Int. J. Electrochem. Sci., 6 **(2011)** 3017 - 3035

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Corrosion Resistance of Magnesium Alloy (AZ31E) as Orthopaedic Biomaterials in Sodium Chloride Containing Antioxidantly Active Compounds from *Eichhornia crassipes*

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Received: 2 May 2011 / *Accepted:* 8 June 2011 / *Published:* 1 July 2011

Eichhornia crassipes (Water hyacinth) is a hydrophyte which invaded all Egyptian water bodies causing serious problems of navigation and water quality deterioration. Known weight of the sample was successively extracted with hexane (E_1), ethyl acetate (E_2) and methanol (E_3). Antioxidant activity of extracts was performed by two complementary test system , 2.2'diphenyl-1- picryl hydrazyl (DPPH) and 2,2'- azino-bis (ethylbenzthiazoline-6- sulfonic acid (ABTS) methods which revealed the highest activity of E_3 followed in descending order by E_2 and E_1. Fractionation of the promising methanol extract E_3, produced five antioxidantly active fractions (a-e). Identification of the five fractions was performed using chromatographic and spectroscopic methods. Electrochemical impedance spectroscopy (EIS) experiments were carried out in artificial sea water (3.5% NaCl) to study the corrosion inhibition effect of *Eichhornia crassipes* (Water hyacinth) extracts (E_1, E_2, E_3) and E_3 fractions (a-e) on extruded AZ31E surfaces. The obtained results indicated that, the type of extract, the extract concentrations and immersion time affect the inhibition efficiency (IF %). Corrosion rate for E_3, E_2 &E_1 decreases with increasing extract concentration or immersion time. Fractions (a-e) exhibited anticorrosion activities with the order of: a > d > b > E_3> c > e.

Keywords: Magnesium alloy implant, corrosion, water hyacinth, antioxidant activity

1. INTRODUCTION

Water hyacinth, *Eichhornia crassipes* (Mart) Solms.is a hydrophyte (Pteridophyte), originated in the state of Amazonas, Brazil ,spread to other regions of south America and was carried out by humans through the tropics and subtropics .It is now widespread and recognized as one of the top 10 weeds in the world [1]. The large, dense monoculture of water hyacinth cover the Egyptian water

bodies particularly the River Nile, irrigation and drainage canals [2]. It produces serious problems in navigation, increase the water loss and alteration of the water quality [3]. Reactive oxygen species (ROS) were known to cause the oxidation of the common unsaturated fatty acids constituting the lipids of biomembranes. This led to many pathological effects to humans as cardiovascular disease, cancer and brain dysfunction as well as aging processes .It also led to the development of food rancidity and off-flavors [4,5]. Free radical scavengers (antioxidants) protect both the human body and food from the adverse effects of ROS. Synthetic antioxidants as butylated hydroxy toluene (BHT) and butylated hydroxyl anisol (BHA) are used in both sectors to inhibit free radical chain reactions. Recently they have been shown to cause pathological, enzyme, lipid alterations and have carcinogenic effects [6,7]. The development of alternative antioxidants of natural origin (plant,freshwater ,marine organisms and hydrophytes) is of great importance for our health and holds considerable commercial potential . Antioxidants from natural sources (α-tocopherol ,phenolic compounds, carotenoids and flavonoids) have high bioavailability and therefore high protective efficiency against ROS and free radicals [8]. Free radicals and singlet oxygen scavengers (antioxidants) ,were found to have metal and alloy anticorrosive effect which depend to a great extend on the structural feature of the antioxidant added and to its accepting – donating hydrogen or electron behaviors [9]. Reduction of oxygen availability in the corroding system and presence of a barrier (physical , chemical , biological)between the electrode surface and oxygen , retarding or even inhibiting the rate of metal (or alloy) corrosion [10]. Marine ,freshwater algae ,plants and hydrophytes (as water hyacinth) have increasingly been shown to provide rich source of natural bioactive compounds with antimicrobial , antitumoral ,antiviral and antioxidant activities.

