



ORIGINAL ARTICLE

Evaluation of crude methanolic mangrove leaves extract for antibiofilm efficacy against biofilm-forming bacteria on a cooling tower wastewater system



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Abstract The present study demonstrated microbial corrosion protection of MS 1010 on cooling tower water using plant-based inhibitors derived from methanolic extraction of dry mangrove leaves (*R.mangle* and *A.marina*), and its assessment of antibacterial activity against corrosive bacteria (*B.megaterium*) was investigated. FT-IR and GC-MS analyzed the inhibitors component and corrosion behavior of MS 1010 on cooling water, with and without inhibitors were analyzed by EIS and Tafel studies. GC-MS spectra confirmed the presence of Myo-Inositol, 4-C-methyl and chromene as major constituents presented on the *R.mangle* whereas Lupeol, trifluoroacetate and beta-amyrin compounds were found on the *A.marina*. In the cooling water, these two inhibitors demonstrated outstanding antibacterial activity and controlled biofilm growth. As plant-based inhibitors were used in cooling water systems, EIS data showed a significant increase in R_{ct} value when

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compared to the control system. Tafel plot indicates inhibitors have mixed inhibitory effects and for the systems with and without inhibitors, the i_{corr} value was 1.5649A and 2.0875A, respectively. At the optimal dose of 25 ppm, the inhibitory efficiency of MERM and MEAM was 81% and 80%, respectively. The overall discussion reveals that inhibitor substances can be absorbed on the metal surface and then act as a dual role in inhibiting corrosive bacterial growth and barrier to the corrosion process in the cooling water system.

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1. Introduction

Mild steel (MS) is the most favorable metal used for various industrial sectors such as oil, gas pipelines, building construction, cooling tower plants, etc. For industrial purposes, MS pipelines are usually used to cool tower plants for circulation and transport the water to specific operating conditions (Kumar Yadav & Soni, 2013; Rajasekar et al., 2007). The cooling tower is damaged due to the metal composition and regular water flow. This water contains rich in presence of microbes that have secreted extracellular polymeric substances and formed biofilm layers on the metal surface that involve metal deterioration is called Microbially Influenced Corrosion (MIC). (Fayyad et al., 2021; Granero et al., 2009). *Bacillus megaterium* (*B.megaterium*) is a gram-positive, aerobic spore-forming huge size bacteria, the name *megaterium* means 'big animal' (Greek), and even this microorganism is the biggest of all bacilli (Vejar et al., 2016). In the process of bacterial communication known as quorum sensing (cell signalling hormones), it can release the extracellular polymeric substance (EPS). Acyl homoserine lactones (AHLs) are responsible for the secretion, synthesis and sense of signal molecules known as autoinducers, as well as communicating the signal to other microbes, which subsequently interact to exchange electrons and assemble to create biofilm layers (Marchand & Collins, 2016; Pusparizkita et al., 2020). In the cooling tower water plant, bio-treatment activities of wastewater treatment units have gathered a large number of microbes allowing for simple and healthy bacterial growth. By increasing heterogeneity on the metal's surface, it can influence metal distraction through the development of biofilm layers. *B.megaterium* will begin to utilise iron (Fe) as an electron donor, which is oxidized to Fe^{2+} and Fe^{3+} to provide the energy needed for the metabolic pathways. Direct transfer electrons can carry electrons through the cell wall to the cyto-plasma via protein cell membranes (Feng Lin et al., 2013). Beneath the biofilm, oxidized metal on the anode might interact with acid compounds generated by *B.megaterium* to induce corrosion (pitting) products (Rajasekar & Ting, 2011). Corrosion is a significant trouble in the cooling tower system because of the wastewater composition, which includes salt, pH, temperature, impure particles, microbes, and chemical compounds (hydrogen sulfide, sodium chloride), which all impact the metals susceptibility to corrosive substances. The corrosion rate is increased by sodium chloride which prohibits the formation of a protective FeS layer on the iron surface. Green inhibitors are a concentrated mixture of organic compounds including oxygen, sulphur, nitrogen and hydrogen atoms that inhibits microorganism from interacting with metal compounds (Kokilaramani et al., 2021a; Shah et al., 2011). Many researchers are working to develop effective green corrosion inhibitors for a wide range of metals in multiple configurations utilizing various components of plant (organic) materials including fronds, seeds, or bark extracts (Kamaruzzaman et al., 2021; Rahim et al., 2007).

Mangrove leaves include bioactive and secondary compounds, which are non-poisonous, ecologically friendly, organic molecules derived from natural sources and the mangrove leaves extracted were reported to be an effective inhibitor for corrosion (Bothi Raja & Sethuraman, 2008). The mangrove tree is a global, fast-growing species found along tropical and subtropical coastlines and it has been noted for its bio-potentials and distinctive chemical compositions, which are attributed to its therapeutic uses (Cui et al., 2019). *Rhizophora mangle*

(*R.mangle*) often known as red mangroves, is a kind of mangrove tree that grows in tropical evergreen trees that look like a stilt root and form dense thickets along coastal coastlines. It has thick bark, grey-brown in color, with a smooth edge of oval shape, yellow flowers were blooms in spring and top of the trees has a deeper shade of green than its bottom (Biber, 2006; Okeniyi et al., 2019b; Tan & Kassim, 2011). Another one is the *Avicennia marina* (*A.marina*) often known as white mangrove, which has light grey or whitish bark in stiff, brittle, thin flakes and their upper leaves are robust, shiny, and deep green, while the lower leaves are grey or silver-white with tiny hairs and they are the global, fastest-growing species located along with tropical and subtropical areas. (Amaral et al., 2011; Fouda et al., 2017; Shebany, 2012). Table 1 shows the classification of the mangrove plants *R.mangle* and *A.marina*. In corrosive environments, plant extracts are preferable to use as an inhibitor owing to their simple synthesis, biocompatibility, widespread availability, and economical efficiency. High inhibitor concentrations allow more surface molecules to be adsorbed, which enhances inhibitor efficiency and slows down the corrosion rate. In that adsorption provides a smooth interface between inhibitors and a metal surface and the metal surface, which is mostly ascribed to the heteroatom in an organic molecule with unsaturated p-bonds, effectively blocks the metal dissolution. The antimicrobial activity of mangrove leaves highly inhibit bacterial growth, it is easily adsorbed on the metal surface and creates protective layers for preventing metal corrosion and microbe communication (Chauhan et al., 2021; Musthafa et al., 2013).

