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Antibacterial activity and corrosion inhibition of mild steel in 1.0 M hydrochloric acid solution by *M. piperita* and *M. pulegium* essential oils

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Abstract

The inhibitive effect of *M. piperita* and *M. pulegium* essential oils on the corrosion behavior of mild steel in 1M HCl was studied using the weight loss measurements, polarization curves, and electrochemical impedance spectroscopy techniques. The inhibition efficiency was found to be dependent on the concentration of the essential oil obtained from both plants. Results showed that these essential oils act as a mixed-type inhibitor. The charge transfer resistance increases, while the double layer capacitance decreases by increasing the essential oil concentration of both plants. The presence of such essential oil may have enhanced their adsorption on the metal surface and thereby blocked the surface and protected the metal from corrosion. The results of the electrochemical methods were in good agreement with the weight loss measurement results. These essential oils antibacterial activity was evaluated using the microdilution method against one gram positive and one Gram negative bacteria. *M. piperita* and *M. pulegium* essential oils showed satisfactory activities against *S. epidermis, E. coli DH5α.*

1. Introduction

Mild steel is a unique metal among other metallic materials. This is due to its wide applications and its usefulness in many areas such as: domestic, services, construction, marine, industrial and engineering purposes. However, it has a challenge of being subject to corrosive degradation by time. The use of chemical inhibitors is one of the means to mitigate this destructive phenomenon. Chemical inhibitors are chemical compounds that are adsorbed on the metal surfaces to control, prevent and/or minimize the destructive corrosion reactions.

Green inhibitors are the plant extracts that are used for corrosion inhibition of metals/ alloys in different test environments. Many researchers have recently shown interest in the use of these extracts for corrosion inhibitive control [1-5]. Corrosion inhibitive effect of the extracts of plants has been attributed, in many cases, to the various complex chemical constituents of the extracts such as tannins and polyphenols [6-10]. These extracts of plants used as inhibitors are environment friendly.

Essential oils are composed of a number of compounds of different biosynthetic origins ranging from terpenoid hydrocarbons to sulfur compounds [11], and such compounds are naturally present in different concentrations [12]. The essential oils of the *M. piperita* and *M. pulegium* have a high commercial value [13].

Within the broad spectrum of the essential oils action, one can highlight the study of their antibacterial activities [14]. Several methods have been used with the intention of obtaining products uncontaminated by these microorganisms. Essential oils, constitute a viable alternative, because of their low molecular weight, are volatile and usually exhibit low toxicity to mammals. Bacteria have caused many problems mainly due to the proliferation of resistant microorganisms that represent a major threat to public health and to the success of antibacterial treatment. Recently, the emergence of antibiotic-resistant bacterial strains has increased dramatically, so that the search for natural substances to control micro-organisms is of paramount importance [14-16]. The objectives of this study were firstly, to determine the chromatographic analysis of the extracts oils of *M. piperita* and *M. pulegium*. Secondly, to evaluate their antibacterial activity and finally, to investigate them as a corrosion inhibitors for mild steel in 1.0 M HCl solution using the weight loss measurements, polarization curves, and electrochemical impedance spectroscopy methods.

2. Materials and Methods

2.1. Inhibitors

2.1.1. Plant material

Pouliot mint (*Mentha pulegium* L.), locally known as "Fliou", is also known as pouliot, royal pouliot, flea grass, chip hunting, Saint Laurent grass or fretillet. It grows in the humid areas of plains and mountains up to 2200 meters of altitude. Belonging to the Labiatae family, *Mentha pulegium* is an herbaceous plant that measures 10 to 55 cm of high. Its stems are square section, upright or lying and densely ramified. Flowering occurs from July to the end of September and flowers are pinkish-purple with four lobes (Figure 1).

Figure 1. The *Mentha pulegium* plant

The peppermint (*Mentha piperita*), local name: "Naanaa l'abdi", is also called English mint. It is a sterile hybrid, resulting from a cross between *Mentha aquatica* and *Mentha viridis*. This plant grows in all wet and fresh grounds of clay and natural limestone. It is often found at the edge of ditches or streams and in the woods. It grows in temperate and subtropical zones, and propagates by stolon. It measures 10 to 55 cm of high. Originally from the Middle East, this perennial plant with rhizome tracing and short purple quadrangular stems has a flowering period between August and September depending on the region (Figure 2).

