PHENOLICS COMPOSITION, ANTIOXIDANT AND CORROSION INHIBITION EFFICIENCY, CAPACITY OF F*raxinus Excelsior* EXTRACTS

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ABSTRACT

Objective: In this study, we will determine the antioxidant properties of *Fraxinus excelsior* extracts and will correlate the values with total levels of polyphenolic compounds. And the ethyl acetate extract (EAE) was investigated as eco-friendly corrosion inhibitor of carbon steel in 1M HCl has been studied by both electrochemical impedance spectroscopy (EIS) and Tafel polarization measurements. **Methods:** Antioxidant activity was evaluated by DPPH, $ABTS^{+}$, FRAP and enzymatic test. Total polyphenols and total flavonoids contents were evaluated by spectrophotometric assays. And the corrosion inhibition was studied by polarization and electrochemical impedance spectroscopy methods. **Results:** The total polyphenols and flavonoids contents were in the order EAE > ChE > BolE > MetE > AqE > PEE. The DPPH of MetE, PEE, EAE, ChE, BolE and AqE extract activity expressed as IC_{50} values were in the order 125.15 ± 2.5, 3722.5 ± 31.82, 23.1 ± 0.48, 101.86 ± 1.64, 106.06 ± 2.97 and 363.41±1.38 µg / ml. The results of $ABTS^{\cdot+}$, FRAP and the enzymatic test showed that the *Fraxinus excelsior* extracts have a potential antioxidant activity in the same order obtained by using the DPPH test, which the antioxidant activity followed a decreasing order: EAE > ChE > BolE > MetE > AqE > PEE. The ethyl acetate extract (EAE) exhibits good inhibition properties for the corrosion of carbon steel in 1M HCl solution. **Conclusions:** In conclusion, *Fraxinus excelsior* extracts contain active compounds which have antioxidant effects and can be useful in the treatment of pathologies where these activities are needed. And the corrosion inhibition data considered this extract (EAE) as an efficient corrosion inhibitor.

Keywords: *Fraxinus excelsior,* $ABTS^{+}$, FRAP, DPPH, Antioxidant Capacity, Corrosion inhibitor.

1. INTRODUCTION

Oxidative stress is a result of imbalance between the antioxidant systems and the formation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and free radicals such as superoxide ions ($O_{2}^{\cdot}$) and hydroxyl radicals ($OH^{\cdot}$) [1,2]. It is believed to damage cell membranes and DNA, as well as membrane lipid peroxidation with subsequent decreases in membrane fluidity [3,4]. Oxidative damage may cause cell injury, death and exacerbate the development of several age-related chronic diseases including cancer, Alzheimer's disease, Parkinson's and heart disease [5]. Therefore, antioxidants are widely used in the food industry as potential inhibitors of lipid peroxidation [6]. Many synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may accumulate in the body, resulting in liver damage and carcinogenesis [7].

For this reason, more attention has been paid to natural non-toxic antioxidants in an effort to protect the human body from free radicals and retard the progress of many chronic diseases. Many medicinal plants contain large amount of antioxidants such as polyphenols which can play an important role in adsorbing and neutralizing free radicals [8,9]. Ash (*Fraxinus excelsior*), belonging to the Oleaceae family, locally known as "l'ssane l'ousfour", is a native shrub widely distributed throughout regions of Algeria. *Fraxinus excelsior* is worldwide known as an antioxidant) [10] anti-inflammatory, anti-rheumatic, analgesic and antipyretic plant [10,11]. have reported that aqueous extract of *Fraxinus excelsior* possess hypoglycemic activity.

Acid solutions are generally used for the removal of undesirable scale and rust in several industrial processes. Hydrochloric and sulphuric acids are widely used in the pickling processes of metals. Use of inhibitors is one of the most practical methods for protection against corrosion especially in acid solutions to prevent metal dissolution and acid consumption [12]. Several methods are available to prevent or retard corrosion of metallic materials, the use of inhibitors is one of the best technique to ensure their protection in contact with aggressive media such as hydrochloric acid medium. Oil and plant extracts have become a source of inhibitors, ecological guarantee high efficiency at a cheaper price. These types of inhibitors do not contain heavy metals or toxic compounds and they are biodegradable. This review presents a synthesis of the majority of the contributions published in the literature during these last ten years, on the use of oil and extracts of plants as inhibitor of corrosion in the hydrochloric acid medium [13,14].. They showed that all the reported plant extracts were found to inhibit the corrosion of mild steel in acid media. Indeed, the extract *Andrographis paniculata* has a better inhibition performance (98%) than the other leaves extract. *Strychnos nuxvomica* showed better inhibition (98%) than the other seed extracts. *Moringa oleifera* extract is reflected as a good corrosion inhibitor of mild steel in 1M HCl with 98% inhibition efficiency among the studied fruits extract. *Bacopa monnieri* extract showed its maximum inhibition performance to be 95% at 600 ppm among the investigated stem extracts.