This research work was aimed to evaluate the efficiency of mangrove leaves as a green corrosion inhibitor, as well as their adsorption behavior in *B.megaterium* strain isolated from cooling tower water using MS 1010 and the studies inhibition efficiency of methanolic extraction of mangrove leaves was evaluated by Minimal Inhibition

Table 1 Classification of mangrove plant *Rhizophora mangle* and *Avicennia marina*.

Names	<i>Rhizophora mangle</i>	<i>Avicennia marina</i>
Empire	Eukaryota	Eukaryota
Kingdom	Plantae	Plantae
Subkingdom	<u>Viridiplantae</u>	<u>Viridiplantae</u>
Infrakingdom	<u>Streptophyta</u>	<u>Streptophyta</u>
Superdivision	<u>Embryophyta</u>	<u>Embryophyta</u>
Division	<u>Tracheophyta</u>	<u>Tracheophyta</u>
Subdivision	<u>Spermatophytina</u>	<u>Spermatophytina</u>
Phylum	Spermatophyta	Tracheophyta
Subphylum	Angiospermae	Spermatophytina
Class	Dicotyledonae	Magnoliopsida
Subclass	Dictyotophycidae	Fucophycidae
Superorder	<u>Rosanae</u>	<u>Asteranae</u>
Order	Rhizophorales	Lamiales
Family	Rhizophoraceae	Acanthaceae
Genus	Rhizophora	Avicennia
Species	<i>Rhizophora mangle</i>	<i>Avicennia marina</i>

Concentration, Biofilm Inhibition Assay, and Weight Loss measurement (WL). Future analysis Electrochemical measurements (Tafel and Polarization), surface studies such as GCMS (Gas chromatography-mass spectrometry), and FTIR (Fourier transform infrared spectroscopy) was done. This is the first report, on using mangrove leaves (green inhibitors) to be considered a potential inhibitor for microbial corrosion (biocorrosion) in the cooling tower water. Recently, many research papers have reported the successfully used of natural products like plant leaf, flower, stem extracts as effective corrosion inhibitors against metal corrosion in acidic and alkaline media. Peach pomace (Vorobyova & Skiba, 2021), Fucus spiralis (Afrokh et al., 2022), purple rice bran (Pal & Das, 2022), Moroccan, Mauritania, and Senegalese gum (el Azzouzi et al., 2022) was investigated for their corrosion inhibition potential. The corrosion prevention mechanism this natural plant extract on mild steel metal demonstrates favorable adsorption and physisorption. This study shows that the leaf extracts from these natural plants *R. mangle* and *A. marina* are suitable for corrosion prevention in cooling tower water environment as a green/environmentally friendly inhibitor.

2. Materials and methods

2.1. Bacterial strain and media composition

The bacterial strain, *B. megaterium* SKR7 was identified from wastewater of the cooling water system. GenBank accession number MT211511.1 was assigned to the 16S rDNA gene sequence submission described by Kokilaramani 2020 (Kokilaramani et al., 2020). The SKR7 strain was recovered at 37 °C for 24 h in Luria-Bertani (LB) medium (5 g yeast extract, 10 g sodium chloride, 10 g tryptone, 15 g agar and final pH (at 25 °C) 7.5 ± 0.2 g/L (Himedia, Mumbai, India)). The well-grown bacterial inoculums were prepared to inoculate a single pure isolated colony in LB broth and shaking it for 24 h in a rotary shaker at (150 rpm) 37 °C.

2.2. Extraction of inhibitors (Mangrove Leaves)

The greenish raw mangrove plants of *R. mangle* and *A. marina* leaf samples were gathered from Parangipettai, Tamil Nadu, India (southeast coast of India, $11^{\circ}29'25.6''$ N and $79^{\circ}45'57.1''$ E) during January 2020. The leaves were cleaned with sterile double distilled water to detach dirt before being sent to the laboratory and the samples are left shadow environment at room temperature (RT) for a few days to dry. After the drying process, the leaves are shredded by mixer and followed for Soxhlet extraction, which entails mixing 10 g of powdered mangrove leaves added into 300 mL of methanol for 3 h at RT. From the obtained methanolic extraction of *R. mangle* (MERM) and *A. marina* (MEAM) the methanol was evaporated at 64.7 °C then the vaporized samples were stored in a refrigerator for future study (Punniyakotti et al., 2020; Zaher et al., 2022).

2.3. Minimal inhibitory concentration assay

The agar diffusion technique was used to estimate the minimal inhibitory concentration, that suppressed bacterial growth following overnight incubation at 37 °C. In the Mueller Hinton Agar (MHA) plate (Himedia) bacterial culture of SKR7 was swapped and profuse concentrations (10, 25, 50, and 100 ppm) of MERM and MEAM were added to the individual

well of the test and control samples were then incubated at 37 °C for 16 h in an incubator. The zones of the sample plates are evaluated after the incubation time (Li et al., 2018).

2.4. Enumeration of total viable count

The SKR7 bacterial cells had been cultured overnight and it was spread on the nutrient agar plates at 37 °C for 24 h with and without inhibitors (MERM and MEAM) at various doses (10, 25, 50, and 100 ppm) and the number of colonies was calculated using the following equation to estimate the TVC values as a colony-forming unit after the inhibition period (Li et al., 2018).

