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Effectiveness of an alkaloid fraction on carbon steel corrosion inhibition evaluated by green chemistry biotechnological approach

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Outline:

Introduction

Corrosion phenomena is a natural process that results in considerable waste of industrial investment. Corrosion inhibitors are common for protecting steel structures and their alloys in industry. This phenomenon can easily be found in different types of surfaces causing major economic losses in the industrial sector. Chemicals used as corrosion inhibitors are very toxic even in small concentrations, leading to environmental agencies requesting prohibition. Corrosion control involves different aspects such as environmental, economical and technical resulting in major advances of science and biotechnology. Considerable attention has been focused on corrosion inhibitor handling and its chemical residue aimed health and environmental safety. Hence, there is a growing demand for environmentally appropriate inhibitors such as vegetal inhibitors. It is estimated that more than 30 % of the steel produced worldwide is used for spare parts, pieces of equipment and facilities damaged by corrosion. The chemical, electrochemical or electrolytic corrosion processes are spontaneous, leading to modifications in the physicochemical characteristics of materials (Figure 4.1). Corrosion is costly due to any operational downtime necessary for parts replacement. There is also concern about damage to the environment for example the breaking of oil pipelines in the petroleum industry. Corrosion resistant products are in great demand and have been increasing in technological advances.

Recent studies have estimated that annual costs worldwide related to corrosion damage are around 4 % of the Gross Domestic Product (GDP) of an industrialized country [1]. Management practices and corrosion control can reduce 20 % of direct costs [maintenance (protection processes) and/or replacement of parts or equipment] or indirect, such as downtime due to equipment failure, product contamination, production losses and personal and also environmental safety [1,2].

We have been developing studies in green chemistry applied to corrosion phenomena using microemulsions as vehicles of plant extract or synthetic organic compounds aiming to lower the inhibitors concentrations without loss of effectiveness. Saponified coconut oil is a green chemistry surfactant as part of the microemulsion system applied on corrosion inhibition of carbon steel AISI 1020, in saline medium [3-6]. Microemulsions optimize solubilization of water insoluble organic compounds and plant extracts, increasing its adsorption potential due the presence of surfactant, and subsequent expansion of the surface area covered. The microemulsified saponified coconut oil MES-SCO (reported as OCS-ME) effectiveness on carbon steel corrosion inhibition process was evaluated using an electrochemical method of polarization resistance. This microemulsion system showed inhibitors effect 77 % at lower concentrations of the surfactant (0.5 %). Meanwhile, the free surfactant saponified coconut oil (SCO solubilized in H_2O) showed lower efficacy (63 % at 0.20-0.25 % of SCO concentration). The greatest inhibitory effect of MES-SCO was correlated with the rich o/w microemultion system which is very important for adsorption phenomena [3].

In this study, an alkaloid fraction (AF) obtained from the stem bark of the plant species *Croton cajucara* Benth (Euphorbiaceae) was evaluated as a green chemistry corrosion inhibitor. AF loaded in the green MES-SCO system was analyzed in corrosion inhibition of carbon steel AISI 1020, in saline medium. This was followed by a description of phytochemical aspects of AF and its antioxidant and bactericidal influences into MES-AF aiming to finding a more suitable inhibitor effective in both spontaneous corrosion processes and biocorrosion phenomena.

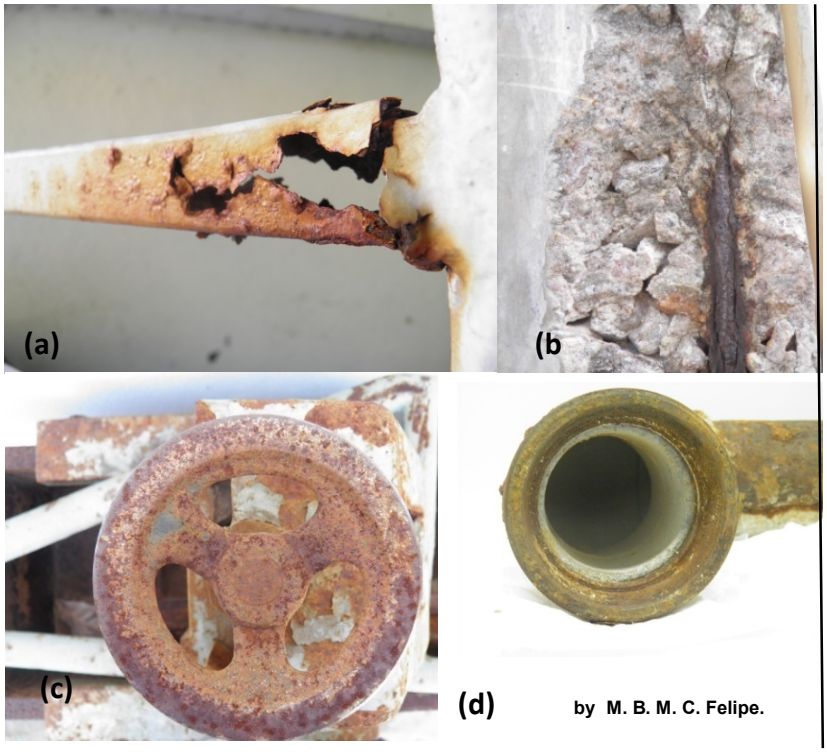


FIGURE 4.1
Metallic structures damaged by corrosion: (a) handrail; (b) metal structure inside a concrete block; (c) tap; (d) tube from an oil pipeline.

