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The Inhibitive Effect of *Gmelina arborea* Fruit Extract on Biocorrosion Induced by Sulphate-reducing Bacteria

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ABSTRACT: Microbial activities contribute significantly to the global economic burden of corrosion. The sulphate-reducing bacteria (SRB) are major cause of biocorrosion in anoxic environments. This study investigated the corrosion inhibition efficiency of *Gmelina arborea* extracts against corrosion of carbon steel caused by SRB isolated from injection and produced water from Bonga oilfield. The SRB isolation, antimicrobial assay and corrosion experiments were carried out in a Coy anaerobic chamber. The isolates were identified based on the microscopic and physiologic characteristics as *Desulfovibrio* spp. The minimum inhibitory concentration (MIC) for *G. arborea* ethanol extract (GAEE) against the isolates was 0.85 g/ml and 0.93 g/ml for *G. arborea* aqueous extract (GAAE). The highest reduction in SRB population was observed with the MIC of extracts potentiated with 50 PPM D-Tyrosine and the least reduction was with half strength of the extract (0.5xMIC). For the 14-day test, the least inhibition efficiencies were 28.88*%I* and 31.01*%I* at 40°C for GAAE and GAEE respectively and the highest were 92.95*%I* and 94.63*%I* at 20°C for GAAE and GAEE potentiated with D-Tyrosine respectively. Similarly, for 28-day test, the least inhibition efficiencies were 22.62*%I* and 24.29*%I* at 40°C and the highest was 85.58*%I* and 90.28*%I* at 20°C for GAAE and GAEE potentiated with D-Tyrosine respectively. Adsorption mechanism of the extracts on the steel obeyed the Langmuir adsorption model at temperature between 20°C - 40°C with extract concentrations range of 0.5-2.5 g/ml. Adsorption mechanism of the extracts on the steel obeyed the Langmuir adsorption model. *G. arborea* contains compounds that inhibit the growth and corrosion activities of SRB. These phytochemicals can be harnessed as green inhibitors in the control of SRB in the oilfield.

Keywords: *Gmelina arborea* extracts, sulphate-reducing bacteria, corrosion inhibition

Introduction

Since sulphate-reducing bacteria (SRB) were first reported to be present in oilfields; interest in this group of microorganisms in the petroleum industry has not waned because of the numerous economic losses associated with their activities. Their activities generally contribute to the acidification of produced fluids. The detrimental effects of SRB activities in the oil and gas industry have been determined to include decrease in economic value of crude, loss in fluidity of crude due to heavy oil formation, bioclogging and biocorrosion (Gregoire *et al*., 2014; Immanuel *et al*., 2015).

An environment rich in sulphate ions, easily degradable organic compounds, moderate salinity and favourable environmental conditions promotes sulphidogenesis by SRB. The SRB are the most often reported biocorrosion culprits. They cause biocorrosion of drilling, processing, transportation, storage equipment or any steel structure for that matter, most notably the mesophilic SRB (Wolicka and Borkowski, 2012; Cortás *et al*., 2012).

SRB can take part in biocorrosion when they are present in biofilms. Biofilms form localized environments on surfaces of metals which greatly affect the underlying corrosion in a number of ways such as by setting up oxygen concentration gradient across the biofilm due both to O_2 diffusion limitations as well as O_2 consumption by aerobic bacteria and by acting as electron shuttle (Sheng *et al*., 2008; Gu *et al*., 2015).

Control of the activities of SRB is a decision that must be taken before the onset of their menace. This is important to forestall the expensive consequences of remediation through chemical and engineering solutions. Batch biocide dosing along with pigging are often employed by the industry to mitigate microbial proliferation in pipelines. These biocides are often required in high concentrations, expensive, ineffective against target microorganism inside the reservoir and in biofilms, coupled with the issue of bacterial resistance as a result of their repeated usage and their environmental toxicity (Vance and Thrasher, 2005; Dinning, 2011). There is the need to find cheap, eco-friendly and effective biocides to control SRB species, hence the use of phytochemicals. Phytochemicals have been reported to be effective as antimicrobial and anticorrosion agents (Korenblum *et al*., 2013; Immanuel *et al*., 2016; Briggs *et al*., 2019). The present study therefore aimed to investigate the efficiency of *G. arbore*a fruit extracts as biocorrosion inhibitors, in the presence of SRB.