Corrosion inhibition effect of *Justicia gendarussa* extract on mild steel in 1 M HCl medium was also studied by [15]. And inhibition efficiency up to 93% was achieved with 150 mg L^{-1} of extract. Aqueous extracts of *Cordia latifolia* and *Curcumin* were investigated as corrosion inhibitors for mild steel in industrial cooling systems by [16]. The extracts showed maximum inhibition efficiency of 97.7% and 60%, respectively. Also, the inhibitive effects of aqueous extracts of *Eucalyptus* (leaves), *Hibiscus* (flower), and *Agaricus* on the corrosion of mild steel for cooling-water systems, using tap water, have been investigated by means of weight loss and polarization methods [16]. All the plant extracts were found to inhibit corrosion of mild steel following and their inhibitive efficiencies were in the order: *Agaricus* (85%), *Hibiscus* (79%), and *Eucalyptus* (74%). For this reason, the aims of our research were: (**1**) to study the relationship among different methods enzymatic (XO), spectroscopic method including UV-visible technique (DPPH, $ABTS^{+}$ and FRAP for measuring antioxdant activity. (**2**) to study the relationship between these methods and phenolic, and flovonoids contents. (**3**) and the corrosion inhibition was studied by polarization and electrochemical impedance spectroscopy methods

2. MATERIALS AND METHODS

2. 1. Extraction of phenolics compounds

Fraxinus excelsior fruits were collected at M'Sila region, Algeria, during 2010 and were identified by Prof H. Laouer, Department of Ecology and Vegetal Biology, Faculty of Nature and Life Sciences, University Setif 1. Fruits parts of the plant were air dried for several days. The dried plant material was ground to a coarse powder using a dry mill. The extractions were carried out using various polar and non-polar solvents according to [17].

2. 2. Determination of total polyphenol and flavonoids contents

The total phenol content was determined by the Folin-Ciocalteu method as described by [18]. In brief, 100 µl of extracts were well mixed with 0.5 ml of the Folin-Ciocalteu stock reagent, after 4 min 0.4 ml of Na_2CO_3 reagent (0.75%) was added to the mixture, incubated at room temperature for 1h 30 min. The mixture absorbance was measured at 760 nm. The amount of total polyphenols was expressed as mg of Gallic acid equivalents per g of extract. Flavonoids were quantified using $AlCl_3$ [19]. One ml of the extract or fractions was dissolved in

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methanol then 1 ml of $AlCl_3$ (2 % in methanol) was added. After 10 min of incubation at room temperature, the absorbance was measured at 430 nm. The amount of flavonoids was expressed as mg of Qurcetine and Rutin equivalents per g of extract.

2. 3. Effects of Fraxinus excelsior extracts on xanthine oxidase activity

The effects of different extracts on XO activity were determined spectrophotometrically by measuring the uric acid production at 295 nm [20]. The enzyme assay was performed in the presence of 100 µM of xanthine dissolved in phosphate buffer (50 mM, pH 7.4), containing 0.1 mM EDTA, supplemented with various amounts of plant extracts. The reaction was initiated by the addition of bovine XO (prepared in the Laboratory of Applied Biochemistry, University Setif 1 with a specific activity of 1176 ηmole/min/mg of enzyme). Allopurinol was used as a positive standard. The inhibition percentage was calculated using the following formula: I % = 100 - (A_{sample} / A_{enzyme}) x100, where A_{sample}: absorbance of the sample, A_{enzyme}: absorbance of the enzyme.

2. 4. Effects of Fraxinus excelsior extracts superoxide anions generation by XO

The XO superoxide anions generation can be measured by following the cytochrome C reduction at 550 nm [20]. The reaction mixture containing: 50 mM phosphate buffer (pH 7.4), 0.1 mM EDTA, 100 µM xanthine, 25 µM cytochrome C and various concentrations of plant extracts. Reaction was started by the addition of XO. The results were expressed as percentage inhibition of cytochrome C reduction. The inhibition percentage was calculated using the following formula: I % = 100 - (A_{sample} / A_{enzyme}) x100, where A_{sample}: absorbance of the sample, A_{enzyme}: absorbance of the enzyme.