General

Great scientific interest has focused on the natural inhibitors from plant sources due to the significant microbiological control and inhibitive property on electrochemical corrosion. Academic researchers need studies using plant extracts as natural effective corrosion bioinhibitors, including biocorrosion process which is influenced by microorganisms. Behpour *et al.* [7] studied the effect of *Punica granatum* in acidic solution (HCl 2 M and H_2SO_4 1 M) through electrochemical impedance spectroscopy and potentiodynamic polarization technique, indicating that this extract could be used as excellent corrosion inhibitor. *Phyllanthus amarus* leaf extract has also demonstrated to protect carbon steel of corrosion deterioration [8]. Anticorrosive properties was also documented for other types of natural compounds *Zanthoxylum alatum, Lawsonia, Occimum viridis, Telfer occidentalis*, *Azadirachta indica* and *Hibiscus sabdariffa* extracts, in acid solution [9]. The methanol extract of *Artemisia pallens* showed 96.5 % of corrosion inhibition efficiency on steel exposed to a 4 N HCl solution [10], among other [12-13].

Atmospheric corrosion is a phenomena derived from condensation of moisture on the metal surface, similar characteristics such as varying of pH, temperature, medium chemical composition, aeration aspects and also the deterioration of a material due to microbiological activity commonly known as biocorrosion or as corrosion influenced by microorganisms (CIM), should all be considered [14]. Since

MIC phenomenon results from interactions between the metal surface with abiotic products in the presence of microbial cells and their metabolites, various environments are advantageous to microbial growth, thus many equipment are subject to biocorrosion [14]. The main types of bacteria associated with metals in aquatic or terrestrial habitat are sulphate reducing bacteria (SRB), sulfur-oxidizing bacteria, manganese-oxidizing bacteria, iron-oxidizing/reducing bacteria, bacteria secreting acids and organic sludge, and algae and fungi [15]. Examples of environments susceptible to microorganism attack include: seawater, rivers and cooling systems, wetlands, and soils containing salts or organic waste.

To assess the prospect of exploiting biomass extracts for the simultaneous control of chemical and microbiologically influenced corrosion, studies have shown that vegetal extracts acting as bifunctional inhibitors on MIC and electrochemical corrosion. The effect of the aqueous extracts of *Piper guineense* (PG) was appraised on low-carbon steel corrosion in acidic medium using gravimetric and electrochemical techniques. The agar disc diffusion method was employed to determine the biocidal effect of the extract on corrosion-associated sulfate-reducing bacteria (SRB), *Desulfotomaculum* species. PG was found to be an excellent inhibitor for both corrosion and SRB growth. The corrosion process was inhibited by adsorption of the extract organic matter on the steel surface, whereas the antimicrobial effect results from disruption of the growth and essential metabolic functions of the SRB [16].

An aqueous methanolic extract of the whole plant of *Artemisia pallens* has shown good antibacterial activity against *Pseudomonas aeruginosa* and *Shigella flexneri*. The crude extract also showed significant anticorrosive efficiency against mild steel, in acidic solution [17].

Hence, the investigation of antibacterial activity and antioxidant property of *Croton cajucara* Benth and its inhibiting action on corrosion aiming development of a green chemistry inhibitor are presented.

Material and Methods

Plant material

The stem bark of *Croton cajucara* Benth was purchased in the Amazon region of Brazil at the free market called Ver-o-peso (Belém, state of Pará) and was chemically identified by phytochemical experimental procedures using standard material [18]. Previously, Nelson A. Rosa performed a botanic identification of this specimen, in which a voucher specimen (no. 247) has been deposited in Herbarium of the Paraense Emílio Goeldi Museum (Belém, Brazil).

Hydroalcoholic extract of Croton cajucara Benth and its phenolic acids and alkaloid fraction

Extraction of the powdered bark (1 kg) of *Croton cajucara* was carried out with aqueous methanol in a Soxhlet apparatus for 48 hr. This hydroalcoholic extract was obtained after solvent removal. Phytochemistry approach was worked out according to previously procedures aiming the plant chemical authenticity and also isolation of phenolic acid compounds and its semi-synthetic derivatives as previously described [18,19]. To obtain the alkaloidal fraction (AF) the extract obtained with a yield of 9.2 % (92 g) was subjected to an open column chromatography on silica gel (230-80 mesh) and 64 fractions were obtained which were eluted with gradient polarity of the mixed solvent. The more polar fraction [eluted with methanol/water (9:1 to 8:2)] after work out in Sephadex and spectroscopic characterization, proved to be a rich source of isoquinoline alkaloids compounds. The total characterization of the natural compounds were performed by spectroscopic methods such as IR, UV,

MS, 1D and 2D-NMR (300 MHz), in which part of that such as alkaloidal fraction chemical identity and its characterization are first reported herein.