2.5. Biofilm inhibition assay

Overnight cultures of SKR7 (2.2×10^6 CFU/mL) were grown in Mueller Hinton Broth (MHB) medium, 1 mL of MHB as control System 1 (S1), System 2 (S2) 1 mL of MHB with 100 μ L of (OD 0.500) SKR7 culture, System 3 (S3 (A)) 1 mL of MHB, 100 μ L of (OD 0.500) SKR7 culture with added 25, 50, 100 ppm of MERM, System 4 (S4 (A)) 1 mL of MHB and 25, 50, 100 ppm of MERM. The well of S3 (B), S4 (B) was similar to S3 (A) and S4 (A) respectively, the *A. marina* was added instead of *R. mangle*. A 96-well microtiter plate was overnight incubated at 37 °C. The inoculum was removed and the 96-well microplate was rinsed in phosphate-buffered saline (PBS) followed by 15 min of incubation with 99% methanol then rinsed with PBS, added 200 μ L of 0.1% crystal violet maintained for 5 mins at RT. Lastly, 200 μ L of 95% ethanol was added and incubate on an orbital shaker for 30 mins then a UV-Visible spectrophotometer was used to absorbance the measured at 595 nm (Kokilaramani et al., 2021b).

2.6. Biocorrosion studies

2.6.1. Preparation of specimen

The mild steel 1010 (MS 1010) has a chemical composition of (wt %) was about C-0.03, Cu-0.093, Fe-99.64, Mn-0.16, Mo-0.002, Ni-0.04, P-0.01, S-0.026, Si- 0.002. The coupons were made out of MS 1010 and cut into small squares the size of $2.5 \text{ cm}^2 \times 2.5 \text{ cm}^2$ with a 2 mm hole in the top for weight loss measurement for hanging purposes and $1 \text{ cm}^2 \times 1 \text{ cm}^2$ for electrochemical analysis (working electrode (WE)). Coupons were polished using various grades of polishing papers to get a smooth and uniform exterior and then 0.3 μ M alumina powder was used to polish the surface mirror finish. Each specimen was washed in ethanol and acetone before being air-dried and placed in a desiccator for further studies then the coupons were sterilized for 30 min using a UV light exposure before the experiment (Kokilaramani et al., 2021b).

2.6.1.1. Weight loss method (WL). The autoclaved cooling tower water (CTW) was utilized as an electrolyte (medium) for the biocorrosion test and the water's chloride level was kept constant then the specimens are weighted for initial weight before the experiment begins. Including both weight loss and electrochemical experiments which were carried out in triplicate, the coupons were placed on a 500 mL flask with 400 mL of CTW and nutrient broth (NB) 1% as noted a control System 1 (S1), System 2 (S2) 1% of NB was added CTW in

400 mL and 1 mL of (OD 0.500) 16 h SKR7 culture about 2.2×10^6 CFU/mL, System 3 (S3 (A)) 400 mL of CTW and 1% of NB with 1 mL of SKR7 and 25 ppm of MERM, System 4 (S4 (A)) 400 mL of CTW and 1% of NB with 25 ppm of MERM. The experimental systems S3 (B), S4 (B) were similar to S3 (A) and S4 (A) respectively, the *A.marina* was added instead of *R.mangle* and incubated at (37 °C) RT for 20 days in an immobile stage. The WL coupons were collected at the end of the biocorrosion study and then soaked in pickling solutions described as (Kokilaramani et al., 2021b) according to ASTM Standard G1-81. It was washed with distilled water before being utilized for the WL study. According to the National Association of Corrosion Engineers (NACE), the experiment of inhibition efficiency (IE) and surface coverage (θ) was evaluated by the following equation (Zulfareen et al., 2016).

$$\text{IE(or) } \eta\% = \frac{W_0 - W_1}{W_0} \times 100$$

$$\theta = \frac{i_{corr} - i_{corr(inh)}}{i_{corr}} \times 100$$

where, W_0 and W_1 - weight loss of MS with and without inhibitor.

where, i_{corr} and $i_{corr(inh)}$ - corrosion current density of MS with and without inhibitor.

2.7. FT-IR

After the weight loss study, biofilm formed coupons were thoroughly dried up and the dried scraped powdered samples were mixed with potassium bromide (KBr) (purified salt) to form a pellet. These pellets were analyzed using FT-IR spectra (Perki-

nElmer Spectrum IR Version 10.6.0) with wave numbers 400–4000 cm^{-1} and 8 cm^{-1} resolution and a 64 scans/spectrum scan rate. FT-IR was used for the analysis to find out the organic functional groups of biofilm layers on the coupons of a metal surface (Rajasekar et al., 2008).

2.8. GC-MS

The examination of inhibitors was attained on an Agilent 7890A GC and 5975C Mass Spectrometer instrument, with 1 μL (volume) of MERM and MEAM taken in the column HP5-MS (Long 30, interior diameter 0.25 μM , thickness 250 μM), the carrier gas is helium.

With 1.0 mL/min flow rate. The oven was programmed between initial temperature: 55 °C for 2 min, increased at 70 °C /min, detector temperature 225 °C, injector volume: 1 μL a carrier gas and total running time of 35.5 min. The compounds were identified by comparing the corresponding mass

Table 2 Weight Loss Measurement for MS 1010 coupons from biocorrosion system.

System	Weight loss (mg)	Corrosion Rate (mm/y)	Inhibition Efficiency (%)
S1	27.66 ± 1	0.102	–
S2	430.89 ± 1	1.598	–
S3(A)	80.33 ± 1	0.298	81
S4(A)	12.57 ± 1	0.044	57
S3(B)	86.80 ± 1	0.322	80
S4(B)	12.13 ± 1	0.046	55