2. 5. DPPH radical scavenging activity

The free radical scavenging properties of *F. excelsior* extracts were measured by decrease in the absorbance (at 517nm) of methanol solution of 2,2'-diphenyl-1- picrylhydrazyl (DPPH) [21]. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$I\,\% = \left(A_{blank} - A_{sample}/A_{blank}\right)x100 \qquad (1)$$

Where A_{sample}: absorbance of the sample, A_{blank}: absorbance of the blank.

2. 6. $ABTS^+$ radical scavenging activity

The method of decolorization of free radical $ABTS^+$ employed was a modified version of that used by R. Samarth et al. [22] and initially reported by R. Re et al. [23] $ABTS^+$ was generated by oxidation of ABTS 7 mM with potassium persulphate 2.45 mM in water, at room temperature for 16 h. For each analysis, the $ABTS^+$ solution was freshly diluted with water in order to obtain an initial absorbance around 0.8 at 734 nm. 20 ml methanolic extract were added to 250 ml of $ABTS^+$ solution. Absorbances were measured at 734 nm after 30 min of incubation in the dark at room temperature.

2. 7. FRAP assay

The ferric reducing antioxidant activity (FRAP) assay of [24] was measured in all samples. Fresh FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6), one volume of 10 mM TPTZ in 40 mM hydrochloric acid and one volume of 20 mM ferric chloride, and then incubating at 37°C for 5 minutes. For each analysis, 30 ml of methanolic solution of extracts were added to 20 ml of distilled water and 250 ml of fresh FRAP solution and mixed thoroughly. The increase in absorbance was recorded at 593 nm after 20 min. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µM Trolox equivalents per gram of *Fraxinus excelsior* extracts) [25].

2. 8. Corrosion inhibition assay

The electrochemical measurements were carried out at 25°C using a PGZ 301 voltlab 40 and Voltamaster 4 software. All the electrochemical studies were carried out in a conventional three electrode set up, open to atmosphere. The counter electrode was platinum sheet (2 cm^2 area) and saturated calomel electrode (SCE) was used as the reference. All potentials are given according to this reference electrode.

The working electrode was mild steel with a chemical composition (in wt%) as follows: 0.09 % C, 0.058 % Si, 0.91 % Mn, 0.014 % P, 0.002 % S, 0.048% V, 0.001 % Ti, 0.056 % Nb, 0.044 % Al, and Fe as balance. The surface area of the working electrode was 0.2 cm^2, connected through a copper wire, isolated with thin polystyrene film. It was abraded with emery papers (220 and 4000 grade) and finally washed with acetone and double distilled water before use. The aqueous electrolyte solution (HCl 1 M) was prepared from analytical grade 37% HCl (Merck) and double distilled water. The measurements were carried out in 1 M HCl in the absence and presence of the extract (EAE) in the concentration range 5- 12.5 mg/ml. Chemicals used in corrosion experiments were analytical grade (Fluka) and used without further purification; ultra-pure water was used to prepare test solutions.

Potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) are the techniques used to examine the corrosion behavior.

The experiments were carried out for the ethyl acetate extract (EAE) in the conventional three-electrode cell with a platinum counter electrode (CE) (2 cm^2 area) and a saturated calomel electrode (SCE) coupled to a fine Luggin capillary as the reference electrode. In order to minimize ohmic contribution, the Luggin capillary was placed close to the working electrode (WE) which was in the form of circular mild steel embedded in polyvinyl chloride (PVC) holder using epoxy resin so that the flat surface was the only surface in the electrode. The surface of the working electrode (mild steel X48) which has a dimension of 0.28 cm^2. Before measurement, the electrode was immersed in test solution (1 M HCl) at open circuit potential (OCP) for 30 minutes to be sufficient to attain a stable state. All electrochemical measurements were carried out at 25 °C using Versa STAT 400 advanced electrochemical system. The potential of potentiodynamic polarization curves system was increased at 0.5 $mV.s^{-1}$ and started from a potential of -250 to + 250 mV versus OCP. The IE_p (%) at different inhibitor concentrations are calculated using this equation [26]:

$$IE_{\mathrm{p}}(\%) = \left(\frac{i^0_{\mathrm{corr}} - i_{\mathrm{corr}}}{i^0_{\mathrm{corr}}}\right) 100 \qquad (2)$$