Materials and methods

Plant collection and extract preparation: *Gmelina arborea* fruits were obtained from Swali Market Road, Yenagoa, Bayelsa State, Nigeria. Extraction followed process described by Stanley *et al.* (2016) with modification. The fruits of *G. arborea* were washed with distilled water and sun-dried for 7 days after which they were oven-dried for 15 minutes at 40 °C. One hundred grammes (100 g) of dried fruit bodies were placed in 250 ml conical flasks containing 150 ml of distilled water for aqueous extraction and 95% ethanol for ethanol extraction. The contents were stirred gently using stirring rod, corked and flasks kept in the laboratory for 72 hours. Filtration was done using Whatman filter paper No.1. The flasks with their contents were transferred into a water bath adjusted to 60 °C to evaporate all the solvent and concentrate the extracts. Extracts were used to prepare the test solutions by dissolving appropriate masses of the extract from 0.1 g - 3 g in 1 ml of distilled water.

Phytochemical screening: Extracts were screened for the presence of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, protein, triterpenoids and steroids following methods described by Shah *et al.* (2014).

Isolation of sulphate-reducing bacteria: Sulphate-reducing bacteria were isolated from injection and produced water obtained from Bonga oilfield in Nigeria. Isolation of SRB was done using modified Postgate B broth tubes as described in Immanuel *et al.* (2016). All tubes were incubated at 30°C for 7 days and observed for blackening by SRB due to H_2S formation. Incubation was done in a Coy anaerobic chamber. Repeated sub-culturing in solid medium was carried out to obtain pure culture of isolates. Isolates were identified based on their morphological and physiological characteristics as described in Immanuel *et al*. (2016).

Antimicrobial assay: A mixed SRB consortium was used for the antimicrobial assay of extracts following the method described by Immanuel *et al.* (2016). The least concentration of extract added that did not result in the formation of a black precipitate in the tube served as the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was determined as the lowest concentration of extract that resulted in no growth of SRB. The enumeration of the SRB population was done using the Most Probable Number (MPN) technique according to FDA (2010).

Gravimetric experiment: A mixed SRB consortium was used for the corrosion experiments following methods described by Immanuel *et al*. (2016). Metal coupons (25±08 g) were conditioned using varying concentrations of extracts, 50 PPM D-Tyrosine and MIC+50 PPM D-Tyrosine for 24 hrs before placement into 100 ml serum bottles containing 90 ml Postgate B. Blank control was untreated coupon in medium not inoculated with SRB cells. Cell control was untreated coupon in medium inoculated with SRB cells. All the metals in the serum bottles were allowed to undergo corrosion at 20, 30, and 40 °C for a period of 14 and 28 days. At the end of the 14- and 28-days test period, metal coupons were cleansed briefly in acid, neutralized with sodium bicarbonate, rinsed in acetone, air dried and reweighed. Changes in weight were recorded. Experiments were performed in triplicate and the mean values of the weight loss were reported. Corrosion rate (CR) was calculated in milli-inch per year (mpy); the inhibition efficiency (IE) and the surface coverage (ϴ) were calculated using equation 1, 2 and 3 respectively:

$$CR = KW/DAT \qquad (1)$$

where K = rate constant 22,300;
W = weight loss in grammes;
D = density in gcm^{-3},
A = exposed area in $in.^2$ and
T = time of exposure in days

$IE = 100 [1 - (W_2/W_1)]$ (2)

where W_1 is the corrosion rate in the absence of the inhibitor,
W_2 is the corrosion rate in the presence of the inhibitor.

$\Theta = IE/100$ (3)

Data obtained from weight loss measurements were used for adso-kinetic studies of the extracts. The adsorption equilibrium constant K_{ads} was deduced from the plot of C/Θ against C, using equation (4).

$C_{inh}/\Theta = 1/K_{ads} + C_{inh}$ (4)

where, C is the concentration of inhibitor.

The Gibbs free energy (ΔG_{ads}) of adsorption was obtained from the relation:

$\Delta G_{ads} = -RTIn(55.5K_{ads})$ (5)

where R is the universal gas constant (8.314 $Jmol^{-1}K^{-1}$),
T is the operating temperature and
55.5 is the molecules of water displaced by adsorded molecules of inhibitor.