Magnesium is the lightest structural metal available as combination of low density and good mechanical strength, resulting in a high strength-to -weight ratio. Magnesium alloys are widely used today in many industries as aerospace compounds, computer parts, mobile phones, aircraft applications as well as passenger cars, automotive trim and camera casing. Since Mg alloys can gradually be dissolved and absorbed after implanting, it has been recently regarded as a potential biodegradable implant material due to its low density, high strength/weight ratio and similar elastic modulus to that of human bone (40–57 GPa) [11,12]. Mg or its alloys are non-toxic to the human body. Mg deficiency has been found to exacerbate the risk of hypertension, cardiac arrhythmias and osteoporosis, etc. [13]. There is growing evidence that if the release of Mg^{2+} is acceptable by human body, it will help to stimulate the healing of bone tissue [14–16]. Moreover, magnesium has a high negative standard electrode potential (−2.37V at 25 ◦C) and thus it corroded relatively faster than other metallic materials, especially in Cl^- containing aqueous environment (NaCl is a part of human digestive and blood fluids), indicating that magnesium can degrade/or be corroded in a human body environment. Thus interface problems (as interface loosening and inflammation) can be resolved and the second removal surgical operation in the case of bone screws and plates can be avoided. Mg alloy can gradually be dissolved and absorbed after implanting [17–20]. After the recovery or healing, the implant should be gradually dissolved, consumed or absorbed by the human body [21]. Extruded Mg alloy as AZ31E is getting more and more widely used because of their considerably high plasticity in comparison with the die-cast Mg alloys [22, 23]. Poor corrosion resistance is one of the major problems that prevent the widespread of magnesium alloys in outdoor applications due to its high

chemical and electrochemical activity compared with other structural metals such as steels and Al alloys. There are two primary reasons for the poor corrosion resistance of magnesium alloys; the first reason is the internal galvanic corrosion by the second phases (β-phase or intermetallic compounds $Mg_{17}Al_{12}$ in the microstructure of AZ series of Mg-alloys) or impurities; the second reason is that the hydroxide film on magnesium is much less stable than passive films that form on metals such as aluminium alloys and stainless steel [24].

To improve the corrosion resistance, further surface treatment is needed in order to protect Mg alloys against corrosion and to achieve the resistance necessary for many applications (as electrochemical plating, anodizing, chemical and physical vapor deposition and plasma polymerization).

It has been recognized that the use of organic inhibitors, particularly the naturally occurring ones of plants and algal origins are viable, and highly beneficial since they are essentially non-toxic, environmentally friendly, readily available, renewable and inexpensive [25,26].

The main goal of this investigation is to search for naturally produced and safe compounds with antioxidant and anticorrosion properties from the hydrophyte *Eichhornia crassipes* as a natural additive to AZ31E magnesium alloy. This study also focused on the protective efficiency and electrochemical behavior of the alloy in sodium chloride solution using Electrochemical Impedance Spectroscopy and polarization techniques. Identification of extract and fractions that record the highest corrosion protection was considered.

2. MATERIALS AND METHODS

2.1. Preparation of E. crassipes extracts

E. crassipes (Mart) Solms .was collected from El-Zomor canal, Giza ,in spring 2009, cleaned from epiphytes ,washed then air dried ,grinded and kept in labeled glass jar till use .A known weight of the air dried grinded sample (100 grams) was successively extracted with organic solvents of different polarities [Hexane (E_1), ethyl acetate (E_2) and methanol (E_3)]. Filtration of extracts and evaporation of solvents by rotary evaporator (at 40 – 45°C) were followed by weighting the extract residues and preparating different concentrations from each extract.

2.2. Dertermination of active compounds with antioxidant activity

2.2.1. Chlorophylls and carotenoids

The lipophilic pigments (chlorophylls and carotenoids) in different extracts were determined according to Holden method [27]. The absorbance of the extract was measured at 663, 645 and 452 nm.

2.2.2. Determination of total phenolic content

Total phenolic contents of different extracts were determined by Folin-Ciocalteu method [28]. The absorbance of the reaction mixture was spectrophotometrically measured at 750 nm against a blank. Gallic acid (GA) was used for the preparation of standard curve.

2.2.3. Determination of total alkaloids

Total alkaloids were determined according to Sabri *et al*. (29). The extract was dissolved in 2 ml of chloroform, then 25 ml of 0.02 N H2SO4 were added, The resulting solution was warmed to driven off the chloroform, cooled and titrated back the excess acid with 0.02 N NaOH solution, using methyl red as an indicator.

2.2.4. Determination of total terpenoids

Ten ml of extract was transferred to wide nick test tube, and then placed in an oven at 100ºC for 1 h. After cooling; 5 ml freshly prepared vanillin reagent (0.7% in 65% H2SO4) was added. Then the tube was heated at 60 ± 1ºC in a water bath for 1 h. after cooling in crushed ice bath the absorbance was measured at 473 nm within 1 min against blankprepared by using distilled water instead of terpenoids solution (30).