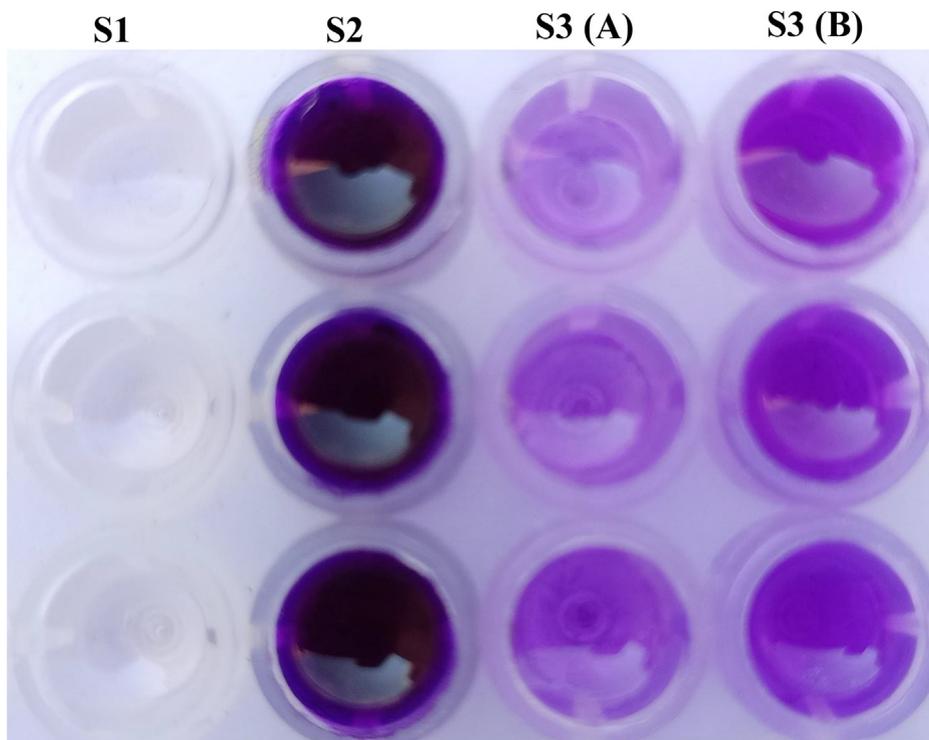


Fig. 1 Biofilm Inhibition assay.

Sample	Peak Values (cm ⁻¹)	Bond	Intensity	Functional groups
S1	3454.36	N-H stretch	weak-medium	Amides R-C(O)-NH-R
	2926.26	C-H stretch	strong	Alkanes & Alkyls
S2	1652.43	C=O stretch	strong, broad	Amides
	1393.30	CH ₃ C-H bend	medium	Alkanes & Alkyls
S3(A)	1532.40	N-O symmetric and asymmetric stretch	strong	Nitro compounds
S4(A)	1054.36	C-O stretch	medium-strong	Alcohols R-CH ₂ -OH (1°) or C=C-CH(R)-OH
	612.57	≡C-H bend	strong broad	Alkynes
S3(B)	1560.81	N-H bend	medium or strong	Amides
	1467.75	C-H bend	strong	Alkanes and Alkyls
S4(B)	1240.55	O=C-O-C stretch	strong-very strong	Esters Acetates

spectra with library data (NIST05.LIB) (ben Harb et al., 2020; Halim et al., 2014).

2.9. Electrochemical method

For electrochemical measurements, the WE is MS 1010 (surface area: 1.0 cm²), whereas the reference and counter electrodes are Ag/AgCl with KCl saturated calomel electrode (SCE) and a Titanium mesh, respectively. The electrolyte (medium) was obtained from the electrochemical experiment system and electrochemical polarization and impedance measurements were performed using the Metrohm Autolab

Version of Nova 2.1.5 software. The frequency range of the applied measurement was 100,000 to 0.1 Hz. The polarization curves were attained by scanning anodically and cathodically from the open circuit potential (OCP) to + 200 and 200 mV SCE at a scan rate of 0.001 V/s. In the AC input, the frequency number is 50, and the amplitude is 0.01 mV, after which the final R_{ct} readings are received. The Tafel polarization was set as a start -0.2 and 0.2 stop potentials and the scan rate was adjusted with a high (1 mA) to low (100 nA) current range by adding constant phase components to the relevant circuit (Kokilaramani et al., 2020).

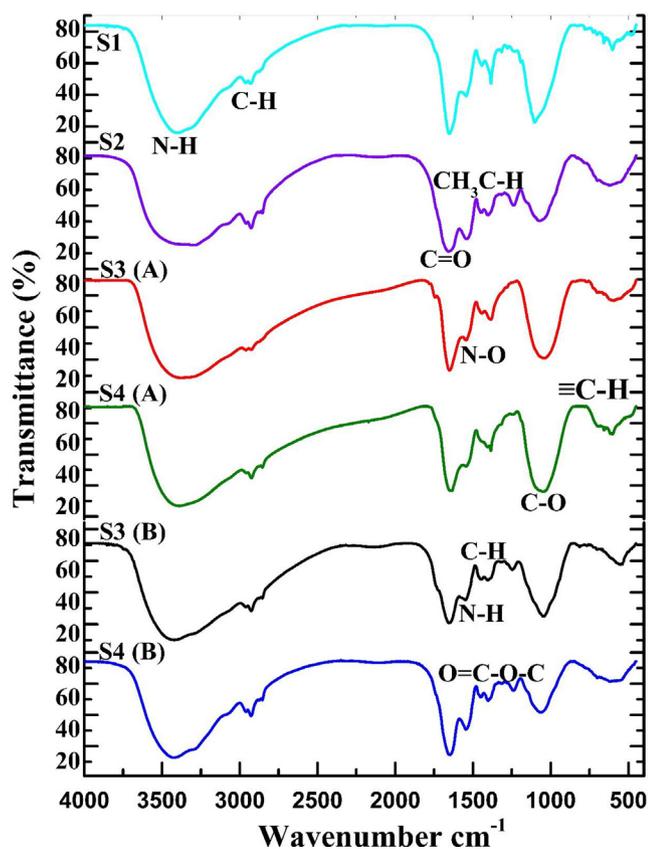


Fig. 2 FTIR analysis for MS 1010 coupons system.