Where i^0_{corr} and i_{corr} represent the corrosion current density values without and with the ethyl acetate extract (EAE), respectively. The electrochemical impedance spectroscopy (EIS) was performing at OCP in the frequency range of 100 kHz– 10 mHz with a signal amplitude perturbation of 5 mV. The inhibition efficiency $IE_R(\%)$ is calculated by using the following equation [27]:

$$IE_{\mathrm{R}}(\%) = \left(\frac{R_{ct} - R_{ct_o}}{R_{ct}}\right) 100 \qquad (3)$$

Where R_{ct_o} and R_{ct} are charge transfer resistance for carbon steel X48 without and with the ethyl acetate extract (EAE), respectively. Nyquist plots were drawn from this experiment.

3. RESULTS AND DISCUSSION

3. 1. Total polyphenols and flavonoids contents

The total phenolic and flavonoids contents of samples were estimated by Folin-Ciocalteu reagent and $FeCl_3$, respectively. The total phenolic contents were expressed as µg gallic acid equivalents per mg dry weight (µg GA-Eq/mg) and total flavonoids contents as µg quercetin and rutin equivalents per mg dry weight (µg Q-Eq/mg and µg R-Eq/mg), (Table 1).

The result obtained shows that the ethyl acetate extract (EAE) contained the highest amount of flavonoids compounds (10.577±0.061µgQ-Eq/mg) and phenolics compounds (157.409±1.346 µgGA-Eq/mg) compared to the others extracts of *Fraxinus excelsior*.

3. 2. Antioxidant capacity

The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each antioxidant component separately. Therefore, in many studies, several intermediate extractions are used to ensure a maximum extraction of the available antioxidants [28]. The antioxidant activity of phenolics is mainly due to their red-ox properties which make them act as reducing as agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metallic chelating potential [29]. The plant extracts were subjected

to screening for their possible antioxidant activity by four complementary test systems namely FRAP assay $ABTS^{+}$, DPPH radical scavenging activity and enzymatic test.

3.3 Effects of extracts of Fraxinus excelsior on XO activity and on superoxide anions generation by XO system

The inhibition of the activity of the XO by the extracts is represented by the presence of one or several compounds reacting on the active sites of the enzyme. This inhibition activity can be allotted to the presence of various bioactive compounds such as polyphenols [30], tannins [31] and flavonoids [32] determined the relation between the chemical structure of the flavonoids and their inhibiting activities of the XO. [33] showed that the glycosylated flavonoids have activities lower than those of the non glycosylated ones; for example, rutin is almost ten times less active than quercetin. [34] showed that there exists a relation between the structure of the flavonoids and their scavenging activity on $O_2^{\bullet-}$ and inhibition of activity on XO where the different structural between flavonols, the flavones, flavanons, and the dihydroflavonols can influence the inhibiting features and scavenging activity of these flavonoids. The presence of the double connection between carbons C2 and C3 and of the hydroxyl groups on carbons C5 and C7 potentiate the scavenger effect on $O_2^{\bullet-}$ of which non-saturation of ring C and the suppression of hydroxyl group on C7 carbon induce a reduction in the scavenger activity of flavonoids [34].

The reactive oxygen species produced by XO can modify the structure and function of macromolecules including proteins, lipids, carbohydrates, and nucleic acids [35]. The inhibitory effect of extracts on XO activity was determined spectrophotometrically by measuring uric acid production at 295 nm. The superoxide anion generated by xanthine/xanthine oxidase system can reduce the cytochrome C^{+3} to cytochrome C^{+2}. The scavenger ability of different extracts of *Fraxinus excelsior* L. on superoxide anions radicals produced by xanthine oxidase (XO) can be measured by following the cytochrome c reduction at 550 nm [20]. All the extracts inhibited the activity of xanthine oxidase and have a potent scavenging activity of superoxide anion in a concentration dependent manner, The results of enzymatic test are presented in the figure 1. With value of IC50 were in the order EAE > ChE > BolE > MetE > AqE > PEE. The difference in antioxidant activities of extracts might be attributed to a difference in total polyphenols and/or flavonoids contents.