2.3. Antioxidant activity

2.3.1. By DPPH method

The antioxidant activity of water hyacinth extracts was evaluated by using the 2.2'diphenyl-1-picryl hydrazyl (DPPH) assay [31]. The extracts and its fractions (50-200 µg/ml) were added to 1 ml of a 0.004% (w/v) of DPPH in methanol (100% v/v). After 30 min incubation period at room temperature the absorbance at 517 nm was compared to DPPH in methanol without an extract sample (blank), and butylated hydroxyl anisole (BHA) was used as positive control. The percent inhibition of free radical formation (I %) was calculated as I% = (A blank _ A sample / A blank) × 100 Where; A blank is the absorbance of the control reaction (containing all reagents except the extract), and A sample is the absorbance of the mixture containing the extract. The experiment was carried out in triplicate.

2.3.2. By ABTS method

This assay was based on the ability of different substances to scavenge 2,2'- azino-bis (ethylbenzthiazoline-6- sulfonic acid ($ABTS^+$) radical cation in comparison to a standard (BHA, 50-200 µg/ml). The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 hrs. until the reaction was completed and the absorbance was stable. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.700

± 0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 ml of $ABTS^+$ solution and 0.1 ml of tested samples (at 50 and 100 μg/ml) and mixed for 45 s; measurements were taken immediately at 734 nm after 1 min. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation: E%= ((Ac-At)/ Ac) x 100, where: At and Ac are the respective absorbances of tested samples and ABTS+, was expressed as μmol [32].

2.3.3. Fractionation of the crude methanolic extract

Using precoated thin layer chromatographic plates (TLC_{F254}), the methanolic crude extract of *Eichhornia crassipes* was fractionated using different combinations of Hexane/ethyl acetate as mobile phase (9:1 , 8.5:1.5 , 8:2 , 7.5:2.5 , 7:3 , 6.5:3.5 , 5:5 v/v). It was found that the combination 7.5:2.5 v/v was the best one for the separation of compounds. The separated fractions (a-e) seen under the Ultraviolet lamp were scratched, eluted by the same mobile phase, filtered in preweighted vials, then solvents were evaporated and vials were reweighted to determine the weight of each fraction. Antioxidant activity of each fraction was carried out using DPPH and ABTS methods as it was preformed to crude methanolic extract.

2.4. Electrochemical studies on E. crassipes.

2.4.1. Specimen preparation

An electrode of magnesium alloy (AZ31E) was donated from department of Mining ,Metallurgy and Materials Engineering, laval University, Canada ,with chemical composition (Wt%) : 2.8Al ,0.96Zn , 0.28 Mn ,0.0017 Cu ,0.0111Fe ,0.0007 Ni ,0.0001Be and balance Mg for AZ31E.The sample was divided into small coupons . Each coupon was welded to an electrical wire and fixed with araldite epoxy resin in glass tube leaving cross –sectional area of the specimen 0.196cm^2. The surface of the test electrode was mechanically polished by emery papers with 400-1000 grit to ensure the same surface roughness, degreasing in acetone, rinsing with ethanol and drying in air. The cell used was a typical three electrode one fitted with a large platinum sheet of size 15x20x2mm as a counter electrode (CE), saturated calomel (SCE) as a reference electrode (RE) and the alloy as the working electrode (WE).

2.4.2. Solutions and extract concentrations

The solution used was 3.5% NaCl solution (artificial seawater) which was prepared by triply distilled water. Different concentrations (20, 40 and 80 ppm) from each extract (E_1, E_2, E_3) were prepared and the promising concentration of the pronounced corrosion inhibiting extract was used for the electrochemical studies of fractions (a-e).

2.4.3. Electrochemical measurements

The impedance diagrams were recorded at the free immersion potential (OCP) by applying a 10mv sinusoidal potential through a frequency domain from 10KHZ down to 100 mHZ . The instrument used was the electrochemical workstation IM6e Zahner-elektrik,Gmbh , (Kronach,Germany) .The electrochemical measurements were always carried inside an air thermostat which was kept at 25^{o} c ,unless otherwise stated . All potentials were measured and given with respect to SCE (E=0.241 V/SHE). The open circuit potential (OCP) was measured in 3.5% NaCl solutions by mass. The anticorrosion behaviors were evaluated using the polarization curves and electrochemical impedance spectroscopy (EIS).

2.5. Statistical analyses

Data were subjected to an analysis of variance. Combined analysis was used for the two annual seasonal cycles. Means were compared by calculating the Least Significant Difference (LSD) values at alpha 1% ($P < 0.01$) according to Snedecor and Cochran [33].

3. RESULTS AND DISCUSSION

3.1. Effect of pigments and phenolic contents in extracts on antioxidant activity

Table 1. Determination of pigments (Chlorophyll a,b & total chlorophylls, carotemoids) and total phenolic contents (as mg/g and gallic acid equivalents (GAEs)).