Enthalpy change (ΔH_{ads}) and entropy change (ΔS_{ads}) of adsorption were deduced from the linear plot of ΔG_{ads} against T, using equation 6:

$\Delta G_{ads} = \Delta H_{ads} - T\Delta S_{ads}$ (6)

Results

Phytochemical screening: Qualitative screening of *G. arborea* extracts indicated presence of flavonoids, alkaloid glycosides, phenolic compounds, tannins, triterpenoids and steroids as shown in Table 1.

Table 1: Phytochemical present of *Gmelina arborea* fruit extracts

Phytochemical	**Test**	**Observation**	
		Aqueous	Ethanol
Alkaloid	Mayer’s test	-	+
	Wagner’s test	-	+
	Dragendorff’s test	+	+
Proteins	Million’s test	-	-
	Biuret test	-	-
Glycosides	Legal’s test	+	+
	Keller-Killiani test	+	+
Flavonoids	Shinoda test	+	-
	Alkaline reagent test	-	+
Triterpenoids and Steroids	Libermann-Burchard’ test	+	+
	Salkowski test	+	+
Phenolics and Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+

Characteristics of isolates: Microscopic, morphological and physiological characteristics of isolates are given in Table 2. The isolates were motile, non-spore forming, curved rod mesosophiles that gave a negative Gram reaction. The isolates were identified as belonging to the genus Desulfovibrio.

Table 2: Morphological and physiological characteristics of isolates

Characteristic	**IW1**	**PW1**	**PW2**
Gram reaction	Negative	Negative	Negative
Morphology	Curved rod	Curved rod	Curved rod
Motility	Motile	motile	Motile
Sporulation	Absent	Absent	Absent
Salinity range (%NaCl)	2.5 -4.5	2.5-4.5	2.5-4.5
pH range	7-9	7-9	7-9
Temperature range	20-40	20-40	20-40

Characteristic	IW1	PW1	PW2
ELECTRON ACCEPTORS			
Sulphate (10mM)	+	+	+
Sulphite (5 mM)	+	+	+
Thiosulphate (5 mM)	+	+	+
Manganese oxide (20 mM)	-	+	-
Nitrate (0.2 mM)	-	+	-
ELECTRON DONORS			
Acetate (20 mM)	+	+	+
Lactate (20 mM)	+	+	+
Pyruvate (10 mM)	+	+	+
Fumerate (10 mM)	+	+	+
Cystein (5mM)	+	+	-
Yeast extract (0.1%)	+	+	+
Phenol (5 mM)	-	-	-
Alanine (10 mM)	-	-	-
Ethanol (5 mM)	+	+	+
Probable organism	*Desulfovibrio* spp.	*Desulfovibrio* spp.	*Desulfovibrio* spp.

Effect of Extracts on SRB Population: Both GAEE and GAAE demonstrated inhibitory effects against SRB at 0.5-2.5 times their MICs which was determined to be 0.85 g/ml and 0.93 g/ml respectively. The MBC for GAEE was 2.13 g/ml and 2.79 g/ml for GAAE. Figure 1 show the effect of GAEE on SRB population in Postgate B broth tubes, with MPN values varying from 0.86 to 6.3 log MPN/ml for GAEE, where 0.85 g/ml of extracts potentiated with 50 PPM D-Tyrosine gave the highest reduction and 0.5xMIC gave the least reduction. Figure 2 show the effect of GAAE on SRB population. MPN values varied from 1.04 to 6.54 log MPN/ml for GAEE, where 0.93 g/ml of extracts potentiated with 50 PPM D-Tyrosine gave the highest reduction and 0.5xMIC gave the least reduction. There was statistically significant difference ($p<0.05$) in SRB cells reduction between MIC+50 PPM D-Tyrosine and all extracts concentration except 2.5xMIC.

