9]. They demonstrated the corrosion inhibition and adsorption of *Piper guineensis* extracts on the metal surface in varying concentration of HCl and $H_2SO_4$.

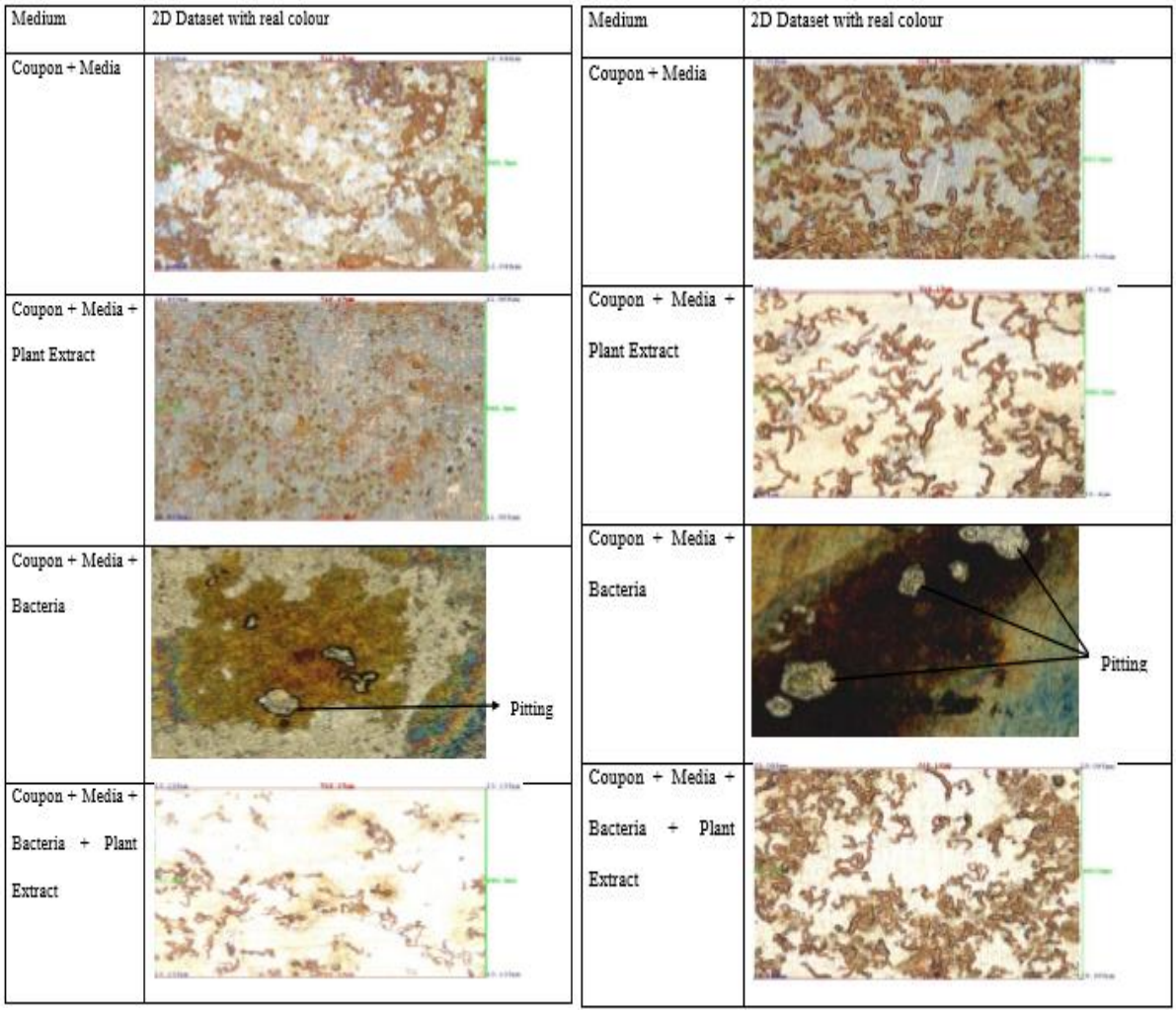


**Fig 1: Two-dimensional surface profile of mild steel coupons from IFM analysis. Arrows indicate pitting formation.**

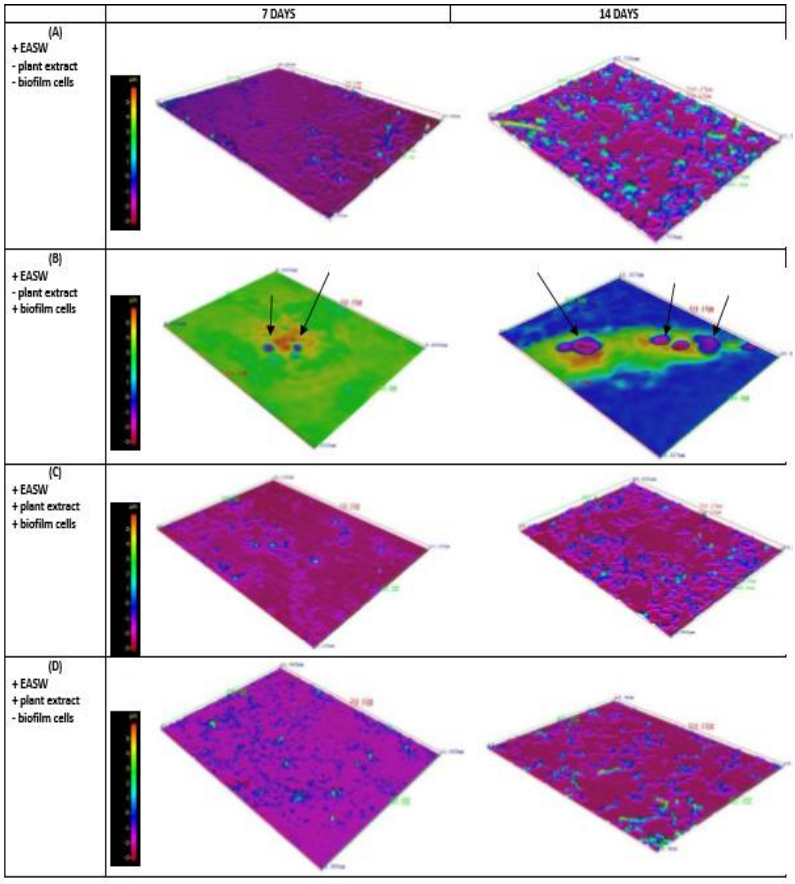