Extract	Total phenol (as GAEs)	Chlorophyll a	Chlorophyll b	T.chlorophyll	T.Carotenoids	Weight of extracts (g)
Hexane(E_1)	28^{b}	0.23^{c}	0.0^{c}	0.23^{c}	0.12^{c}	0.44
Ethyl acetate(E_2)	27^{c}	0.32^{b}	0.08^{b}	0.40^{b}	1.43^{a}	0.41
Methanol (E_3)	40.5^{a}	0.56^{a}	0.57^{a}	1.13^{a}	1.12^{b}	0.89
LS D at 0.01	0.413	0.003	0.003	0.003	0.003	0.004

Values with different superscript letters within the same column are significantly different ($P < 0.01$).
GAEs, gallic acid equivalents

Weight of methanol extract (Table 1) was nearly double those of hexane and ethyl acetate (0.89 ,0.44 and 0.41mg respectively) ,also chlorophyll a,b and total chlorophyll contents of methanol extract (E_3) were highly pronounced than those of ethyl acetate (E_2) and hexane extracts (E_1) (1.13 ,0.40 and 0.23 mg/g respectively) as illustrated in Table 1 .While carotenoids content of ethyl acetate

was slightly greater than that of methanol extract and carotenoids of hexane was very low (1.43 ,1.12 and 0.12 mg/g respectively) . Concerning phenol content (Table 1), methanol extract showed the highest level (40.5 GAE) followed in descending order by that of hexane (28GAE) and ethyl acetate (27GAE).

Table 2. Antioxidant activity (%) of successive extracts from *Eichhornia crassipes* against DPPH and ABTS radicals

Extracts	Scavenging activity %			
	DPPH method		ABTS method	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
Hexane(E_1)	25.11[d]	43.15[d]	40.20[d]	55.95[d]
Ethyl acetate(E_2)	40.30[b]	59.71[b]	41.81[b]	54.80[b]
Methanol(E_3)	38.53[c]	55.42[c]	43.60[c]	56.93[c]
BHA (Standard)	63.40[a]	81.85[a]	74.60[a]	89.94[a]
LSD at 0.01	0.0326	0.0326	0.0326	0.0326

Values with different superscript letters within the same column are significantly different ($P < 0.01$).

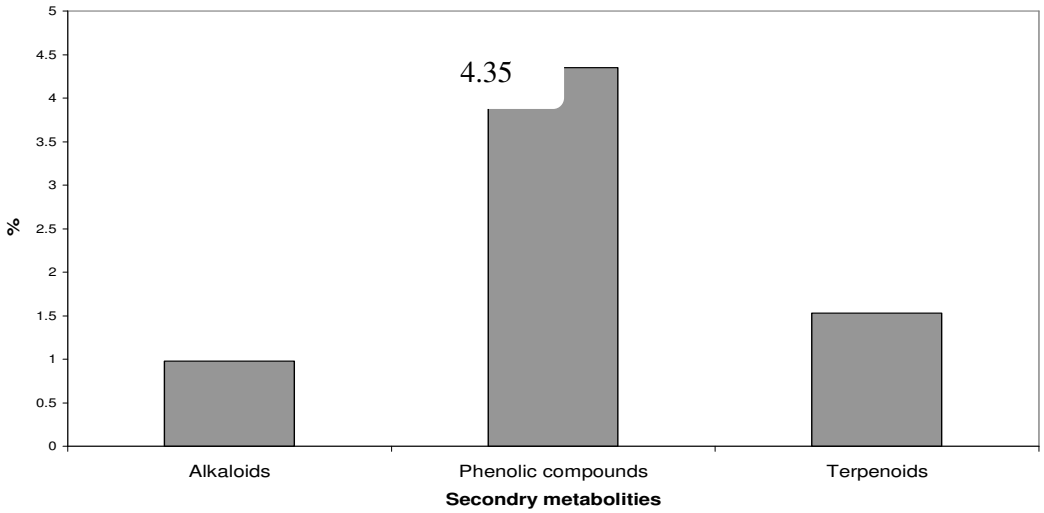

Figure 1. Phytochemical analysis of major secondary metabolites (as %) in methanolic extract of *E. crassipes*.