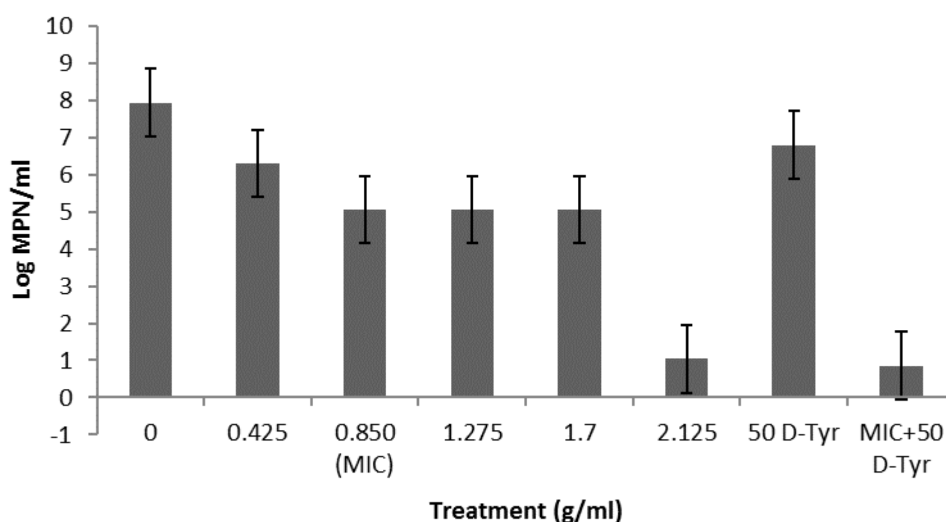

Figure 1: Count of SRB with *Gmelina arborea* ethanol extract (GAEE) and D-Tyrosine treatments

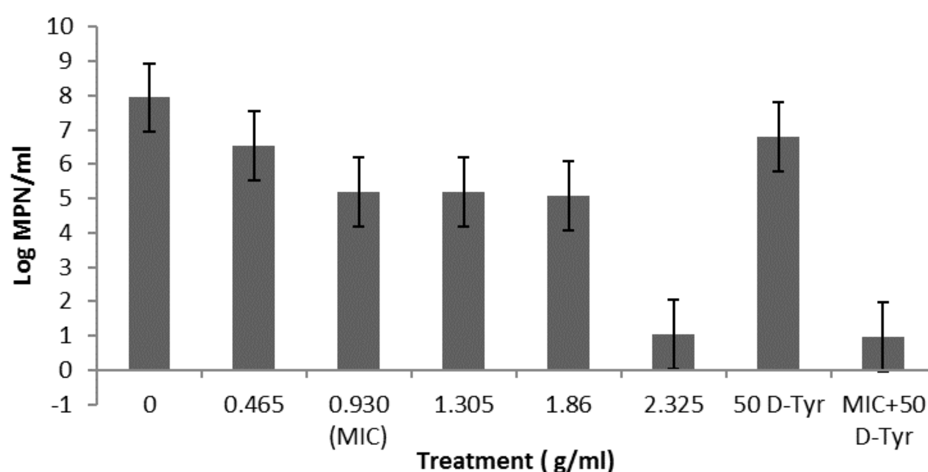

Figure 2: Count of SRB with *Gmelina arborea* aqueous extract (GAEE) and D-Tyrosine treatments

Corrosion Rate: Results for corrosion rate with and without extracts at different temperatures are shown in Figure 3-6. All concentrations of extracts showed some degree of activities. Results indicated that corrosion rate decreased with increase in concentration of extracts and increased with increase in temperature. Corrosion rate varied from 0.113-147 mpy, 0.298-0.374 mpy, 0.016-0.258 mpy 0.021-0.266 mpy for blank control, cell control, GAEE and GAAE respectively, for the 14-day test, while for the 28-day test, it varied from 0.14-0.182 mpy, 0.319-0.420 mpy, 0.031-0.318 and 0.046-0.077 mpy for blank control, cell control, GAEE and GAAE in that order.