Figure 2. The *Mentha piperita* plant

The fresh aerial parts of *Mentha piperita* and *Mentha Pulegium* were harvested respectively from the National Institute of the Medicinal and Aromatic Plants (NIMAP) garden in Taounate city (34°32′11″ N, 4°38′24″W, Altitude: 600 m) and Oued Laou region (35°26'24" N, 5°4'48" W, 69 m altitude) in Morocco. The botanical identification was performed, then voucher specimens were deposited at the Herbarium of National Institute of the Medicinal and Aromatic Plants, Morocco.

2.2. Essential oils extraction

The fresh aerial parts of *M. pulegium* and *Mentha piperita* (leaves and stems) were hydro-distilled for 3 hrs. using a Clevenger-type apparatus. The essential oils collected were kept in dark at 4° C for further use.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis conditions

The essential oils were analyzed using GC-MS (Polaris Q ion trap MS) according to the protocol previously described by Chraibi et al. [17]. Hence, analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (flame ionization detector), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30 m ×0.25 mm ×film thickness 0.25 µm). The temperature was programmed from 50 °C after 5 min initial hold to 200 °C at 4 °C/min. Chromatography carrier gas was N2 (1.8 mL/min); split mode was used with a flow rate of 72.1 mL/min and a ratio of 1/50. The temperature of injector and detector was 250 °C with final hold time of 48 mins. The machine was led by a computer system type "HP Chem Station", managing its functioning and allowing the evolution of chromatographic analyses. Diluted samples (1/20 in methanol) of 1 µL were injected manually.

2.4. Target strains

Tested bacteria include two isolates of *Escherichia coli* DH5α and *Staphylococcus epidermis*. Before use, strains were revivified by subcultures in Luria-Bertani (LB) plates at 37 °C for 24 hrs.

2.5. Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined in 96 well-microplate using the microdilution assay according to the protocol previously described by Chraibi et al., (2016) [17]. Bacteriological agar at 0.15 % (w/v) was used as an emulsifier of the essential oil in the culture medium. The essential oil was serially diluted in Muller Hinton broth supplemented with agar to obtain final concentrations ranging between 8% and 0.007% (v/v). The 12^{th} well was considered as growth control (free-essential oil control). Then, 50 µL of bacterial inoculum, previously prepared and adjusted to 0.5 McFarland, was added to each well to reach a final concentration of 106 CFU/mL. After incubation at 37° C for 24 hrs., 10 µL of resazurin was added to each well as bacterial growth indicator. After further incubation at 37 °C for 2 hrs., the bacterial growth was revealed by the change of color from purple to pink. Experiments were carried out in triplicate.

2.6. Determination of Minimal Bactericidal Concentration (MBC)

The MBC corresponds to the lowest concentration of the essential oil at which the incubated microorganism was completely killed. It was determined by spotting 3µL from each negative well on (LB) plates and incubating at 37 °C for 24 hrs. Tests were performed in triplicate.

2.7. Mild Steel and HCl solution

The chemical composition of the mild steel used in this experiment is 0.22 % C, 0.79 % Mn, 0.022 % P, 0.030 % S, 0.21 % Si, 0.02 % Ni, 0.030 % Al, and the remainder is iron.

The aggressive solution of 1.0 M HCl was prepared from the analytical grade reagent 37 % HCl by dilution with distilled water. All chemicals used for preparing the tested solutions were of analytical grade, and the experiments were carried out at room temperature.

The 1.0 M HCl solutions with the extract oil of *M. piperita* or *M. pulegium* were freshly prepared before each experiment. The concentrations of extracts oil's were 0.1 to 1g/L.