These results were confirmed by the great content of phenolic compounds in methanol extract as recorded by its phytochemical analyses (Fig.1). The antioxidant activity of the three extracts E_1, E_2 , E_3 by both DPPH and ABTS methods (Table 2) revealed that , the activity is concentration dependant

and it was increased with doubling the extract concentration (50, 100 µg / ml) . Using DPPH method, the antioxidant activity (at higher concentration, 100 µg /ml) of ethyl acetate (E_2) was greater than those of methanol (E3) and hexane (E1). ABTS method was known to be more sensitive than that of DPPH, methanol extract exhibited higher antioxidant scavenging activity followed by that of hexane and ethyl acetate (56.9, 55.9 and 54.8 % respectively). This higher activity may be due to synergistic effect of its total components of chlorophylls, carotenoids and phenolic compounds. The crude methanolic extract (E_3) containing higher contents of pigments (Chl a ,b ,carotenoids) ,phenolic compounds (Table 1,Fig 1) as well as alkaloids and polysaccharides (not determined here) .Each of these components manifest certain and specific free radical scavenging activity as recorded in many literatures [34,35]. Natural antioxidants as carotenoids, chlorophylls, phenolic compounds exhibit potent scavenging activity against ROS [36]. The phenolic compounds extracted from different sources were known to exert potent antimicrobial and antioxidant activities [37].

It is well known that the scavengers (antioxidants) of the reactive oxygen species may have inhibition efficiency of metals (or alloys) corrosion. As the successive extracts of *Eichhornia crassipes* (E_1,E_2,E_3) where shown to exhibit antioxidant activity of the order $E_3 > E_2 > E_1$, it was interesting to test electrochemically the anticorrosion behavior of these extracts on the widely applied AZ31E Mg alloys.

3.2. Electrochemical measurements

Figure 2, represented the potentiodynamic polarization curves of *Eichhornia crassipes* in blank solution without and with 80ppm of *Eichhornia crassipes* extract.

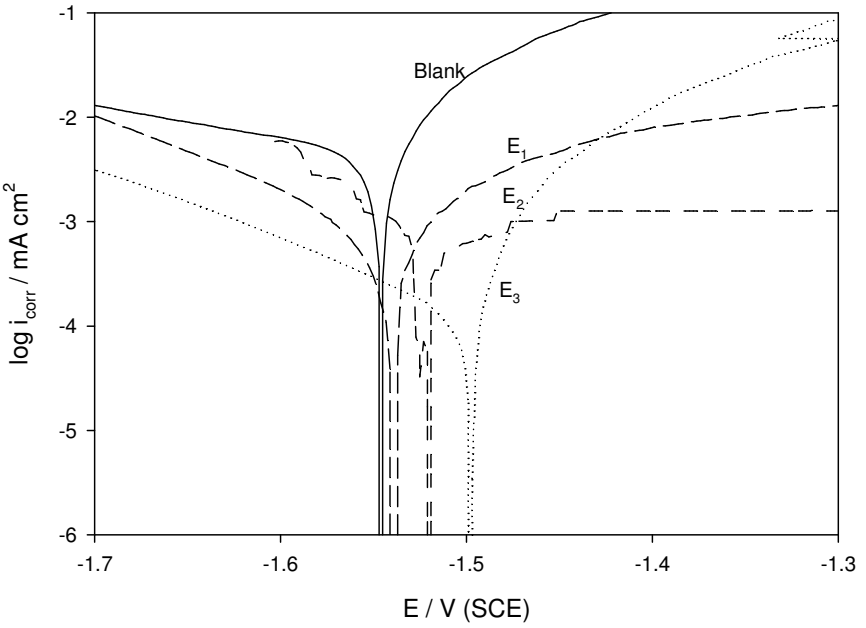

Figure 2. Equivalent circuit model representing two time constants for an electrode/electrolyte solution interface.