Determination of total antioxidant capacity by phosphomolybdenum method

The total antioxidant activity of AF in a water solution (AF-WS) was determined by green phosphomolybdenum complex formation. Triplicates of 100 µL of AF-WS (1 mg/mL of solvent) and standard (ascorbic acid) were added to 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 ºC for 90 min [20]. The absorbance of the solution was measured at 695 nm using a spectrophotometer (Shimadzu, UV-1650Pc) against blank after cooling to room temperature. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of solution.

Antimicrobial assay

Minimal inhibitory concentration (MIC) of *C. cajucara* Benth hydroalcoholic extract (CC-HE) was determined by broth microdilution method [21] using 96-well microtitre plates. The pre-inoculum culture consisted of a bacterial colony cultivated in 15 mL LB medium, for 16-18h at 37 °C at 180 rpm. After this period, 150 µL of pre-inoculum was transferred to 150 mL of LB and incubated at 37 °C at 180 rpm until optical density of 0.8 at 600 nm measured by UV-VIS (Quant, Biotek) was achieved. Concentrations of CC-HE extract were tested in the range of 0.06-1.0 mg/mL against gram-negative bacteria, *Escherichia coli* ATCC25822, *Chromobacterium violaceum*, *Pseudomonas aeruginosa* ATCC27853, and gram-positives, *Bacillus cereus* ATCC11778, *Enterococcus faecium* and *Staphylococcus aureus* ATCC25923 cultures. Ampicilin (2.0 µg/mL) e chloramphenicol (12.5 µg/mL) were used as positive controls and appropriate controls with no extracts and only solvent were done. The experiments were done in triplicate.

Microemulsion system approach

The microemulsion system MES-SCO was used as vehicle to dissolve the plant extract resulting in the MES-AF formulation. MES-SCO containing saponified coconut oil in lower concentration as surfactant and butan-1-ol as cosurfactant, was performed with large microemulsion region. Specifically, the MES-SCO was obtained from the titration methodology and mass fractions in pseudoternary phase diagram containing a mixture of 40 % C/S (cosurfactant/surfactant), 5 % of kerosene as the oily phase and 55 % of double-distilled water as the aqueous phase [3-6].

Efficiency of corrosion inhibition

The MES-AF formulation efficiency was evaluated in saline medium (3.5 % NaCl) by polarization resistance methodology using PGSTAT 300 potentiostat coupled with GPES version 4.9 software aiming calculate corrosion parameters. An electrochemical cell consisted of a reference electrode (Ag/AgCl), a graphite auxiliary electrode, and a working electrode was used. The working electrode was constructed using a cylindrical piece of carbon steel AISI 1020 with exhibition area of 1.77 cm^2. The concentrations of the tested alkaloidal fraction ranged from 50 to 400 ppm. The polarization curves were recorded at a scanning rate of 0.05 V/min with varying potential from the observed open circuit potential after one hour of immersion. The corrosion parameters were obtained by extrapolating the Tafel curves and

used to calculate corrosion inhibition efficiency (IE) according to the following equation:

$$IE(\%) = \frac{Ecorr - Ecorr(inh)}{Ecorr}$$

where Ecorr and Ecorr(inh) are the corrosion potentials of the steel coupons in the absence and presence of MES-AF, respectively.
To evaluate the adsorption process of MES-AF on the metal surface, Langmuir and Frumkin adsorption isotherms were obtained by the equations presented in Table 4.1 [22].

TABLE 4.1
Equations for the isotherms of Langmuir and Frumkin.

Isotherm	Equation
Langmuir	θ/(1- θ) = KC
Frumkin	Log (θ/(1- θ).C) = logK + g θ

The surface coverage (θ) was calculated from the rates of corrosion inhibition obtained by polarization resistance data at 25 °C, in which "C" represents the inhibitor concentration in ppm and "K" is the adsorption equilibrium constant. Finally "g" is the degree of lateral interaction among the adsorbed molecules.

Results and Discussion

The species *Croton cajucara* Benth (Figure 4.2) commonly known as *sacaca* (which means spell in a specific indigenous language) is a native tree (4 to 6 m tall) from Amazon region of Brazil. Studies point out that the stem bark (Figure 4.3) of this plant is largely used as tea or pills to combat diabetes, diarrhea, stomach upset and to control high levels of cholesterol [23]. Despite the enormous potential of plants around the world only a minor fraction of globe's living species has ever been tested for any bioactivity. This is not the case of *Croton cajucara* Benth with has a large historic of multidisciplinary researches, confirming its medicinal properties on a progressive biotechnological development. There is a number of *Croton cajucara* Benth devoted to its chemical, biochemical, pharmacological and potential advantages of molecular incorporation into drug delivery systems [23-26]. Phytochemical studies carried out with this specie had involved plants ageing from 1 ½ to 6 years old (native and cultivated plants) showing stem barks as a rich source of clerodane-type diterpenes. Among them, the biocompound *trans*-dehydrocrotonin (DCTN) detected in trees ageing up to 3 years old, which have shown pharmacological results that lead to the phytotherapy validation of *Croton cajucara* Benth. The encapsulation of DCTN in liposomes enhanced its antitumor activity [26].

FIGURE 4.2
Croton cajucara Benth.