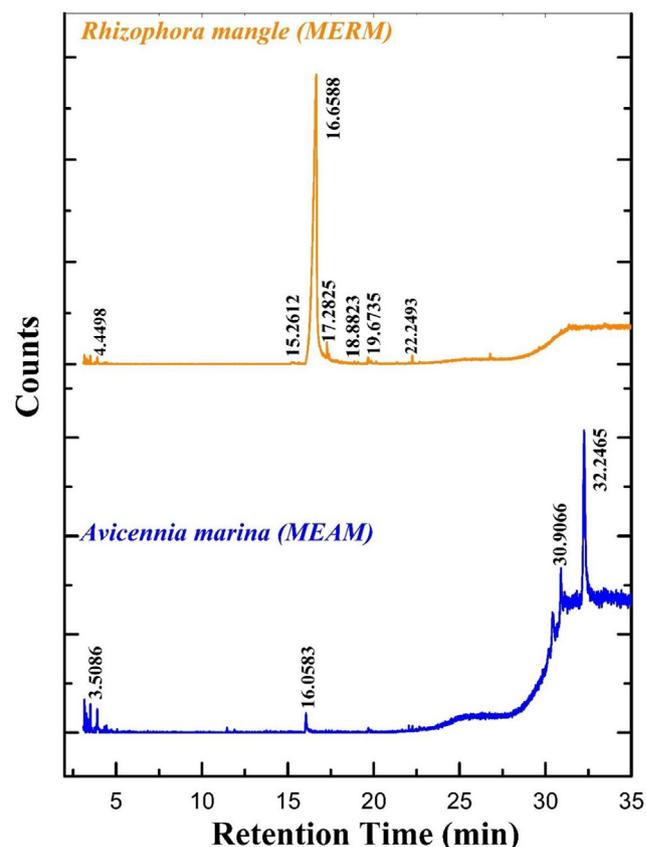
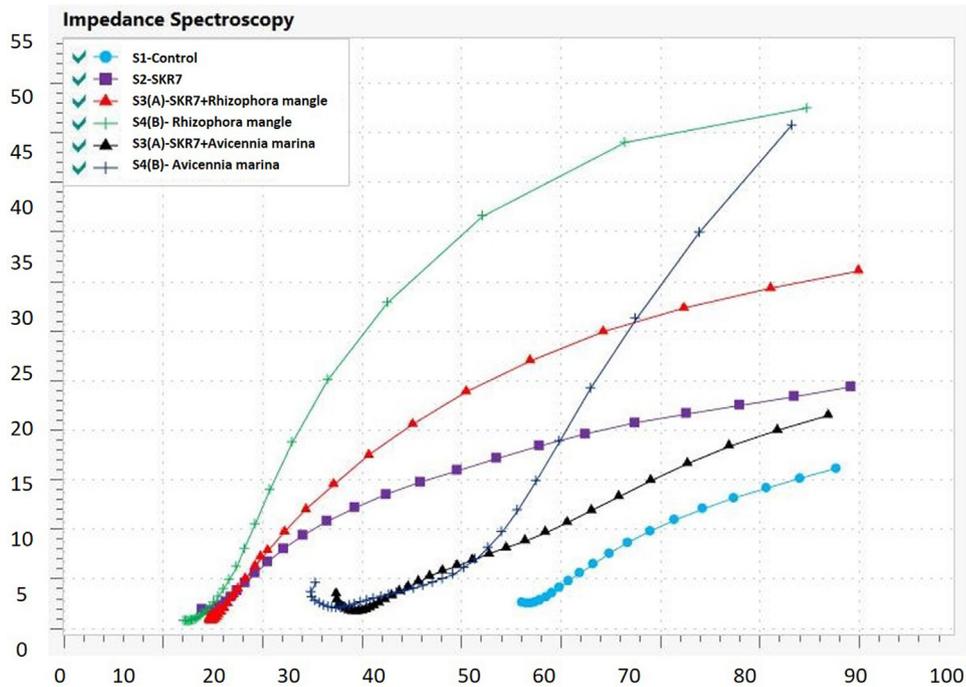


Fig. 3 GCMS analysis for green inhibitors of mangrove plant *Rhizophora mangle* and *Avicennia marina*.

Table 4 Electrochemical impedance parameters for MS 1010 coupons.

System	R_s (Ωcm^2)	R_{ct} (Ωcm^2)	CPE Y_0	CPE N
S1	32.9	116	0.006682	0.8561
S2	32.3	76.3	0.004856	0.5114
S3(A)	42.5	547	0.007907	0.5355
S4(A)	46.0	226	0.010707	0.2965
S3(B)	55.7	438	0.003155	0.4022
S4(B)	62.6	218	0.003393	0.3605

R_s – Solution Resistance; R_{ct} - Charge transfer resistance; CPE - Constant Phase Element; $Y_0 = C =$ The Capacitance; N - a constant value that is less than unity, $0 < n < 1$.

**Fig. 4** Impedance Spectroscopy for MS 1010 coupons collected from biocorrosion system.

3. Results

3.1. Minimal inhibition concentration (MIC) assay

The agar well diffusion technique was used to evaluate the minimal inhibitory concentration of MERM and MEAM leaf components. Various inhibitor doses (10, 25, 50, and 100 ppm) were tested, with MERM 2.92 ± 0.1 mm and MEAM 2.81 ± 0.1 mm of 25 ppm observed to be the most effective zone of inhibition. Both mangrove leaves were highly effective against the SKR7 bacterial growth. The total viable count (TVC) was revealed to be 25 ppm of MERM and MEAM suppressed bacterial growth in a random manner when compared to the other concentrations. In 25 ppm of MERM and MEAM, the lowest colonies were counted, whereas the control plate showed no colony growth.

3.2. Biofilm inhibition assay

For the biofilm inhibition assay, 25 ppm of inhibitors MERM and MEAM were employed with SKR7 strains in the biofilm studies using the crystal violet method in a 96-well microtiter plate. Biofilm development on well surfaces was greatly decreased by the presence of inhibitors MERM and MEAM at 25 ppm, this concentration was used for biocorrosion testing. The biofilm formation of SKR7 (S2) is 1.692, and the addition of 25 ppm of biofilm inhibitors MERM S3 (A) and MEAM S3 (B) is 0.534 and 0.619, respectively, according to the data shown in Fig. 1. The violet color fluctuation of wells is visible in the biofilm inhibition assay findings owing to the biofilm retaining crystal violet dye due to the high density of layers. It means that inhibitors can prevent the production of extracellular polymeric substances like proteins, carbohydrates, lipids, and so on (Ibrahim et al., 2021).

3.3. Biocorrosion studies

3.3.1. Weight loss measurement

The weight loss data for MS 1010 coupons placed in cooling tower wastewater with or without inhibitors along SKR7 were summarized in Table 2. Both the mangrove leaf (MERM and MEAM) inhibitors can inhibit the corrosion causing bacteria that have developed biofilm layers in cooling tower wastewater S3 (A) 80.33 ± 1 and S3 (B) 86.80 ± 1 and the S2 430.89 ± 1 sample revealed that the presence of SKR7 can induce severe corrosion on the MS surface. The efficiency of corrosion inhibition at 25 ppm of MERM is 78%, and 25 ppm of MEAM is 75%, respectively.