2.7.1. Gravimetric Study

Gravimetric experiments were performed according to the standard methods [18], the mild steel specimens (1.5 cm × 1.5 cm × 0.05 cm) were abraded with a series of emery papers SiC (120, 600, and 1200 grades) and then washed with distilled water and acetone. After weighing accurately, the specimens were immersed in a 100 mL

of 1.0 M HCl solution in the absence and presence of various concentrations of the essential oil of M. *piperita* or *M. pulegium*.
All the aggressive acid solutions were open to air. After a certain time, (6 hrs.), of immersion in the acidic media, the specimens were taken out, washed, dried, and weighed accurately. In order to get good reproducibility, all measurements were performed three times and the average weight loss values were determined.
The corrosion rate (*v*) is obtained using the following equation:

$$v = \frac{w}{st} (\mathbf{1})$$

Where: W is the average weight loss, S is the total area, and t is the immersion time. With the corrosion rate calculated, the inhibition efficiency (E_w) is determined as follows:

$$Ew\ \% = \frac{V_0 - V}{V_0} X\ 100 \quad (\mathbf{2})$$

Where: $\boldsymbol{v_0}$ and *v* are the values of the corrosion rate without and with the essential oil inhibitor, respectively .

2.7.2. Electrochemical Measurements
The electrochemical measurements were carried out using Volta lab (Tacussel - Radiometer PGZ 100) potentiostat controlled by Tacussel corrosion analysis software model (Voltamaster 4) at static condition. The corrosion cell used had three electrodes. The reference electrode was a saturated calomel electrode (SCE). A platinum electrode was used as auxiliary electrode with a surface area of 1 cm^2. The working electrode was mild steel with 1 cm^2 surface area. All potentials given in this study were referred to this reference electrode. The working electrode was immersed in the tested solution for 30 minutes to establish a steady state open circuit potential (E_{ocp}). After measuring the E_{ocp}, the electrochemical measurements were performed. All electrochemical tests have been performed in aerated solutions at 308 K. The EIS experiments were conducted in the frequency range with high limit of 100 kHz and different low limit 0.1 Hz at open circuit potential, with 10 points per decade, at the rest potential, after 30 mins. of immersion in the acidic media, and by applying 10 mV ac voltage peak-to-peak. Nyquist plots were made from these experiments. The best semicircle can be fit through the data points in the Nyquist plot using a non-linear least square fit so that it intersects with the x-axis.
The inhibition efficiency of the inhibitor was calculated from the charge transfer resistance values using the following equation:

$$\mathrm{E\%} = \frac{R_{ct} - R_{ct}^{\circ}}{R_{ct}} \mathrm{x100} \qquad \mathbf{(3)}$$

Where, R°_{ct} and R_{ct} are the charge transfer resistance in the absence and presence of the essential inhibitor, respectively.

3. Results and discussion

3.1. Chromatographic analysis
From chromatographic analysis, 18 compounds were identified in the essential oil of *Mentha pulegium*.
Pulegone is the major constituent of this oil with a percentage of 75.48% followed by carvone (6.66%) and dihydrocarbon (4.64%) [17]. Other compounds with relatively low levels are the following: p-mentha-3,8-diene (2.44%), limonene (1.88%), pinocarvone (1.27%), α-peperitone (1.13%), and octanol-3 (1.86%) [17]. These results are in good agreement with the previous work of Lorenzo et al (2002) [19] which stipulate that the pulegone was the major component in the essential oil of *M. pulegium* from the North of Montevideo (Uruguay) with a percentage of 73.4%.
Concerning the essential oil of *Mentha piperita*, 27 volatile compounds were detected with a total summation of 99.51% of this oil, and menthol was found to be the major one. This is concomitant with previous research results which showed that Menthol was also dominant in the essential oils of four *M. piperita* plants from Turkey and Germany [20].

3.2. Antibacterial activity

The results of the antibacterial activity of both essential oils against *S. epidermis* and *E. coli* DH5α are shown in table 1. As can be noted in this table, the antimicrobial activity tested in vitro by microdilution method showed that these essential oils have a strong antibacterial effect. Indeed, the MIC values of *M. pulegium* essential oil ranged from 0.25 to 0.062 % (v/v) and for *M. piperita* essential oil from 0.03125 to 0.015 % (v/v) against

studied strains. Hence, *M. piperita* essential oil exhibit a higher antibacterial effect with MIC values 4 fold and 8 fold least compared to *M. pulegium* essential oil against *S. epidermis* and *E. coli* DH5α, respectively. Also, it can be noted that *S. epidermis* and *E. coli* DH5α were more resistant to the *M. pulegium* essential oil than *M. piperita*. In addition it was observed that the gram positive strain, *S. epidermidis,* was more sensitive than the gram negative one (*E. coli* DH5α). The resistance of this latter is due to lipopolysaccharide molecules which forms a barrier against hydrophobic molecules effects [21].