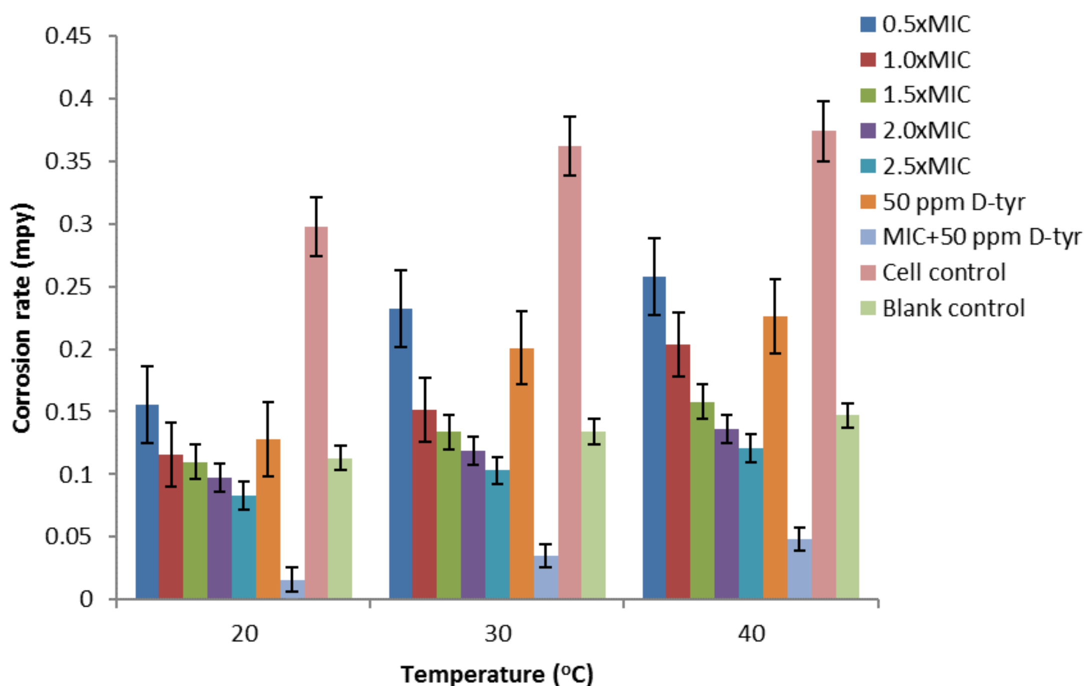

Figure 3: Corrosion rate recorded at different temperatures in the presence and absence of GAEE (14-day test)

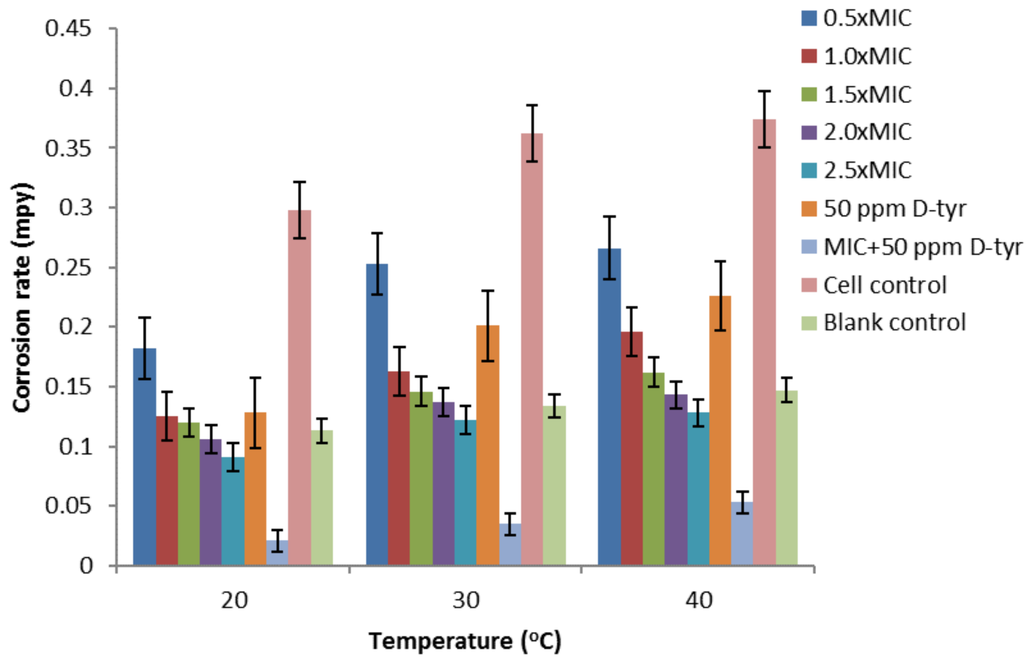

Figure 4: Corrosion rate recorded at different temperatures in the presence and absence of GAAE (14-day test)