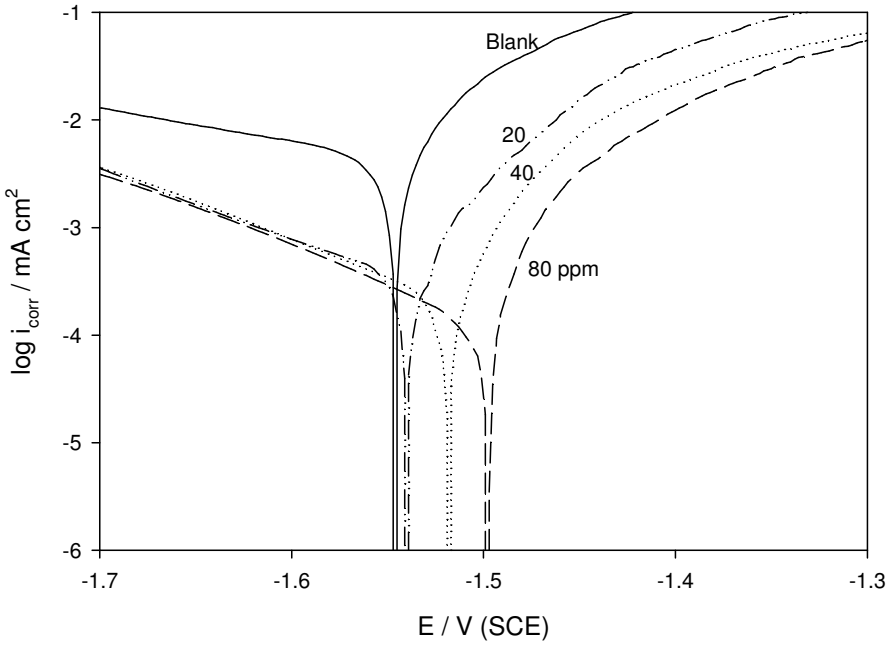

Figure 3. Dependence of R_T on the concentration for AZ31E in 3.5% NaCl containing E1, E2 and E3.

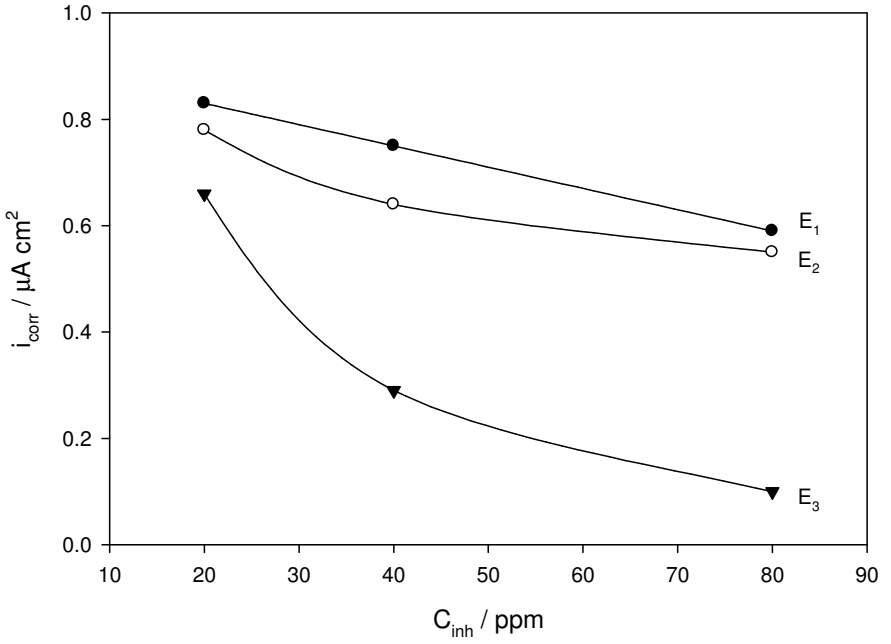

Figure 4. polarization curves of AZ31E in 3.5% NaCl without and with 80 ppm of E_1, E_2 and E_3.

The data showed that, the addition of the three *Eichhornia crassipes* extracts (E_1, E_2, E_3) shifted the corrosion potential (E_{corr}) initially slightly in the positive direction. Figs. 2, 3 represented the polarization curves of AZ31E in blank solution without and with 80 ppm of E_1, E_2, $E_3$and different

concentration of E_3 respectively. Fig.4, illustrated the relation between i_{corr} and the concentration of E_1, E_2 and E_3. It is clear from this figure that in all three components the i_{corr} decreases with increasing the concentration. Also, the order of i_{corr} is: $i_{corr}(E_3) < i_{corr}(E_2) < i_{corr}(E_1)$. Electrochemical impedance (EIS) is a technique with small perturbative signal and the surface damage of the sample is very little. Besides, the corrosion mechanism can be estimated by analyzing the measured electrochemical impedance spectrum. In these experiments, AZ31E alloy was tested in 3.5% NaCl solution without and with 40 ppm of the three extracts: E_1, E_2 and E_3. It can be seen from Fig.5, that these diagrams show resistive regions at high and low frequencies and capacitive contribution at intermediate frequencies. The impedance (|Z|) as well as the phase shift θ for the alloy is clearly found to depend on both the type of three extracts and their concentrations.