FIGURE 4.3
Stem bark of *Croton cajucara* Benth.

Ongoing studies with *Croton cajucara* Benth using classical column chromatography were performed with an aqueous methanol extract. *Croton* fractions eluted with a mixture of hexane/EtOAc (6:4 to 1:1) affording a mixture of phenolic acids (vanillic acid and 4-hydroxy-benzoic acid) and an aminoacid (Figure 4.4) identified as N-methyltyrosine [white powder; mp 240-241 ^{o}C; crystallization from MeOH/H_2O/HCl (8:1.5:0.5); its TLC analysis were performed using n-BuOH/Me_2CO/AcOH/H_2O (2.0:3.5:3.5:1.0), detection performed with ninhydrin reagent, R_f 0.4]. The phenolic acids mixture was subjected to preparative TLC using hexane/EtOAc (6:4) as eluted solvent (it was eluted five times, R_f 0.5) to yield vanillic acid (white needles, mp 209-210 ^{o}C) and 4-hydroxy-benzoic acid (white needles, mp 214-215 ^{o}C).

The polar alkaloid fraction (AF) also obtained from this aqueous methanol extract of *Croton cajucara* was eluted with methanol/water (9:1 to 8:2). In that, alkaloid-type compounds were detected by Dragendorff reagent applied in TLC and also by ^{1}H NMR spectrum analysis. Structure elucidation of those compounds was achieved by spectroscopic measurements including NMR experiments revealing in the AF fraction the presence of isoquinoline alkaloids mixture such as magnoflorine (Figure 4.5) and N,N-dimethyl-lindicarpine (Figure 4.6). This isoquinoline alkaloids mixture presented in the AF fraction, showed b.p. over 280 ^{o}C; TLC-analysis eluted with $CHCl_3$:MeOH:H_2O (6.5:5.0:1.0) Rf=0.5; IR absorptions at 3451, 2922, 1655, 1154 cm^{-1}. ^{1}H NMR spectrum of AF fraction, recorded at 300MHz (CD_3OD/D_2O) allowed hydrogen attribution of magnoflorine: H8 [6.94 d (*J* = 8.0 Hz)]; H9 [6.83 d (*J* = 9.0 Hz)]; H3 [6.58 s]; H6a [3.77 broad t (*J* = 9.8 Hz)], H5 (2.29 – 3.33 m); H7e (2.82 m); H4e (2.55 broad d); H7a (2.41 broad t); OH (9.34 s); OCH_3 - C2 (3.90 s); OCH_3 - C10 (3.89 s); N^+-CH_3B (3.44 s); N^+- CH_3A (3.08 s) and for N,N-dimethyl-lindicarpine: H8 (7.31 d); H9 (7.24 d); H3 (6.83 s); H5e (4.10 m); H7 (3.66 m); H6a (3.68 m); H5a (2.85-2.83 m); H4 (2.85-2.83 m); OH (9.51 s); OCH_3 (3.90 s); OCH_3 (3.89 s); N^+-CH_3 A (3.26 s); N^+-CH_3B (3.44 s).

The structures of the phenolic acids (vanillic acid, 4-hydroxy-benzoic acid and N-methyltyrosine) were identified as previously described [19] using 300 MHz NMR and MS experiments and for the vanillic acid its chemical transformation with diazomethane give the two methylated derivative esters I and II (Figure 4.4). ^{1}H and ^{13}C NMR (DMSO/DCl) data of N-methyltyrosine were in accordance with the authentic sample of 2-amino-3-(4-hydroxyphenyl) propanoic acid (known as tyrosine) obtained from commercial material. The different observed peaks were assigned to the N-Me group of N-methyltyrosine [19].

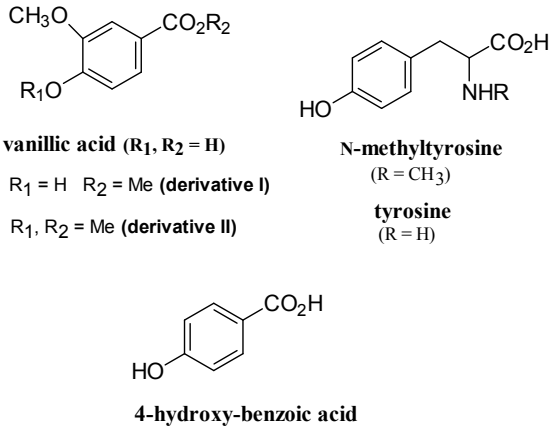


FIGURE 4.4
Chemical structure of phenolic acids from the aqueous methanol extract of *Croton cajucara* Benth.

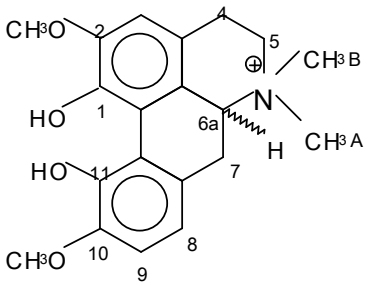


FIGURE 4.5
Chemical structure of the alkaloid magnoflorine obtained from AF fraction.