3.4. FTIR

The FTIR spectrum parameters of corrosion products including mangrove leaves (*R.mangle* and *A.marina*) are identical to alginate standards. Table 3 and Fig. 2 show the FTIR peak results for this experiment. The occurrence of biologically organic substances was validated then peaks were seen at 1054.36 cm^{-1} showing C—O stretch with the moderate intensity of Alcohols R—CH₂—OH (1°) or C=C—CH(R)—OH and 612.57 cm^{-1} indicates the $\equiv\text{C—H}$ bend with a wide, solid density of Alkynes groups are presented, and this resonant peak shows the bacterial suppression (Okeniyi et al., 2019a; Shahin Lefteh et al., 2021). As detected by a biofilm inhibition study, the presence of mangrove leaves compounds absorption with the metal surface while reducing bacterial development.

3.5. GC-MS analysis

The GC-MS analysis of methanolic extracts of mangrove leaves (*R.mangle* and *A.marina*) were identified substances provided in Fig. 3. In the GC-MS study of *R.mangle* the Myo-Inositol, 4-C-methyl was found at a significant constituent with a peak area of 94.36%, followed by 5,5,8a-Trimethyl-3, 5,6,7,8,8a-hexahydro-2H-chromene, Nonanoic acid, Cyclohexane, ethyl, Pyrene, Cyclohexane, 1,2-dimethyl-, trans, Hexane, 3-methoxy was a prominent ingredient. *A.marina* of GC-MS analysis the Lupeol, trifluoroacetate was significant constituent with a peak area of 65.35% followed by beta.-Amyrin, Hexane, 3-methoxy, Cyclohexane, 1,4-dimethyl were found to be a minor amount. To suppress microbial activity, the Myo-inositol, 4-C-methyl and Lupeol-based compounds are used. The found inhibitory compounds are used to minimize biofilm development and to regulate the corrosion process in the cooling water system (Khatab et al., 2012).

3.6. Electrochemical studies

3.6.1. Impedance spectroscopy

The results of electrochemical impedance measurements are shown in Table 4 and Fig. 4.

The charge transfer resistance (R_{ct}) of the experiential systems is S1 has $116 \text{ }\Omega\text{cm}^2$, while S2 has lower values $76.3 \text{ }\Omega\text{ cm}^2$ absorbs a huge amount of current owing to the addition of SKR7 indicates that the bacteria potentially cause deterioration of the metal, inhibitory system S3 (A), S4 (A) of MERM are $547 \text{ }\Omega\text{ cm}^2$, $226 \text{ }\Omega\text{ cm}^2$ and S3 (B), S4 (B) of

Table 5 Equivalent circuits for biocorrosion systems.

S1 – MS + CW (Control)			
Element	Parameter	Value	Estimated Error (%)
Rs	R	32.883	2.370
Rp	R	115.9	7.646
CPE	Y0	0.0066829	11.467
	N	0.85614	6.479
	χ^2	0.80532	
S2 – MS + CW + SKR7			
Element	Parameter	Value	Estimated Error (%)
Rs	R	32.294	2.559
Rp	R	76.322	8.266
CPE	Y0	0.0048562	19.362
	N	0.51146	9.474
	χ^2	0.48948	
S3 (A) - MS + CW + SKR7 + MERM			
Element	Parameter	Value	Estimated Error (%)
Rs	R	42.49	2.857
Rp	R	547.43	27.013
CPE	Y0	0.0079077	8.417
	N	0.53554	7.581
	χ^2	0.8695	
S4 (A) - MS + CW + MERM			
Element	Parameter	Value	Estimated Error (%)
Rs	R	46.014	5.198
Rp	R	226.09	53.855
CPE	Y0	0.010707	22.356
	N	0.29654	20.285
	χ^2	0.80767	
S3 (B) - MS + CW + SKR7 + MEAM			
Element	Parameter	Value	Estimated Error (%)
Rs	R	55.717	4.006
Rp	R	438.16	18.437
CPE	Y0	0.0031557	14.488
	N	0.40225	9.432
	χ^2	0.84624	
S4 (B)- MS + CW + MEAM			
Element	Parameter	Value	Estimated Error (%)
Rs	R	62.571	5.586
Rp	R	217.93	20.852
CPE	Y0	0.0033931	26.730
	N	0.36052	16.871
	χ^2	1.1008	

MEAM is $438 \text{ }\Omega\text{ cm}^2$, $218 \text{ }\Omega\text{ cm}^2$ correspondingly. The inclusion of mangrove leaves, MERM and MEAM, can reduce the current consumption level due to reduced corrosion rates because of the formation of protective layers that limit microbial adhesion on the metal surface. The green inhibitors prevent the microbe binding mechanism through a lone pair electron, hence preventing interaction of bacterial community and secretion of polymeric substances. The production of