**Fig 2: Three-dimensional surface profile of mild steel coupons from IFM analysis. Arrows indicate pitting formation.**

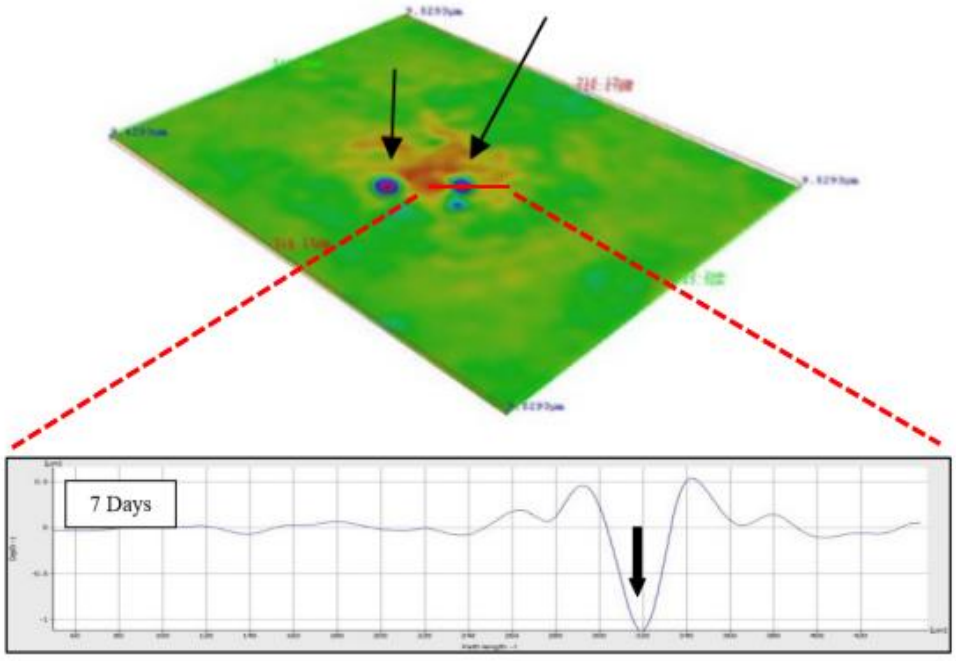


**Fig 3: IFM pit depth profile of mild steel coupon inoculated with *P. aeruginosa* from 7-day experiment. Arrows indicate pitting formation.**