Table 1. The minimum inhibitory concentrations (MIC) of *M. piperita* and *M. pulegium* essential oils against the tested bacterial strains.

E.O	**Strains**	**Concentrations % (v/v)**										
		8	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007
M. pulegium	***S. epidermis***	-	-	-	-	-	-	-	-	+	+	+
	E. coli DH5α	-	-	-	-	-	-	+	+	+	+	+
M. piperita	***S. epidermis***	-	-	-	-	-	-	-	-	-	-	+
	E. coli DH5α	-	-	-	-	-	-	-	-	-	+	+

Table 2. The minimum bactericide concentrations (MBC) of *M. piperita* and *M. pulegium* essential oils against the tested bacterial strains.

E.O	**Strains**	**Concentrations % (v/v)**										
		8	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007
M. pulegium	***S. epidermis***	-	-	-	-	-	-	-	+	+	+	+
	E. coli DH5α	-	-	-	-	-	+	+	+	+	+	+
M. piperita	***S. epidermis***	-	-	-	-	-	-	-	-	-	-	+
	E. coli DH5α	-	-	-	-	-	-	-	-	+	+	+

Regarding the MBC values of both essential oils tested (Table 2), these values were found to be similar to those of the MICs of the essential oils values against *S. epidermis* and 2 fold higher toward *M. pulegium*. In fact, the MBC values of *M. piperita* and *M. pulegium* essential oils were 0.0625 %; 0.015 % and 0.5 %; 0.125 %; (v/v) against *E. coli DH5α* and *S. epidermis*, respectively.

3.2. Potentiodynamic polarization measurement

Figures 3 and 4 represent the anodic and cathodic Tafel polarization curves of mild steel in different concentrations of essential oil of *M. piperita* or *M. pulegium* in 1.0 M HCl solutions. By extrapolating the Tafel anodic and cathodic linear parts until they intersect as straight lines and show the corrosion current density (I_{corr}) and corrosion potential (E_{corr}). A steady state of corrosion current density (I_{corr}) occurs when the measured curve becomes horizontal [22].

The corrosion inhibition efficiency of the studied essential oils was more pronounced by increasing their concentration until a concentration of 1g/l, where the efficiency reached its maximum value. The corrosion current density decreases with increasing the inhibitor concentration [23, 24].
According to several authors this may be due to the adsorption of the inhibitor on mild steel/acid solution interface [25, 26]. Here, it shows that the decrease of current density of all essential oils in comparison with 1.0 M HCl for both anodic and cathodic site, may indicate a mixed type of corrosion inhibition. Basically, anodic polarization is the shift of anode potential to the positive (noble) direction whereas cathodic polarization is the shift of cathode potential to the negative (active) direction. In literature [27, 28], it has been reported that if the displacement in Ecorr is >85mV the inhibitor can be considered as a cathodic or anodic type inhibitor and if the displacement of E_{corr} is <85 mV, the inhibitor can be considered as mixed type inhibitor. In this study, the maximum displacement in E_{corr} value was 12 mV and 19 mv for *M. piperita* and *M. pulegium* essential oils, respectively. This indicates that both inhibitors act as mixed type inhibitors with predominant cathodic effectiveness.
A study of corrosion prevention and protection have supported that mixed type of inhibitors are generally represented by organic compounds with donor atoms Se, S, N or O instead of having reactive functional groups which latch on to the metal [29, 31]. For this reason, it was confirmed by potentiodynamic polarization curves that the essential oils of *M. piperita* and *M. pulegium* are mixed-type inhibitors with a highest efficiency value at a concentration of 1 g/l. Electrochemical corrosion parameters obtained from the Tafel analysis of the polarization curves from Figure 3 and 4 were given in Table 3.