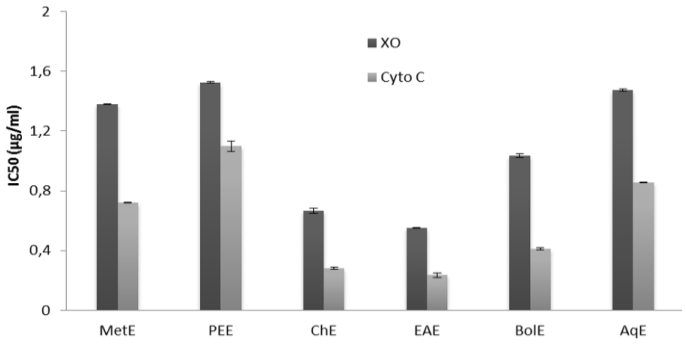

Figure1. Effects of extracts of *F. excelsior* on XO activity and on superoxide anions generation by XO system.

The correlation coefficient between XO activity and total phenolic contents of Algerian plants is R^2=0.911 (Table1), whereas for the correlation between the total flavonoids and antioxidant activity this coefficient is determined to be R^2= 0.729. This result suggests that the antioxidant capacity of *F.excelsior* plants is due to the contribution of phenolic and flavonoids compounds. Regression analysis between antioxidant power (cyto c) and total phenols and flavonoids content shows a linear correlation (Figure 2). Coefficients of R^2= 0.785 and 0.691 have been obtained for polyphenol and flavonoids compounds, respectively. These results suggest that the antioxidant power is especially due to phenolic and flavonoids compounds that are the major constituents having reducing power.

3. 4. $ABTS^{+}$ and DPPH radical scavenging activity

The total antioxidant capacity of extracts was carried out using a spectrophotometer by the improved 2,2-azino-bis-(3-ehylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{+}$) radical cation method as described by Y. Z. Cai et al. [36] and J. S. Bao et al. [37]. The reaction mixture was kept at room temperature and the absorbance was immediately recorded at 734 nm. And the $DPPH^{\cdot}$ (2,2'-diphenyl-1-picrylhydrazyl) is a stable radical widely used to monitor the free radical scavenging abilities (the ability of a compound to donate an electron) of various antioxidants. The $DPPH^{\cdot}$ radical has a deep violet color due to its impaired electron, and radical scavenging can be followed spectrophotometrically by the loss of absorbance at 517 nm, as the pale yellow non radical form is produced [38,39]. The scavenger effect on the free radicals depends on the presence of free OH groups in particular 3-OH on ring C, with a configuration 3',4'-dihydroxyl on ring B in the polyphenols [40,41]. All extracts show antioxidant activities proving their capacity to scavenge the $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical (Figure 2 and Figure 3). In fact, the ethyl acetate extract of *Fraxinus excelsior* showed the highest antioxidant activity with IC_{50}=23.1±0.48µg/ml, 4.98±0.08µmolTrolox-Eq/g respectly. The difference in antioxidant activities of extracts might be attributed to a difference in total polyphenols and/or flavonoids contents. Several studies showed a correlation between antioxidant activity and phenolics and flavonoids content [42].

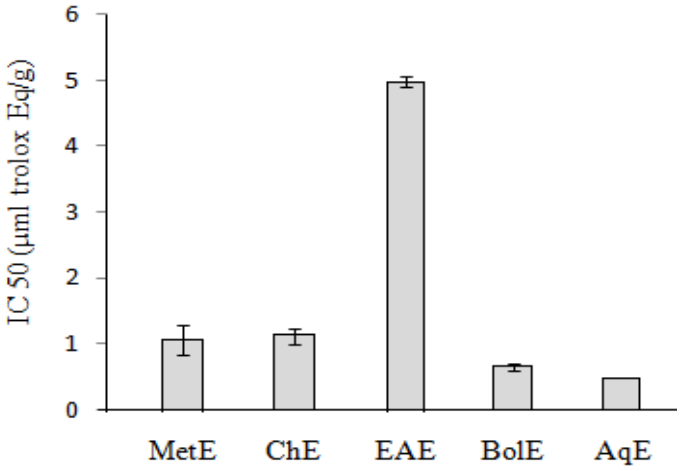

Figure 2. $ABTS^{+}$ cation radical scavenging activity of *Fraxinus excelsior* extracts.