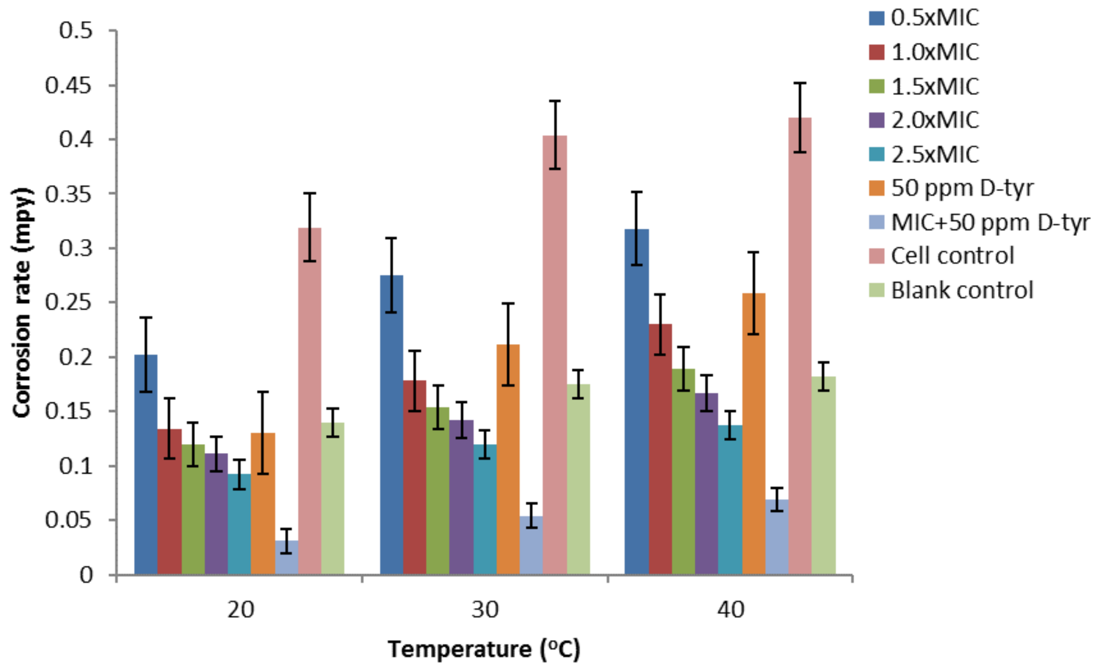

Figure 5: Corrosion rate recorded at different temperatures in the presence and absence of GAEE (28-day test)

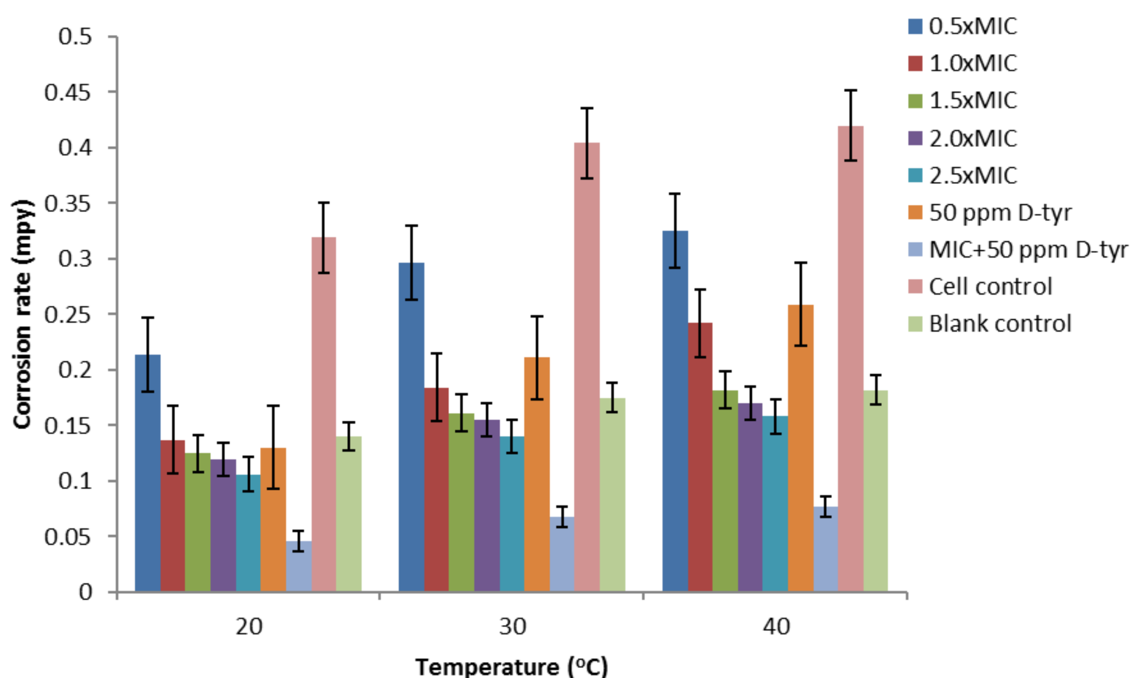

Figure 6: Corrosion rate recorded at different temperatures in the presence and absence of GAAE (28-day test)