Table 3. The polarization parameter values obtained for the corrosion of mild steel in 1.0 M HCl solution containing different concentrations of essential oil of *M. piperita* or *M. pulegium*.

Inhibitor in 1.0 M HCl	Concentration (g/L)	$-E_{corr}$ (mV/SCE)	$-\beta_c$ (mV/dec)	I_{corr} ($\mu A/cm^2$)	E (%)
Blank (1.0 M HCl)	-	450	193	1386	-
M. piperita	1	455	161	200	**86**
	0.5	461	157	354	74
	0.2	459	152	488	65
	0.1	462	167	512	63
M. pulegium	1	464	161	140	**90**
	0.5	469	165	234	83
	0.2	463	158	389	72
	0.1	460	159	471	66

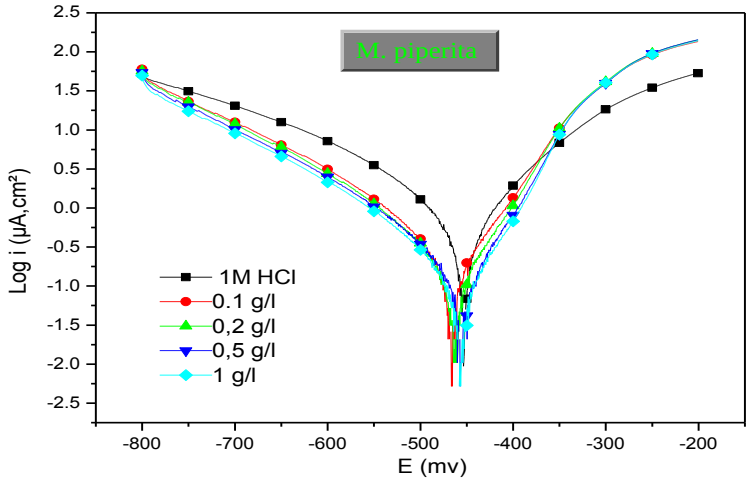

Figure 3. Tafel curves for mild steel in 1.0 M HCl solution in the absence and presence of various concentrations of essential oil of M. piperita.

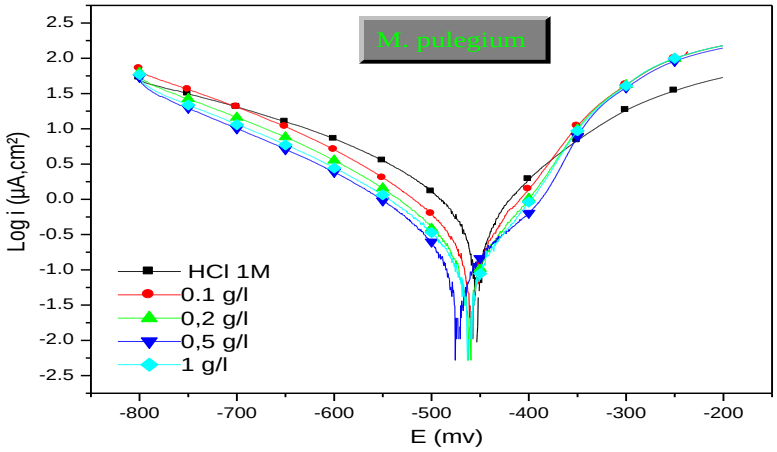

Figure 4. Tafel curves for mild steel in 1.0 M HCl solution in the absence and presence of differen concentrations of essential oil of *M. pulegium*.

3.3. Electrochemical impedance spectroscopy (EIS) study

Figures 5 and 6 show the Nyquist plots acquired at the open-circuit potential after 30 mins. of immersion in acidic media. The analysis of the Nyquist diagrams, show that these curves consist of a depressed capacitive loop semicircle for all the studied essential oils extracted from *M. piperita* and *M. pulegium*.