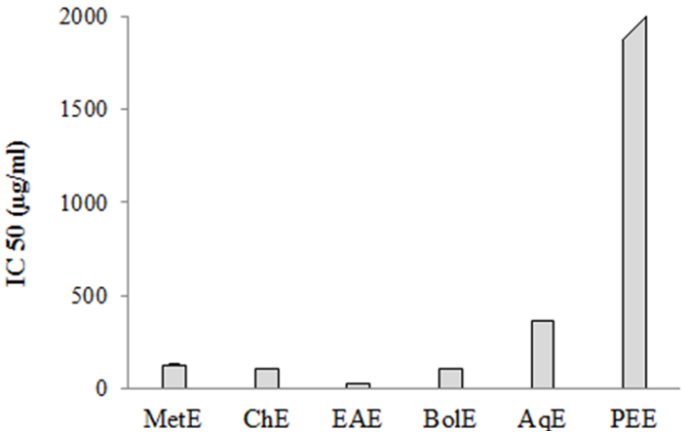

Figure 3. DPPH radical scavenging activity of *Fraxinus excelsior* extracts.

3. 5. FRAP assay

The FRAP assay is based on the measurement of the ability of the substance to reduce Fe^{3+} to Fe^{2+} and was initially proposed to measure the total antioxidant capacity of plasma [24] and then applied by the same authors to other substrates. Fe^{2+} is measured spectrophotometrically via determination of its coloured complex with 2,4, 6-Tris (2-pyridyl) triazine (TPTZ), which has a high absorbance at 593 nm.

Since the antioxidant activity of a substance is usually correlated directly to its reducing capacity, the FRAP assay provides a reliable method to study the antioxidant activity of various compounds [24]. This method has been frequently used for a rapid evaluation of the total antioxidant capacity of various food and

beverages [43,44] and also different plant extracts containing flavonoids [45]. Furthermore, it has been applied to measure the antioxidant activity of dietary polyphenols and a limited number of flavonoids *in vitro* [46]. The results of FRAP assay are presented in the figure 4. The AqE showed the lowest activity by other extracts with 138.14 ± 4.00 µmol Trolox-Eq/g.

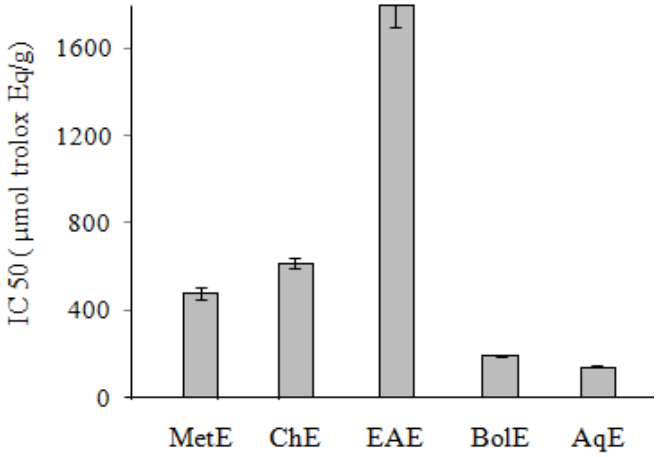

Figure 4. FRAP assay of *Fraxinus excelsior* extracts.

In table1 the total phenolic and flavonoid content and the antioxidant power of the *F. excelsior* plant evaluated by the ABTS^{+}, FRAP method and by the DPPH assay are reported. The data obtained by the ABTS^{+}, FRAP method were substantially confirmed by the DPPH assay since a good correlation was observed between the methods (Table 1).

Total polyphenols and flavonoids contents						
	MetE	**PEE**	**ChE**	**EAE**	**BolE**	**AqE**
µg REq/mg	06.84±0.40	01.37±0.98	11.67±0.41	16.42±0.45	08.05±0.45	02.83±0.00
µg QEq/mg	04.79±0.53	01.23±0.03	05.93±0.05	10.58±0.06	04.99 ±0.07	01.58±0.00
µg GEq/mg	49.75±1.54	26.74±0.52	109.9±0.19	157.41±1.34	67.9±0.1.06	34.74±1.27

Antioxidant capacity			
ABTS (µmol T Eq/g)		**FRAP (**µmol T Eq/g)	**DPPH (**µg/ml)
MetE	1.05 ± 0.23	477.20±30.26	125.14 ± 2.50
PEE	-	-	3722 ± 31.82
ChE	1.11 ± 0.12	615.55 ± 21.50	101.86 ± 1.64
EAE	4.98 ± 0.08	1826.64 ± 127.4	023.10 ± 0.48
BolE	0.63 ± 0.05	191.81 ± 3.17	106.05 ± 2.97
AqE	0.48 ± 0.01	138.14 ± 4.00	363.41 ± 1.37
Correlation			
R^2/ falavonoid	0.736	0.835	0.884
R^2/ phenolic	0.579	0.787	0.891