Corrosion Inhibition: Results for inhibition efficiency in the presence and absence of extracts monitored at different temperatures for the 14- day are shown in Table 3. GAEE alone gave inhibition efficiency range of 31.01*%I*-72.15*%I* with highest at 2.5xMIC when temperature was 20ºC and the least at 0.5xMIC when temperature was 40ºC. GAAE alone gave inhibition efficiency range of 28.88*%I*-69.46*%I* with highest at 2.5xMIC when temperature was 20ºC and the least at 0.5xMIC when temperature was 40ºC. 50 ppm D-Tyrosine alone gave inhibition efficiency range of 44.48*%I* – 57.04*%I* and MIC+50 ppm D-Tyrosine blends gave inhibition efficiency ranging from 85.83*%I* to 94.63*%I*.

Table 4 shows corrosion inhibition results for the 28-day test. GAEE alone gave inhibition efficiency range of 24.29*%I*-71.16*%I* with highest at 2.5xMIC when temperature was 20ºC and the least at 0.5xMIC when temperature was 40ºC. GAAE alone gave inhibition efficiency range of 22.62*%I*-71.16*%I* with highest at 2.5xMIC when temperature was 20ºC and the least at 0.5xMIC when temperature was 40ºC. 50 ppm D-Tyrosine alone gave inhibition efficiency range of 38.33*%I* – 59.25*%I* and MIC+50 ppm D-Tyrosine blends gave inhibition efficiency ranging from 81.67*%I* to 90.28*%I*.
Inhibition efficiency increased with increase in concentration for all extracts and decreased with increase in temperature. There was no statically significant difference (p>0.05) in corrosion inhibition efficiency at day 14 and 28.

Table 3: Variation in inhibition efficiency with temperature in the presence and absence of extracts (14-day test)

Treatment	**Inhibition efficiency (%I)**					
	GAEE			**GAAE**		
	20°C	**30°C**	**40°C**	**20°C**	**30°C**	**40°C**
0.5 x MIC	47.65	35.91	31.01	38.93	30.11	28.88
1.0 x MIC	61.07	58.01	45.45	58.65	54.97	47.59
1.5 x MIC	63.09	62.98	57.57	59.73	59.67	56.68
2.0 x MIC	67.45	67.13	63.64	64.42	62.15	61.76
2.5 x MIC	72.15	71.62	67.65	69.46	66.30	65.78
50 ppm D-Tyrosine	57.04	44.48	47.59	57.04	44.48	47.59
MIC + 50 ppm D-Tyrosine	94.63	90.33	87.17	92.95	90.33	85.83

Table 4: Variation in inhibition efficiency with temperature in the presence and absence of extracts (28-day test)

Treatment	Inhibition efficiency (%I)					
	GAEE			GAAE		
	20°C	30°C	40°C	20°C	30°C	40°C
0.5 x MIC	36.68	31.93	24.29	32.92	26.73	22.62
1.0 x MIC	61.60	55.94	45.24	57.05	54.46	42.38
1.5 x MIC	62.38	61.88	55.00	60.82	60.15	56.67
2.0 x MIC	65.20	64.85	60.24	62.70	61.63	59.52
2.5 x MIC	71.16	70.30	67.38	66.77	65.35	62.38
50 ppm D-Tyrosine	59.25	47.77	38.33	59.25	47.77	38.33
MIC + 50 ppm D-Tyrosine	90.28	86.63	83.53	85.58	83.17	81.67

Adsorption and thermodynamic characterization: Table 5 shows adsorption and thermodynamic kinetics of the extracts. The coefficient of determination (R^2) values for GAEE and GAAE at 20ºC, 30ºC and 40ºC ranged between 0.968 and 0.996, highest at 20ºC for GAAE and least at 30ºC for GAEE.