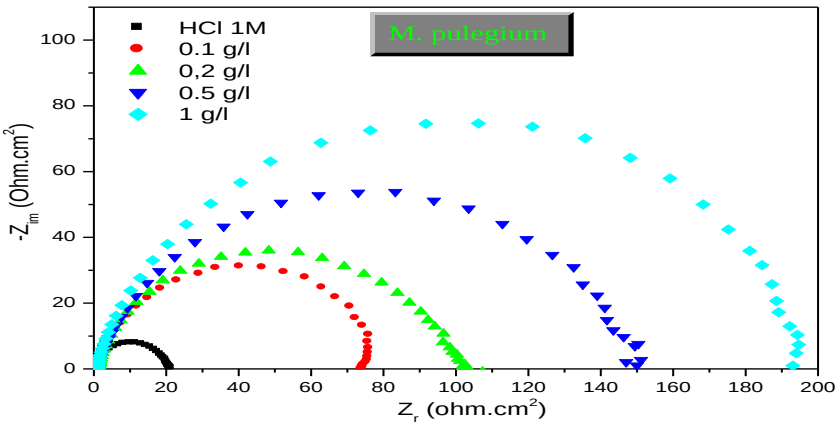

Figure 5. Nyquist plots for mild in various concentrations of essential oil of *M. pulegium* in 1.0 M HCl solutions at 308 K.

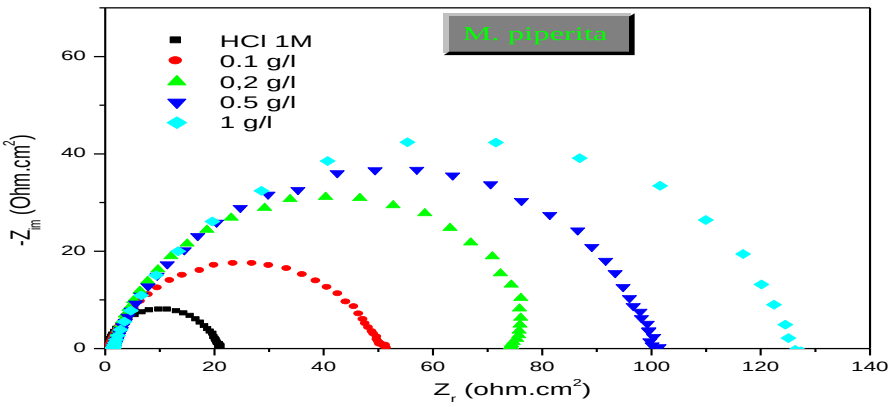

Figure 6. Nyquist plots for mild steel in various concentrations of essential oil of *M. piperita* in 1.0 M HCl solutions at 308 K.

The form of the capacitive loops shows that the corrosion process was controlled by charge transfer. It is clearly noted that the obtained impedance responses were significantly changed by addition of different concentrations of essential oils. The diameter of these capacitive loops increases gradually with the rise of the concentration of essential oil of both *M. pulegium* and *M. piperita*, indicating strengthening of inhibitive film. The Nyquist plots are evaluated in terms of the equivalent circuit composed with a constant phase element (CPE). The capacitive loops obtained in the absence and presence of both inhibitors are not perfect semicircles due to a frequency dispersion which has been attributed to the surface heterogeneity [32-33]. The main parameters extracted from the Nyquist diagrams in 1.0 M HCl with and without various concentrations of essential oils are given in Table 4.

Table 4. Electrochemical impedance measurements for mild steel immersed in 1.0 M HCl for 30 mins. in the absence and presence of different concentrations of essential oil of *M. piperita* or *M. pulegium*.

	Conc (M)	R_{ct} (Ω cm^2)	C_{dl} (μF cm^{-2})	E (%)
Blank	1M	19	200	-
	0.1	52	130	63
M. piperita	0.2	78	120	76
	0.5	100	83	81
	1	145	66	**87**
	0.1	77	119	75
M. pulegium	0.2	105	81	82
	0.5	148	62	87
	1	190	58	**90**

The CPE comportment can be quantified by plotting the imaginary part of the impedance as aversus frequency in logarithmic coordinates. The parameters associated with Nyquist plots were determined by the equivalent circuit presented in Figure 7. This circuit gives an exact fit to all experimental impedance data for both studied inhibitors. The equivalent circuit is composed of charge transfer resistance (R_{ct}), constant phase angle (CPE) and a solution resistance (R_s). The regression results are regrouped in Table 4.