3.6. Corrosion Inhibition Studies

3.6.1 Potentiodynamic polarization measurements

Polarization curves of the carbon steel electrode in 1 M HCl without and with addition of various concentrations of EAE are shown in figure 5. Electrochemical kinetic parameters (corrosion potential (E_{corr}), corrosion current density (i_{corr}), and cathodic Tafel slope ($\boldsymbol{\beta}_c$), anodic Tafel slope ($\boldsymbol{\beta}_a$), surface coverage (θ) and inhibition efficiency IEp (%)) determined from these experiments by extrapolation method, are reported in table 2.

From table 2, it is clear that the values of i_{corr} decrease considerably with the increase of the concentration of EAE, however,E_{corr} values (Table 2) in the presence of EAE are shifted to negative way. These results indicate that EAE behave as mainly cathodic inhibitor [47]. In other words, compound EAE inhibits the anodic dissolution of mild steel in 1 M HCl and delays the cathodic reduction reaction associated with the evolution of hydrogen gas. The variation of the values of the slope of anodic Tafel, $\boldsymbol{\beta a}$ in the presence of inhibitor can be attributed to the adsorption of chloride ion molecules or inhibitors on the surface of mild steel, or to the appearance of certain redox process involving the Fe Inhibitor Complex on active sites of steel [48,49]. The values of the Tafel slopes also vary with concentrations of the inhibitor. These remarks indicate that inhibitive effect of EAE includes some changes in the mechanism of the corrosion reactions. The inhibition efficiency increases with concentration of the inhibitor and reaches a maximum value of 89.58 % at 12.5 mg/ml

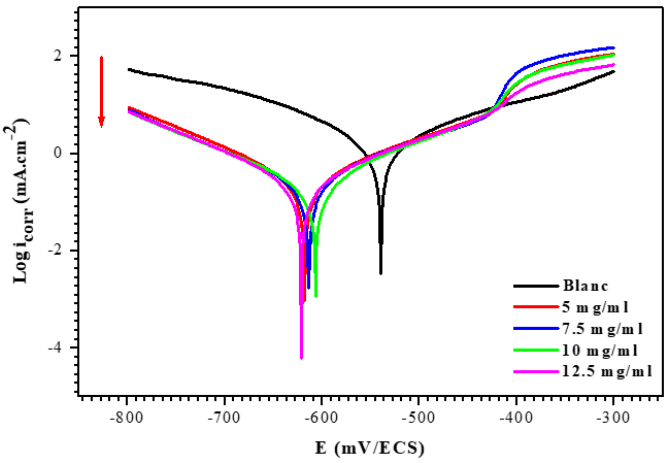

Figure 5. Polarization plots of the mild steel electrode obtained in 1 M HCl solution containing different concentrations of EAE at 25 °C.

Table 2. Polarization data and the corresponding inhibition efficiency for the mild steel in 1 M HCl with and without addendum of different concentrations of EAE at 25 °C.

Inhibitor	**Cm (mg/ml)**	**-E_{corr} (mV/ECS)**	**-β_c (mV/dec)**	**β_a (mV/dec)**	**i_{corr} (mA/cm^2)**	**EI_P (%)**	**θ**
Blank	0	540	127.8	160.8	1.670	-	-
EAE	5	617	116.4	129.4	0.269	83.89	0.838
	7.5	613	123.5	112.2	0.227	86.41	0.864
	10	606	125.3	105.4	0.204	87.78	0.877
	12.5	621	93.2	104.8	0.174	89.58	0.895

3.6.2 Electrochemical impedance spectroscopy (EIS)

The corrosion of MS in 1 M HCl solution in the presence of compound EAE was investigated by EIS method. Nyquist plots of uninhibited and inhibited acid solutions containing various concentrations of the inhibitor are presented in figure 6**.** It was found that, all Nyquist plots show a single capacitive loop, both in uninhibited and inhibited solutions. This would be attributed to charge transfer of the corrosion process. The impedance spectra showed the single semicircle and the diameter of semicircle increases with increasing concentration of the inhibitor [50]. The semicircles show slight irregularity which may be attributed to the roughness, impurities, dislocations, or non-homogeneous nature of the metal surface [51-56]. The capacitance loop intersects the real axis at higher and lower frequencies. At high frequency end the intercept corresponds to the solution resistance (R_s) and at lower frequency end corresponds to the sum of R_s and charge transfer resistance (R_{ct}). The technique behavior can be well explained by pure electric models which could verify and enable to calculate numerical values corresponding to the physical and chemical properties of electrochemical system under examination.