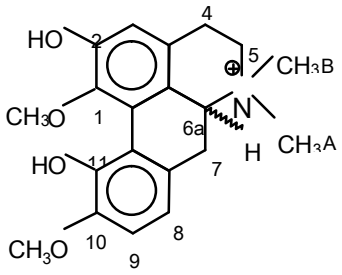


FIGURE 4.6
Chemical structure of the alkaloid N,N-dimethyl-lindicarpine obtained from AF fraction.

The microemulsion system MES-SCO was obtained from the titration methodology and mass fractions in pseudoternary phase diagram containing saponified coconut oil (SCO) in lower concentration as surfactant and butan-1-ol as cosurfactant (C/S=1) showed a large microemulsion region (Figure 4.7). The quinoline alkaloid fraction AF loaded in MES-SCO resulted in a MES-AF formulation which was evaluated in the presence of carbon steel AISI 1020, in saline medium.

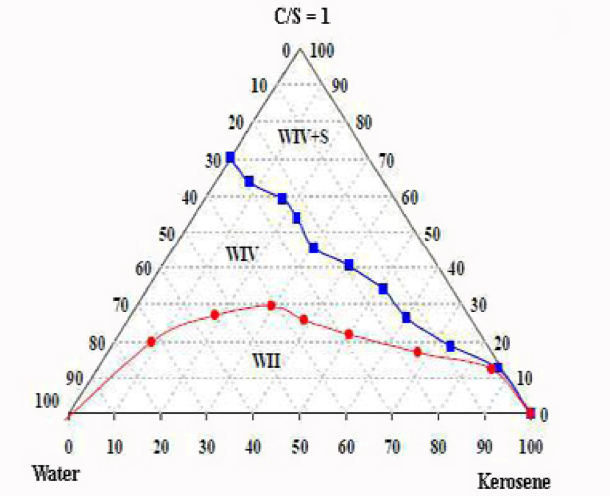


FIGURE 4.7
Schematically representation of MES-SCO system.

The effective solubilization of the alkaloidal fraction (AF) in the microemulsion system (MES-SCO) was evaluated by measurement of absorbance in ultraviolet region, being observed that volume of 1 mL of MES-SCO dissolved 10.50 mg of AF fraction. Surface tension measurements of MES-AF were taken at constant temperature of 25 °C varying the concentration of the system in two different medium such as distilled water and saline solution (3.5 % NaCl). The Critical Micellar Concentration (CMC) was estimated in the region where the curve changed abruptly, corresponding to saturation of the surface. The CMC value calculated was 0.0148 mol/L in pure distilled water and 0.0084 mol/L in saline solution. Scattering coefficients remained constant with approximate values of 38.60 (pure water) and 38.00 mN/m (saline solution) and surface tensions were plotted against small concentrations of surfactant (Figure 4.8). The observed results suggested satisfactory stability of the micellar system since the surface tension decreases with increasing concentration of MES-AF without abrupt changes in the structure of micelles after reaching the CMC.

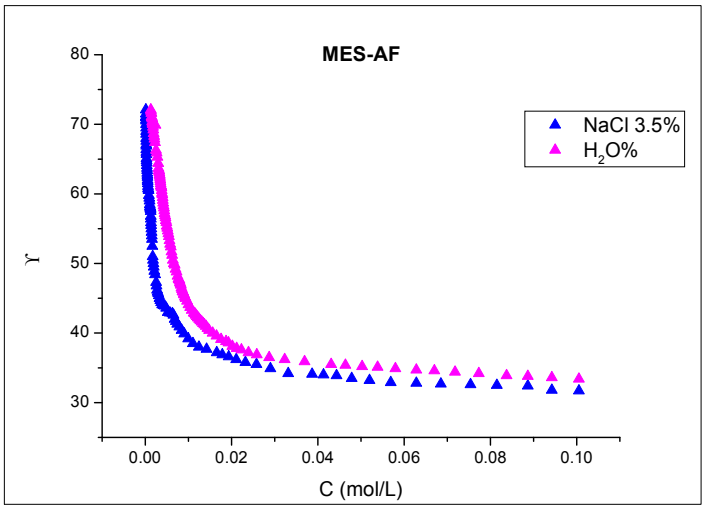


FIGURE 4.8
Surface tension of saponified coconut oil in the micellar system MES-AF in pure distilled water and saline solution.

The total antioxidant activity is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity can be calculated by the method described by Prieto *et al.* [20]. In the ranking of the antioxidant activity obtained by this method, the isoquinoline alkaloid fraction in a water solution (AF-WS) showed higher phosphomolybdenum reduction, followed by MES-AF and MES-SCO as shown in Figure 4.9. This study reveals that the antioxidant activity of the alkaloid mixture presented in the AF fraction, increase with the increasing concentration of AF regardless of the type of solution, which is 0.2 mg/mL (200 µg/mL) for AF-WS. Therefore, AF solubility evaluation on MES-SCO was of limited solubility 10 mg of AF/1 mL of MES-SCO affording the tested MES-AF formulation applied in the corrosion experiments. Water dilution of MES-AF resulted in 2.06 µg/mL of the load AF. For the antioxidant analysis MES-AF showed poor efficiency as evidenced in Figure 4.9.