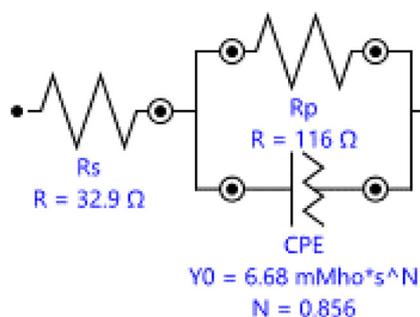
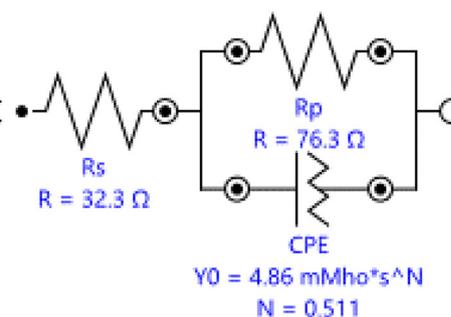
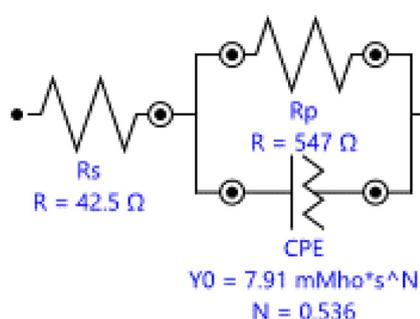
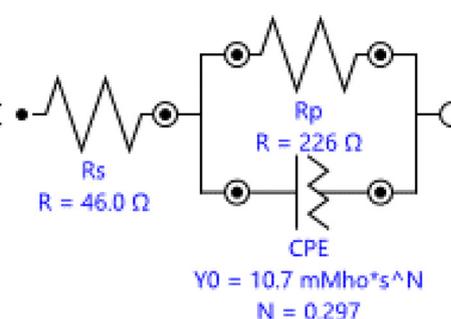
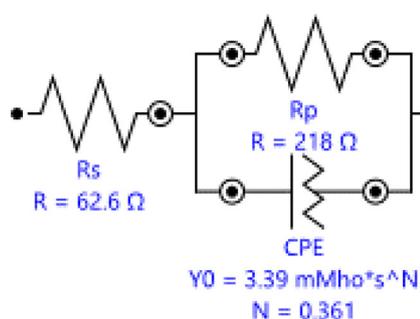
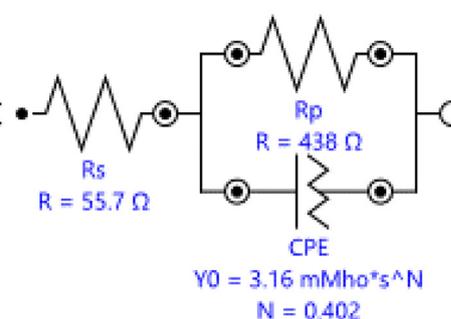
S1 – MS+CW (Control)**S2 – MS+CW+SKR7****S3 (A) – MS+CW+SKR7+MERM****S4 (B) - MS+CW+MERM****S3 (A) - MS+CW+SKR7+MEAM****S4 (B) - MS+CW+MEAM**

Fig. 5 Equivalent Circuit for MS 1010 coupons collected from biocorrosion.

insoluble complexes as a result of interaction between iron cations caused by adsorption at the electrode/solution interface is attributed to the mechanism of action.

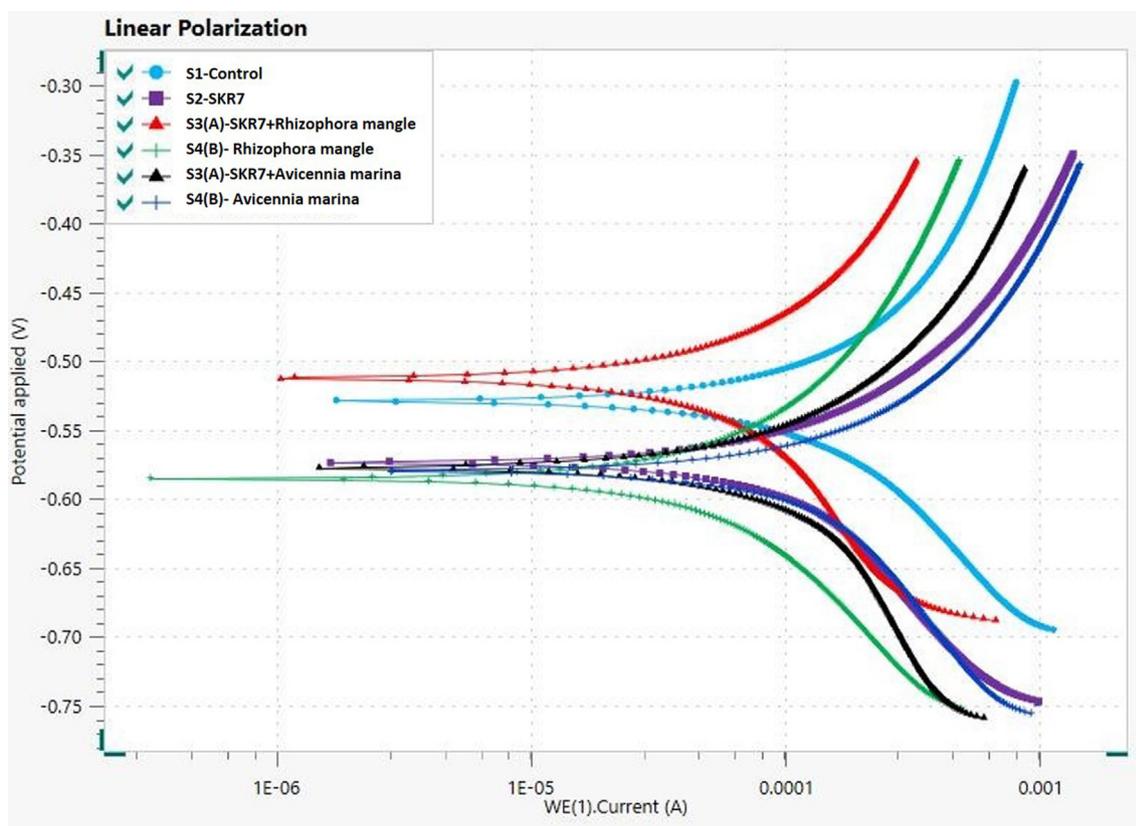
Both MERM and MEAM are mixed (anodic and cathodic) type inhibitors, according to the adsorption mechanism electron atoms of inhibitors (free lone pairs of electrons) such as nitrogen, oxygen, phosphorus and sulfur are vital in forming

protective layers on the metal products (vacant-d-orbital). Table 5 and Fig. 5 are the result of Equivalent Circuit for MS 1010 coupons. The polarization absorption of electrochemical coupon S2 rapidly lowers the circuit rate, whereas S3 (A) and S3 (B) increased. Constant phase elements (CPE) have become widely used for generating acceptable impedance spectra. (Pino et al., 2001).

Table 6 Potentiodynamic polarization parameters for MS 1010 coupons.