Table 5: Thermodynamic parameters for adsorption of GAEE on carbon steel at different temperatures from Langmuir adsorption isotherm

	Temperature (K)	R^2	K_{ads}	ΔG_{ads} ($kJmol^{-1}$)	ΔH_{ads} ($kJmol^{-1}$)	ΔS_{ads} ($Jmol^{-1}K^{-1}$)
GAEE	293	0.996	2.611	-12.122	-40.910	-98.000
	303	0.991	1.555	-11.230	-40.910	-98.000
	313	0.997	1.054	-10.159	-40.910	-98.000
GAAE	293	0.993	1.698	-11.074	-34.000	-21.000
	303	0.968	1.205	-10.588	-34.000	-21.000
	313	0.989	0.978	-10.390	-34.000	-21.000

Discussion

Phytochemical screening of *G. arborea* ethanol extract (GAEE) and *G. arborea* aqueous extract (GAAE) revealed the presence of alkaloids, glycosides, triterpenoids, tannins and phenolics as constituents. Alkaloids, flavonoids, phenolic compounds and glycosides have been reported to be responsible for inhibiting acid corrosion (Nnanna *et al*., 2010; Lebrini *et al*., 2011). They possess antimicrobial properties, which could make them effective in inhibiting microbially influenced corrosion. They can do this by inhibiting cell wall synthesis, causing severe leakage of cellular material, stagnating the growth or even lead to cell death (Helander *et al*., 1998; Marcucci *et al*., 2001). Triterpenoids are reported as common secondary metabolites found in *Ganoderma* sp. with diverse range of biological activities (Zjawiony, 2004; Xia *et al*., 2014). *G. arborea* extracts are rich in tannins which are known to be antimicrobial (El-Mahmood *et al*., 2010). Isah *et al*. (2011) reported a reduction in rumen bacteria of goats fed with *G. arborea*. Nayak *et al*. (2012) reported that ethanol extract of *G. arborea* fruits showed antimicrobial activities against pathogenic bacteria.

GAEE and GAAE treated vial had up to 5-log reduction in SRB population. This suggests that the extracts did not completely kill the microorganisms with time. For plant extracts to be able to cause irreversible damage to bacteria, they should be able to alter or cross their membrane barrier, which is particularly difficult with Gram negative bacteria such as SRB.

The *G. arborea* fruit extracts showed some anticorrosion activities, which increased significantly with increase in concentration from 0.5xMIC to 2.5xMIC. For microbial induced corrosion, the MIC can reduce the concentration of planktonic and sessile bacteria (Immanuel *et al*., 2016). However, higher concentration can increase the inhibition efficiency as the molecules adsorbed onto the metal surface (Stanley *et al*., 2016). Nwaedozie *et al*. (2015) reported the inhibition effect of *G. arborea* fruits extract on armoured steel plate corrosion in HCl solution. Their investigation showed evidence of plant material adsorption on the metal surface to provide protection in the presence of the acid. The inhibition efficiencies of the extracts were seen to reduce with exposure time and at higher temperatures.

Gmelina extracts where able to inhibit corrosion because they contain alkaloids, flavonoids, phenolic compounds and glycosides which have reported to be responsible for inhibiting corrosion (Nnanna *et al*., 2010; Lebrini *et al*., 2011). Besides, the chemical structure of some of the extracts as revealed by FTIR spectroscopy

have anchoring functional groups which contained one or more hetero atoms such as N and O, as in hydroxy, epoxy, amino and carbonyl, which could allow for binding onto the metal surface.

The Langmuir adsorption isotherm plots showed a good fit with R^2>0.9. The lower temperature appears to favour the adsorption of the extract to the metal that the higher temperature, as shown by higher linearity at 20ºC for the 14 and 28-day test. The Gibb's free energy values around -20kJ/mol or lower. The negative ΔG_{ads}, values obtained in this study suggest a spontaneous adsorption process (Nwaedozie *et al*., 2015) and that the mechanism adsorption was by physisorption (Obot *et al.,* 2009; Solomon *et al*., 2010; Odozi *et al*., 2015). This implies that the extracts could prevent microbial attachment to steel surface (Immanuel *et al*., 2016). The positive K_{ads} values indicate conditions for good adsorption and strength of adsorption (Ansari *et al*. 2015; Nwaedozie *et al*. 2015). Obtained values for heat of adsorption in the presence of GAEE and GAAE were all negative. This established that the adsorption reaction was exothermic at study temperatures.

Conclusion

This study has demonstrated the anticorrosion efficiency of *Gmelina arborea* extracts against corrosion induced by SRB and could have important application in controlling of biocorrosion under low temperature condition.

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