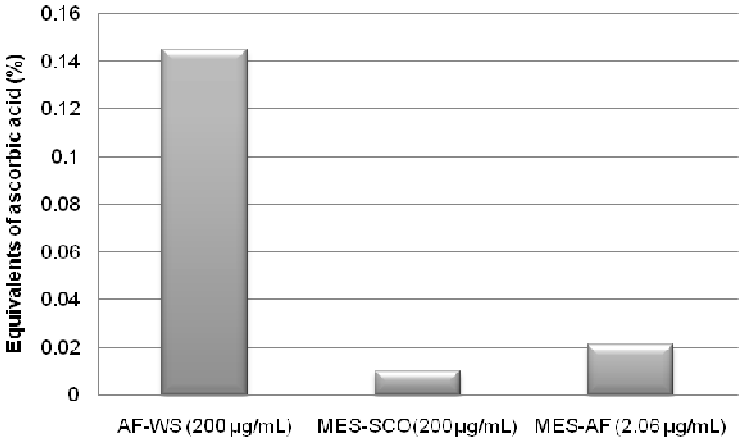


FIGURE 4.9
Antioxidant activity of AF expressed as the number of equivalents of ascorbic acid.

Previously, we investigated the effectiveness of the hydroalcoholic extract of the plant species *Croton cajucara* (CC) dissolved in the microemulsion system MES-SCO (reported as MES-CC) as well as dissolved in DMSO, as corrosion inhibitor on carbon steel AISI 1020 in saline medium. Comparatively, in that study, according to the obtained results using a potentiodynamic technique and Tafel extrapolation, the maximum inhibition efficiencies were observed for plant extract loaded in MES-SCO (93.84 %) with predominant control of cathodic reaction [4].

Generally, a specific inhibitor is classified as effective when electric current that flows in a given specific system is significantly reduced [1-5,9]. In the present work, Figure 4.10 shows the polarization curves for carbon steel in NaCl 3.5 % in the absence or in the presence of AF loaded in the microemulsion formulation MES-SCO (named MES-AF). The observed results showed that MES-AF systems (AF loaded in different concentrations) presented displacement of the corrosion potential for positive values which is correlated with concentration increases of AF dissolved in the microemulsion system MES-SCO.

The corrosion inhibition efficiency (IE%) was obtained from extrapolation of Tafel region (Table 4.2). The increase in concentration for the system evaluated (MES-AF) caused a reduction in current densities, indicating that the inhibitor are acting on the steel surface, slowing down the corrosion process. Conclusively, corrosion rates calculated proved MES-AF as effective in inhibiting corrosion of mild steel in brine (maximum efficiency 92.20 %).

Generally, the application of organic and inorganic corrosion inhibitors is one of the most common practices for the protection of steel structures and their alloys in industry. Inhibitors are mostly organic compounds rich in nitrogen, sulfur and oxygen atoms, and aromatic type-compounds [1-5,9]. Plant extracts rich in alkaloids, phenolic substance, terpenoids, or biomolecules (carbohydrates, lipids and proteins) also act as corrosion inhibitors [11]. Considering the chemical structures of these components, the effect of MES-AF in corrosion inhibition may result from AF as well as a synergistic effect of the MES-SCO.

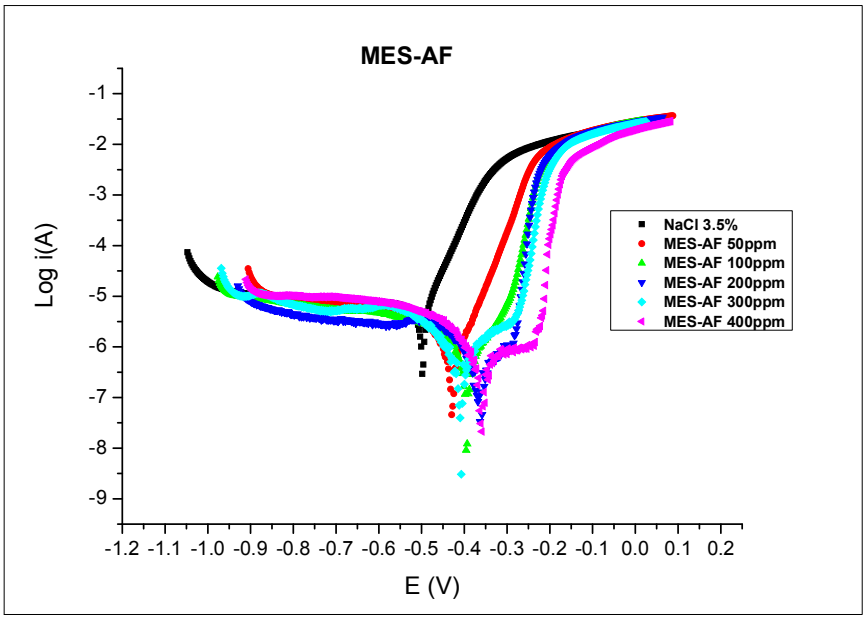


FIGURE 4.10
Tafel plots for carbon steel AISI 1020 in 3.5 % NaCl solution, ranging concentrations MES-AF.