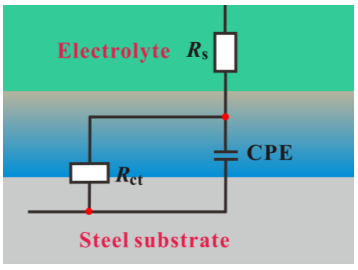

Figure 7. The equivalent circuit model of EIS.

The results of Table 4 show that the charge transfer resistance (R_{ct}) and efficiency (%E) increase with the increase of the concentration of essential oil obtained from the studied plants. However, the value of double layer capacitance (C_{dl}) decreases, because it is inversely proportional with the charge transfer resistance in all concentrations. This result may be attributed to the formation of a protective layer on the electrode surface [34-36]. The results show that the essential oils of *M. pulegium* and *M. piperita* inhibit the corrosion of mild steel in 1.0 M HCl solution with all the studied concentrations, and the efficiency increases with increasing the inhibitor concentration at 308 K.

3.4. Inhibitors efficiency

The inhibition efficiency data for both the studied plants are shown in Figure 8. After 21 days, a maximum inhibition efficiency of about 87% and 82% were obtained in 1.0 M HCl at a concentration of essential oil 1g/L of *M. piperita* and *M. pulegium*, respectively.

This shows the essential oil extracted from both plants possesses a very good corrosion inhibition in 1.0 M HCl solution for mild steel at 308 K. All the inhibition efficiency results obtained for both the studied plants follow the same trend as the results obtained with the weight loss and potential measurements.

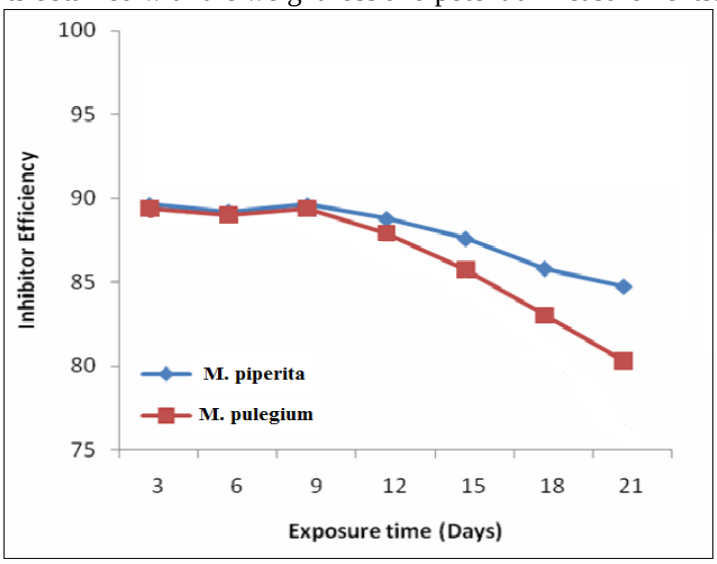

Figure 8. Variation of inhibiton efficiency with exposure time for the mild steel specimen immersed in 1.0 M HCl containing of 1g/l of essential oil of *M. pulegium* or *M. piperita*.

Conclusion

The chromatographic analysis revealed about 18 compounds in *Mentha pulegium* essential oil with Pulegone as the major constituent and 27 volatile compounds in *Mentha piperita* essential oil with menthol as the major compound. The antimicrobial activity evaluated by microdilution method showed that these essential oils have a strong antibacterial effect especially against the gram positive strain *S. epidermidis*. The MIC values ranged from 0.25 to 0.062 % (v/v) for *M. pulegium* essential oil and from 0.03125 to 0.015 % (v/v) for *M. piperita* essential oil. So, it suggests that there is a high potential for application of the essential oils tested as natural preservatives, and that they may have wide applications in food and cosmetics industries, among others.

Working at the temperature 308 k, the essential oils extracted from both *M. piperita* and *M. pulegium* gave an effective corrosion inhibition performance in 1.0 M HCl solutions. Although, all the studied concentrations of both inhibitors gave good results, the best result was obtained with the concentration of 1g/l essential oil in 1.0 M HCl.

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