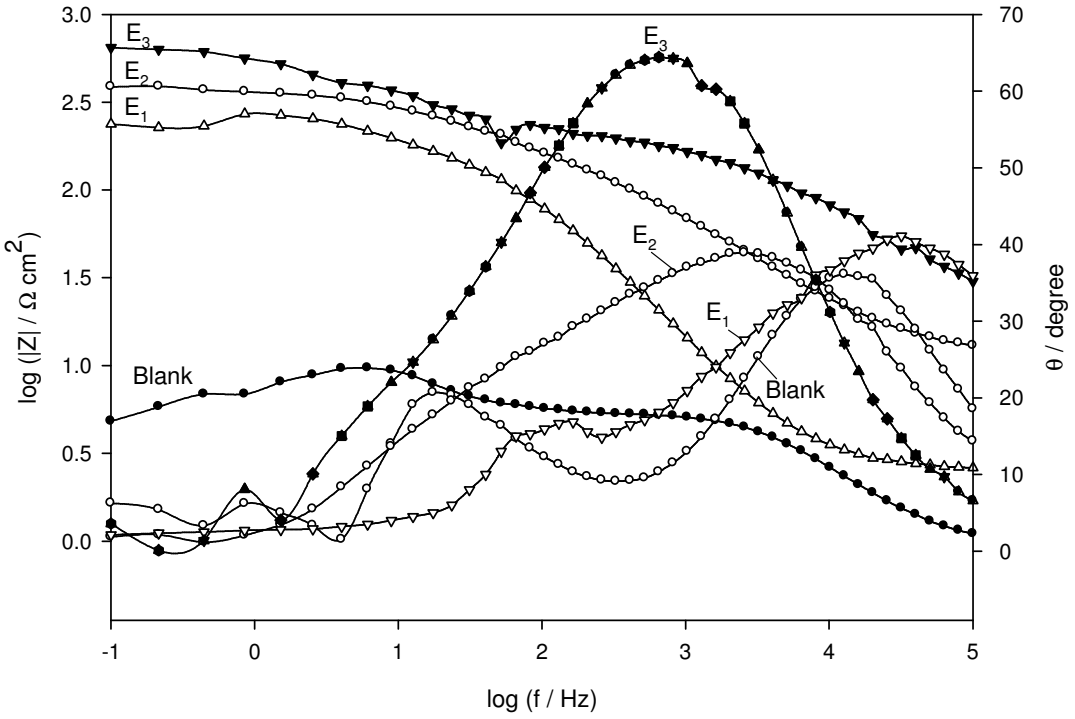

Figure 5. polarization curves of AZ31E in 3.5% NaCl without and with different concentrations of E_3.

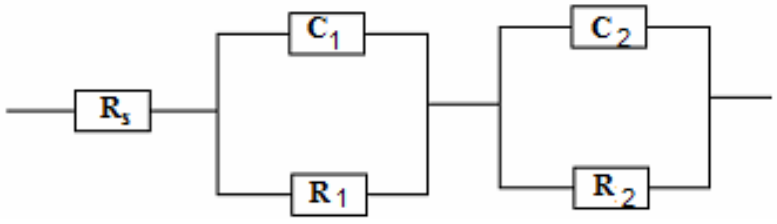

Figure 6. Relation between corrosion current and concentrations of E_1, E_2 and E_3.

The Bode format of Fig. 5 confirms the presence of two time constants as there are two maximum phase lags appears at medium frequencies (MF), and low frequencies (LF). On the other hand, for the impedance diagrams with two time constants the appropriate equivalent model, shown in Fig.6 , consists of two circuits in series from R_1C_1 and R_2C_2 parallel combination and the two are in series with R_s. In this way C_1 is related to contribution from the capacitance of the outer layer and the faradaic reaction therein and C_2 pertains to the inner layer, while R_1 and R_2 are the respective resistances of the outer and inner layers constituting the surface film, respectively [23, 38]. Analysis of the experimental spectra was made by best fitting to the corresponding equivalent circuit using Thales software provided with the workstation where the dispersion formula suitable to each model was used [38]. In this complex formula an empirical exponent (α) varying between 0 and 1, is introduced to account for the deviation from the ideal capacitive behavior due to surface inhomogeneties, roughness factors and adsorption effects [39]. In all cases, good conformity between theoretical and experimental was obtained for the whole frequency range with an average error of 3%. For this model the electrode impedance is represented by the following transfer function [40,41]:

$$Z(\omega) = R_o + \frac{R_1}{1+R_1C_1(j\omega)^{\alpha_1}} + \frac{R_2}{1+R_2C_2(j\omega)^{\alpha_2}} \quad (1)$$