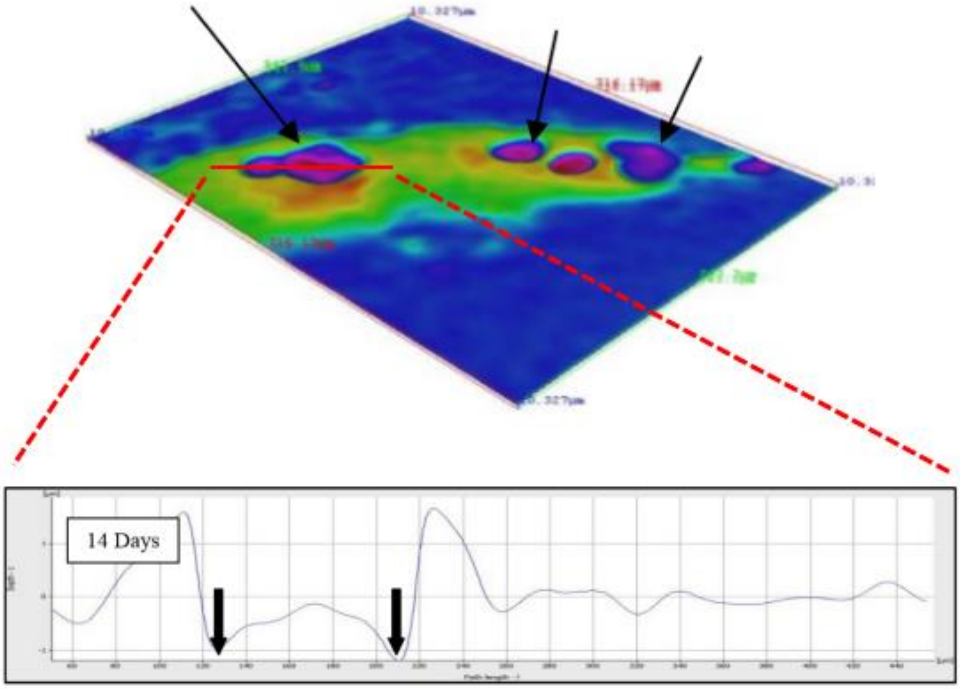


**Fig 4: IFM pit depth profile of mild steel coupon inoculated with *P. aeruginosa* from 14-day experiment. Arrows indicate pitting formation.**

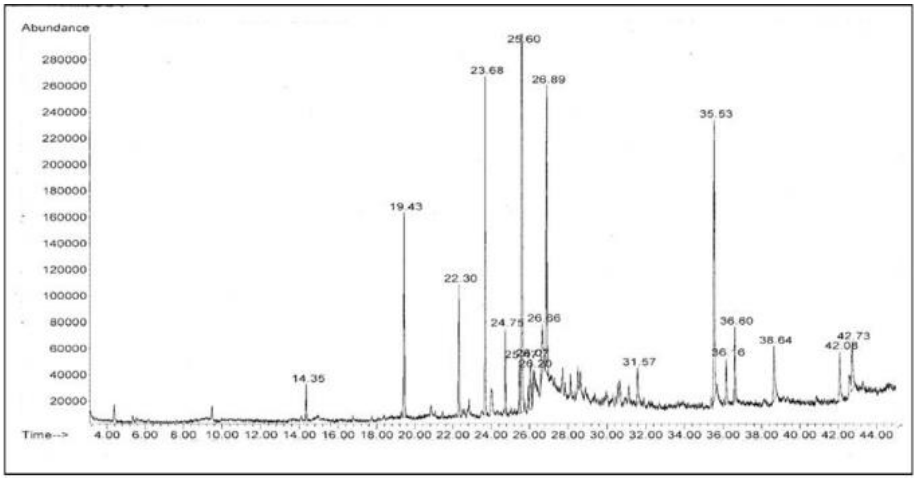


**Fig 5: GC-MS phytochemical profile of COEE**

**Pit depth profile**

Figure 3 and 4 show the pit depth profile of mild steel coupons inoculated with *P. aeruginosa*. The fluctuations noted in the surface profiles indicate the corrosion depth, corrosion width and height of corrosion residue. The corrosion pit of mild steel coupon from 7-day experiment was characterized by approximately 2.8 µm corrosion in depth and 50 µm corrosion width. On the other hand, the corrosion pit of mild steel coupon from 14-day experiment was characterized by approximately 6 µm corrosion depth and 75 µm corrosion width. Corrosion pit is an important indication of biocorrosion. It is normally formed when electrochemical oxidation-reduction takes place within the localized area on the metal surface and is difficult to detect. Metabolic products from surface-attached microbial community often chemically disrupt passivating film under anaerobic environment and promote pitting formation at the metal-biofilm interface. The result from the present study corroborates [10] demonstrating the pit formation on carbon steel following 7-day incubation with *Desulfovibrio vulgaris.* Characterization of corrosion pit may also involve other analytical

methods. In 2018, Zhang et al. [11] studied corrosion behaviors of aluminium alloy in ethylene glycol using three-dimensional confocal laser scanning microscope (3D CLSM). They found that the pit depth of aluminium alloy immersed in 3.5% NaCl solution was greater than that of aluminium alloy immersed in $(CH_2OH)_2$