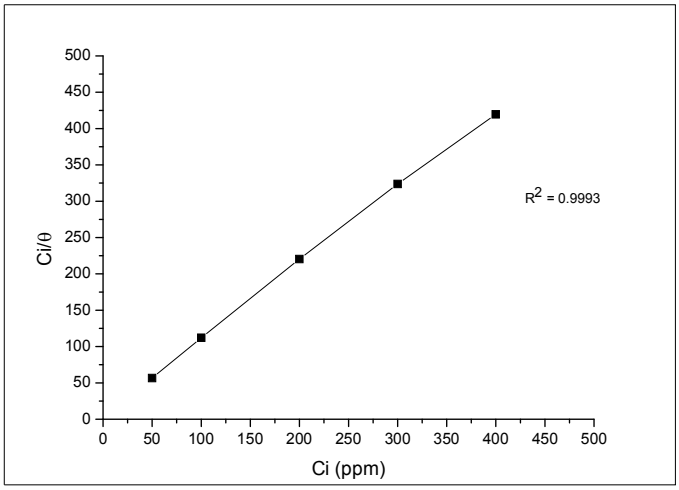
Concentration (ppm)	I_{corr} (A/cm)	IE (%)
0	2.40 x 10^{-5}	0
MES-AF		
50	4.18 x 10^{-6}	82.60
100	3.14 x10^{-6}	86.92
200	3.27 x10^{-6}	90.12
300	2.02 x10^{-6}	91.58
400	1.87 x10^{-6}	92.20

FIGURE 4.2
Parameters obtained from polarization curves for carbon steel AISI 1020 in saline solution containing MES-AF.

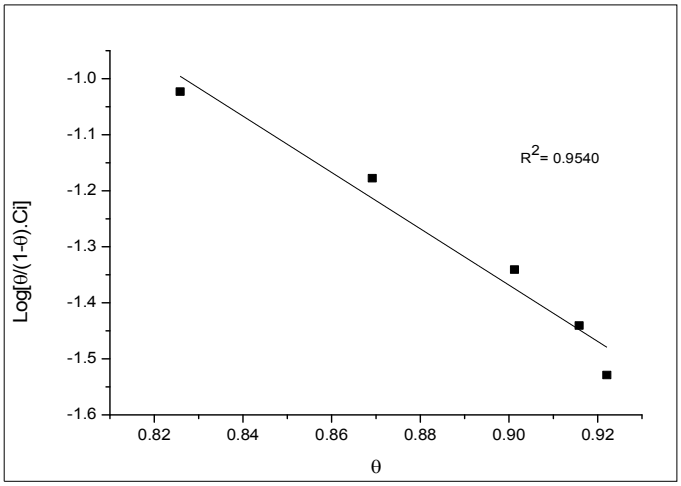
Concerning to the adsorption phenomena, isotherms were plotted in order to evaluate the adsorption behavior of the microemulsion MES-AF to the metallic surface. From isotherm equations it was possible to estimate the value of adsorption equilibrium constant (K), which leads to the calculation of the standard free energy of adsorption (ΔGads), using the equation,

$$K = \frac{1}{55.5} \times e^{-\Delta G_{ads}/RT}$$

The value of 55.5 refers to the concentration of water in mol / L, and the equilibrium constant adopted for the calculation of the ΔGads was Langmuir isotherm, since it is more consistent with the usual equilibrium constant [22]. For MES-AF, two adsorption isotherms were tested: Langmuir and Frumkin. The best fit was obtained for the Langmuir isotherm (R^2 = 0.9993) (Figure 4.11a), suggesting a model for the adsorption of the inhibitor adsorption phenomena on the metal surface occurs with monolayer. The heat of adsorption (ΔGads) calculated was -6.14 kJ/Mol (MES-AF), the negative value indicates that the process is exothermic and spontaneous, as well as considered a physical process since is under 20 kJ/Mol. As suggested above, inhibitor probably promotes the formation of a protective film on the metal surface acting as a barrier to the transfer of mass and charge.



(a) Langmuir



(b) Frumkin

FIGURE 4.11
Isotherms (a) Langmuir e (b) Frumkin for carbon steel at 25 ° C exposed to 3.5 % NaCl solution and MES-AF.

Concerning to the tested microorganisms, the results of the bioassays showed that the *C. cajucara* hydroalcoholic extract exhibited positive antimicrobial activity against all of the tested microorganisms, except *E. faecium* and *P. aeruginosa* cells. The Gram-negative *Chromobacterium violaceum*, and gram-positives, *Bacillus cereus* ATCC11778, and *Staphylococcus aureus* ATCC25923 were inhibited only in higher concentrations of the extract (MIC value 2.0 mg/mL) while gram-negative *E. coli* showed to be more affected when treated with lower concentration of *C. cajucara* (MIC values 0.06-0.25 mg/mL) (Table 4.3). Solvent controls results are shown on Table 4.4.

The mechanism of action of *C. cajucara* hydroalcoholic extract as an antimicrobial agent is not known. Antimicrobial agents can act by different manners, such as interfering in cell membrane wall, protein synthesis inhibition, nucleic acid synthesis blockage, metabolic pathway inhibition and others [27]. The inhibitor effect observed for CC-HE can be attributed to the presence of alkaloids and/or terpenoids in the hydroalcoholic extract mainly due to their cytotoxicity generated by interaction with cell membrane [28,29].