System	E_{corr} (V)	i_{corr} ($\mu\text{A}/\text{cm}^2$)	β_a (mV/dec)	β_c (mV/dec)	R_p ($\text{K}\Omega\text{cm}^2$)	Corrosion Rate (mm/year)
S1	-0.57946	2.2114	0.043291	0.046952	304.59	0.1647
S2	-0.59815	6.0514	0.028579	0.031072	211.88	1.8065
S3(A)	-0.58472	1.5649	0.054324	0.060331	793.28	0.1843
S4(A)	-0.57651	1.2044	0.026398	0.028673	495.63	0.2425
S3(B)	-0.51204	2.0875	0.062683	0.096332	790.02	0.3545
S4(B)	-0.5285	1.4187	0.02232	0.026475	370.9	0.3731

E_{corr} - Corrosion Potential; i_{corr} - Corrosion Current Density; β_a - Anodic Beta coefficient; β_c - Cathodic Beta coefficient; R_p - Polarization Resistance; mV/dec - Millivolt/Decade.

**Fig. 6** Linear polarization of electrochemical studies for MS 1010 coupons.

3.6.2. Linear polarization

The linear polarisation of MS 1010 coupons on cooling water is shown in Table 6 and Fig. 6. The bacterium SKR7 was tested on the MS coupons with and without inhibitors for anodic and cathodic polarisation curves.

The Tafel polarization method is used to evaluate the surface coverage (θ) values of inhibitors to know the corrosion inhibition mechanism and adsorption between mild steel and inhibitor (Preethi Kumari et al., 2017). The corrosion current density (i_{corr}) of S1 is $2.2114 \mu\text{A}/\text{cm}^2$, while S2 SKR7 has a $6.0514 \mu\text{A}/\text{cm}^2$ reached a high corrosion rate of 1.8065. The i_{corr} value was lower in the presence of green inhibitors S3 (A), (B) showing $1.5649 \mu\text{A}/\text{cm}^2$, $2.0875 \mu\text{A}/\text{cm}^2$ and the corrosion rate was partially controlled at 0.1843 and 0.3545. As a result, the inhibitors can prevent both biofilm growth as well

as corrosion. The S4 (A), (B) readings of i_{corr} are $1.2044 \mu\text{A}/\text{cm}^2$, $1.4187 \mu\text{A}/\text{cm}^2$ and corrosion rate is 0.2425 and 0.3731 respectively. The S2 E_{corr} and i_{corr} values have the potential to rise to result in pitting corrosion. Based on the values of activation energy and free energy of adsorption, the Langmuir adsorption isotherms appear to represent the mechanism of adsorption. MERM and MEAM have been identified as efficient biofilm inhibitors and preventers. MERM is the best inhibitor of inhibiting efficiency than MEAM (Kokilaramani et al., 2020).

4. Discussion

The researchers are successfully finding green inhibitors to inhibit metal corrosion in various environments. Gelatin, gram

flour, onion, organic honey, potato, plant roots, leaves, seeds, and floral gums have all been described as potential metal corrosion inhibitors (Argyropoulos et al., 2021). Mangrove plants have natural bioactive compounds that are effective against a variety of microbial pathogens and exhibit an alternate source of antimicrobial agents to combat biofilm formed by microbes. It has biochemically distinct, producing a varied spectrum of novel natural products as well as bioactive compounds that defend against microbial pathogens. The MERM and MEAM have been demonstrated to inhibit the activity of virulence factors such as LasA protease, LasB elastase, protease, and pyocyanin pigment. Furthermore, bioactive proteins deactivate bacteria's Quorum Sensing (QS) signals and prevent bacterial communication. (Marzorati et al., 2019). (Ejikeme et al., 2013; Haldhar et al., 2021; Michael, 2014; Okeniyi et al., 2019a; Perencatan et al., 2011; Shah et al., 2011; Tan & Kassim, 2011; Umoren et al., 2018) are all reported, that the mangrove plants worked against corrosion and electrochemical studies provide a good inhibition efficiency result and the SEM, FTIR, GCMS analysis are detailed studies about the inhibitors compound. It was the first report to use mangrove leaves extract to reduce bacterial corrosion on MS 1010 in cooling tower wastewater.

5. Conclusion

In conclusion, this was reported to examine the microbial corrosion inhibition of mangrove trees of *R.mangle* and *A.marina* leaves against the *B.megaterium* strain. MERM and MEAM inhibitors effectively reduce bacterial accumulation by inhibiting biofilm formation caused by forbidden cell-cell communication. According to the results of the FT-IR studies, extract inhibitors are primarily responsible for the adsorption on the metal surface and suppress bacterial growth, which leads to avoiding the corrosion process. A weight loss study has confirmed that the SKR7 bacterium generates high corrosion on metals and plant-based substances prevent corrosion through an adsorption mechanism in which metal d-orbitals interact with inhibitory lone pair electrons. The mechanism process is based on the formation of a layer between the external corrosive medium and the substrate, which is generally provided by the coating structure by its chemical contents. Controlling the transit of corrosive ions becomes important to postpone and, hence, avoid a corrosive reaction once the corrosive medium has penetrated the coating over time. In the results of GCMS, plant compounds exhibit excellent antimicrobial efficacy against microorganisms. EIS and Tafel plots indicated that inhibitors substance to adsorption with metals surface and suppress both anodic and cathodic sites. A comprehensive study concluded that plant-based inhibitors act as excellent antibacterial activity against corrosion-causing bacteria and prevent the corrosion process in the cooling water system.

CRedit authorship contribution statement

Seenivasan Kokilaramani: Writing – original draft, Methodology. **Jayaraman Narenkumar:** Validation, Writing – review & editing. **Mohamad S. AlSalhi:** Resources, Funding acquisition, Writing – review & editing. **Sandhanasamy Devanesan:** Validation, Formal analysis, Writing – review & editing. **Parthiba Karthikeyan Obulisamy:** Validation, Writing – review & editing. **Ramasamy Balagurunathan:** Writing – review & editing, Formal analysis. **Aruliah Rajasekar:** Project administration, Supervision, Validation, Writing – review & editing.

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Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable.

Consent for publication

All authors agreed to publish this version of the article.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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