**Phytochemical profile**

Figure 5 and Table 1 show the identified phytochemicals in COEE. Germacrene D, caryophyllene and cadinene were found to be the major phytochemicals in COEE. Besides, palmitic fatty acid was also identified in COEE. Germacrene D, caryophyllene and cadinene are classified as sesquiterpenes which are commonly produced by essential oil-producing plants. They are made from C5 isoprene units and have the molecular formula $C_{15}H_{24}$. Direct effects of these sesquiterpenes (germacrene D, caryophyllene and cadinene) on biofilm-associated corrosion are not well investigated, however, few reports on the antibiofilm activities of plant extracts containing high composition of germacrene D and caryophyllene have previously been published [12, 13]. In general, sesquiterpenes are effective against floating bacteria [14] but their inhibitory effects against biofilms have not yet been determined. Palmitic acid has the chemical formula $CH_3(CH_2)_{14}COOH$ and becomes the major constituent in three varieties of *Labisia pumila* [15]. It shows the highest composition in *alata* variety when compared to *pumila* and *lanceolata* varieties. In addition, *L. pumila* aqueous extract has been shown to exhibit a variable degree of antibacterial activities against eight bacteria Gram-positive and Gram-negative bacteria. The inhibitory effects of palmitic acid against the bacterial biofilms infecting the lungs of cystic fibrosis patients has also been suggested [16].

**Table 1: List of identified phytochemicals in COEE**

| RT | Area (%) | Compound | Other Name | Quality value |
|---|---|---|---|---|
| 22.30 | 6.44 | Copaene | Copaene | 98 |
| 23.68 | 17.30 | Caryophyllene | Caryophyllene | 99 |
| 24.75 | 4.22 | 1,4,7, -Cycloundecatriene,1,5,9,9 tetramethyl-,z,z,z | - | 98 |
| 25.47 | 2.96 | Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-1(1-methylethyl) | γ-Cadinene | 99 |
| 25.61 | 41.19 | 1,6-cyclodecadiene,1-methyl-5methylene-8-(1-methylethyl) | Germacrene D | 96 |
| 26.07 | 3.99 | Cyclohexane,1-ethenyl-1-methyl-2-(1methylethenyl)-4-(1-methylethylidene) | o-Menth-8-ene | 86 |
| 26.20 | 0.52 | Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) | β-Cadinene | 93 |
| 26.89 | 14.47 | Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) | δ-Cadinene | 95 |
| 38.64 | 4.72 | n-Hexadecanoic acid | Palmitic acid | 87 |
| 42.73 | 4.32 | 9,12,15-octadecatriene-1-ol, (z, z, z) | - | 91 |

**Mode of action of COEE**

In the present work, IFM analysis successfully showed that treatment with COEE inhibited biocorrosion of mild steel coupons in EASW. On the other hand, our previous works have demonstrated that pitting corrosion of mild steel coupons results from biofilm formation by *P. aeruginosa* [4] and treatment with COEE is effective against *P. aeruginosa* biofilm under both aerobic and anaerobic environments [13]. Taken together, it is possible that treatment with COEE exhibits protection against biocorrosion by inhibiting biofilm formation. This suggestion is in agreement with [17] claiming that inhibition of biofilm formation is the simplest way of biocorrosion prevention. Recently, Parthipan et al. [18] investigated the effects of *Allium sativum* extract on biocorrosion of carbon steel and mild steel in the presence of *Bacillus subtilis* A1 and *Streptomyces parvus* B7. They demonstrated that *A. sativum* extract inhibited biofilm development in microtiter plate and reduced the rates of biocorrosion rates in a hypersaline environment. Apart from biofilm inhibition, there are also other potential mode of actions performed by the plant extracts in order to protect against corrosion. Garlic extract contains high composition of allyl propyl disulphide which affects the potential cathodic process of steel while *Foeniculum vulgare* extract contains major constituents such as

limonene and pinene which undergo adsorption on metal surface via interaction with the vacant d-orbitals of iron atoms [17]. Moreover, *Ipomoea involcrata* extract contains mainly d-lysergic acid amide (LSA) which form a strong physisorbed layer on the metal surface [17].

## CONCLUSION

We have demonstrated that *C.odorata* ethanol extract possesses antibiocorrosion activity. It also contains a high percentage of antibacterial constituents such as germacrene D, caryophyllene and cadinene. We suggest that, biofilm inhibition may mediate the antibiocorrosion activity of *C.odorata* ethanol extract.

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