Corrosion parameters derived from EIS measurements and inhibition efficiencies, $IE_R(\%)$ are given in table 3. It was found that, the C_{dl} values decreased with increasing inhibitors concentration. The decrease in the C_{dl} values may be due to decrease in the local dielectric constant or an increase in the thickness of the electrical double layer, indicating that the inhibitors adsorbed on the metal surface [57-62]. Thus, the change in C_{dl} values was caused by the gradual replacement of water molecules and by the adsorption of organic molecules on the metal surface, decreasing the extent of dissolution reaction [63, 64].

The impedance results revealed that the inhibition efficiencies of the chiral Schiff bases were different from each other. The maximum inhibition efficiency observed is 93.44% for EAE at 12.5 mg/ml. The variation in the $IE_R(\%)$ might be due to the substituents, molecular mass and molecular sizes of the inhibitor [65], and indicates also that the inhibitor molecules have the capability of forming a compact adsorbed layer over the metal surface.

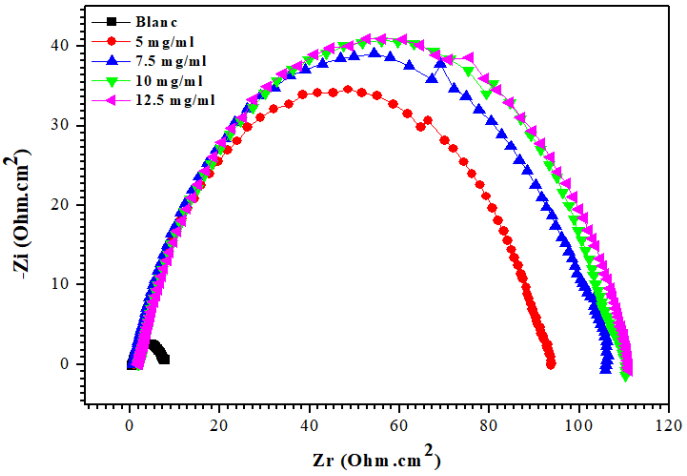

Figure 6. Nyquist diagrams for mild steel in 1 M HCl containing different concentrations of EAE at 25 °C.

Table 3. Electrochemical impedance parameters for mild steel in 1 M HCl, with and without addition of various concentrations of EAE at 25°C.

Inhibitor	Cm (mg/ml)	R_s (Ωxcm^2)	R_{ct} (Ωxcm^2)	C_{dl} ($\mu F/cm^2$)	$EI_R(\%)$	θ
Blank	0	0.512	7.13	1409.0	-	-
EAE	5	0.145	91.79	244.0	92.23	0.922
	7.5	0.809	103.00	204.9	93.08	0.930
	10	2.041	106.80	201.4	93.32	0.933
	12.5	2.046	108.70	166.8	93.44	0.934

The Randles equivalent circuit is one of the simplest and most common circuit models of electrochemical impedance. It includes a solution resistance Rs, in series to a parallel combination of resistor, R_{ct}, the charge transfer resistance and a double layer capacitance, C_{dl}, respectively. The equivalent circuit for the Randles cell is shown in figure 7.

Figure 7. Equivalent circuit model for the studied inhibitor, R_s solution resistance, R_{ct} charge-transfer resistance and C_{dl} double layer capacitance.

CONCLUSION

In this study, it was concluded that the antioxidant activity of Fraxinus excelsior extracts collected from M'Sila was investigated. Different assays employed DPPH, ABTS+, FRAP, enzymatic test and corrosion inhibition assay. All these methods showed that the antioxidant activity is dependant mainly on the phenolic composition.

The inhibition efficiency increases with the concentration of the inhibitor and reaches a maximum value of 89.58 % from potentiodynamic polarization and 93.44 % from electrochemical impedance spectroscopy at 12.5 mg/ml. Our results suggest that Fraxinus excelsior can be used in pharmaceutical products as a source of natural antioxidants and inhibitor of corrosion.

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