TABLE 4.3
Bacteria inhibition rate (%) by *C. cajucara* hydroalcoholic extract (CC-HE).

CC-HE (mg/mL)	Gram-Negative		Gram-positive			
	Ec	*Cv*	*Pa*	*Bc*	*Ef*	*Sa*
NC	0.00±0.0	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.00
0.06	67.32±3.70	6.20±2.93	n.i.	n.i.	n.i.	n.i.
0.12	51.35±9.17	7.01±0.90	n.i.	n.i.	n.i.	n.i.
0.25	55.84±2.55	12.81±2.14	n.i.	n.i.	n.i.	n.i.
0.50	48.08±6,14	12.14±3.25	n.i.	n.i.	n.i.	n.i.
1.00	49.04±3.54	24.72±2.89	n.i.	n.i.	n.i.	n.i.
2.00	50.47±2.26	72.31±6.31	n.i.	76.55±15.28	n.i.	61.32±2.04
PC	98.18±0.16	95.76±0.23	96.43±2.19	90.18±0.03	94.74±1,70	87.16±1.10

Ec, Escherichia coli, Cv, Chromobacterium violaceum, Pa, Pseudomonas aeruginosa, Bc, Bacillus cereus, Ef, Enterococcus faecium, Sa, Staphylococcus aureu.s. NC: Negative Control, PC: Positive Control , n.i.: No inhibition.

TABLE 4.4
Bacteria inhibition rate (%) by DMSO.

DMSO (mg/mL)	Gram-negative			Gram-positive		
	Ec	*Cv*	*Pa*	*Bc*	*Ef*	*Sa*
NC	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.30	n.i	7.40±2.16	n.i	n.i	8.13±2.88	4.83±4.79
0.60	n.i	9.91±1.99	n.i	n.i	13.99±3.96	3.33±0.25
1.25	n.i.	6.09±2.66	n.i.	n.i.	11.38±3.46	5.39±0.91
2.50	n.i	14.98±1.90	n.i	n.i	21.71±4.88	6.29±1.82
5.00	11.11±5.16	41.75±3.67	23.68±8.69	n.i	25.02±1.78	23.77±2.02
10.00	63.04±1.80	84.95±0.23	88.76±4.21	n.i	94.14±2.13	40.37±2.26
PC	96.93±0.18	96.31±0.34	92.97±7.01	90.73±2.83	99.52±0.20	89.36±2.52

Ec, Escherichia coli, Cv, Chromobacterium violaceum, Pa, Pseudomonas aeruginosa, Bc, Bacillus cereus, Ef, Enterococcus faecium, Sa, Staphylococcus aureu.s. NC: Negative Control, PC: Positive Control, n.i.: No inhibition.

Conclusions

The microemulsion system MES-SCO containing saponified coconut oil (SCO) as surfactant dissolved effectively the alkaloid fraction (AF) which was obtained from the stem bark of *C. cajucara* Benth, affording the load system MES-AF. The critical micelle concentration (CMC) of MES-AF occurs at low surfactant concentrations in pure distilled water, and brine (3.5 % NaCl). MES-AF showed good inhibition efficiency of 92.20 % with AF load at low concentration (0,4 mg/mL of MES-SCO). Comparatively, AF missing system (MES-SCO) showed lower efficacy (77 %). The higher efficiency observed for MES-AF may be explained by the presence of the aromatic rings and heteroatoms of the alkaloidal components with conjugated double bonds extended to methoxyl and hydroxyl groups in these organic structures, enhancing the adsorption of MES-AF. In fact, it is known that organic inhibitor molecules with N, O and S atoms as well with carge and π-electrons increases the adsorption in metal surface.

The *Croton cajucara* hydroalcoholic extract was shown to be rich in compounds containing functional eletronegative groups, aromatic rings and π-electrons in conjugated double bonds exhibited both antibacterial activity and quantitative corrosion inhibition (93.84 % with predominant control of cathodic reaction [4]).

To develop green and eco-friendly corrosion inhibitors with low cost, the green microemulsion system MES-AF containing the very cheap saponified coconut oil as surfactant and the polar fraction AF from

Croton cajucara hydroalcoholic extract, represent a promising bifunctional bioproduct against electrochemical corrosion and biocorrosion.
Reinforcing this suggestion, the polar fraction AF is a rich source alkaloid compounds (magnoflorine and N,N-dimethyl-lindicarpine) and the whole hydroalcoholic extract from the stem bark of *Croton cajucara* (from which AF was obtained) present remarkable antioxidant aromatic acids (vanillic acid, 4-hydroxy-benzoic acid and N-methyltyrosine) [23].
These results show the *Croton cajucara* polar extract load into MES-SCO to be a great biotechnological product of strong ecological importance, because the main components of MES-AF are obtained from renewable, biodegradable, easily obtainable and low cost components.

Acknowledgements

The authors are grateful for the financial support provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Agência Nacional de Petróleo, Gás Natural e Biocombustíveis (ANP) and also Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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