The above formula takes into account the deviation from the ideal RC behaviour and considers, for a more realistic approach that each oxide layer as non-homogeneous. Thereby, the impedance associated with the capacitance of each layer is described by a constant phase element (CPE). By this way C_1 is related to contributions from the capacitance of the porous (outer) layer and C_2 of the barrier inner layer while R_1 is the resistance of the outer porous layer and R_2 of the barrier layer. For this model, the total reciprocal capacitance is given by the well-known relation:

$$C_T^{-1} = C_1^{-1} + C_2^{-1} \quad (2)$$

Figs. 7 displays the total resistance ($R_T = R_1 + R_2$) of the surface film as a function of the extract concentration. The obtained results indicated that (Fig.7) both R_T and C_T^{-1} increased rapidly with the increase of concentration, and also their values depend on the type of extract. In general the values of C_T^{-1} and R_T are in the order of: $E_3 > E_2 > E_1$. This result could be due to formation of inhibiting species by the biofilm as suggested by Eashwar et al [42] or to a reduction of the chloride concentration at the surface which was covered by the biofilm. They observed that the impedance for these two materials did not change with time despite formation of a biofilm and suggested that the porous and water-like structure of the biofilm did not produce the characteristic changes in the impedance spectra that result from the application of protective polymer coatings [43].

The higher antioxidant and anticorrosion efficiencies of the promising methanol extract (E_3) were encouraging to go forward for the identification of the active compounds in this extract, so fractionation of E_3 was performed.

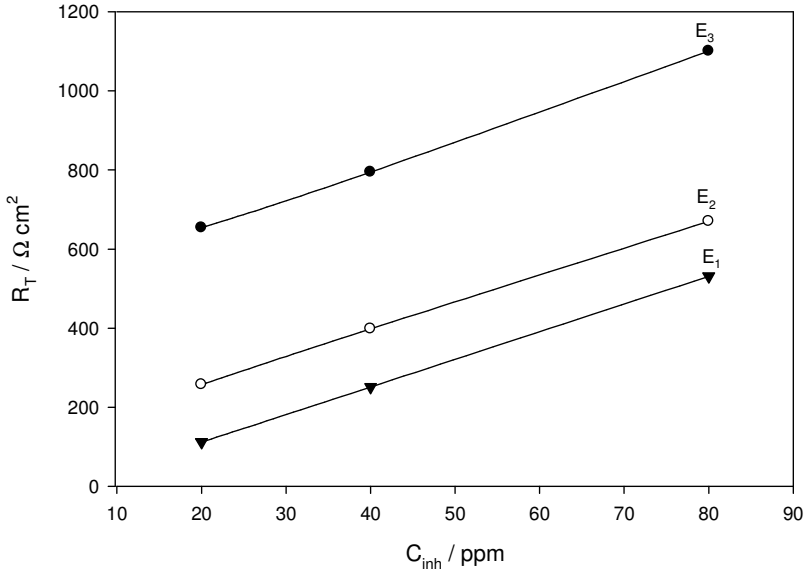

Figure 7. Bode plots of AZ31E in 3.5% NaCl without and with 40 ppm of E1, E2 and E3.

3.3. Fractionation of the promising crude extract and its antioxidant activity

The promising result of methanol extract (E_3) was encouraging to identify the components of this polar extract.

Table 3. Antioxidant activity of the crude extract and its fractions (a-e) using DPPH and ABTS methods.

Fractions .	Antioxidant activity (%)			
	DPPH method		ABTS method	
	100 µg/ml	200 µg/ml	100 µg/ml	200 µg/ml
Crude extract (E_3)	62.6^{b}	77.5^{b}	64.3^{b}	80.0^{b}
a	49.4^{d}	51.3^{d}	49.9^{c}	50.6^{d}
b	48.8^{f}	49.4^{f}	48.5^{f}	52.2^{c}
c	49.0^{e}	52.3^{c}	49.0^{d}	50.1^{e}
d	48.0^{g}	48.8^{g}	48.0^{g}	48.7^{g}
e	49.5^{c}	49.8^{e}	48.8^{e}	48.8^{f}
BHA (Standard)	88.9^{a}	92.3^{a}	86.4^{a}	91.60^{a}
LSD	0.052	0.054	0.045	0.057

Values with different superscript letters within the same column are significantly different ($P < 0.01$).

So fractionation by precoated TLC (Fig .8) revealed the separation of five fractions (a-e). The antioxidant activity (Table 3) of these five fractions (by both DPPH and ABTS) illustrated that all fractions exhibited more or less comparable and moderate activities ranging between 48.0 and 52.3 %

comparing with the standard BHA(86.4-88.9 % at 100 µg/ml and 92.3-91.60 % at 200 µg/ml),while the crude methanolic extract(E_3) using both methods recorded higher activities (62.6 – 80.0 %) than its five fractions depending on the extract concentration (100 -200µg / ml) .

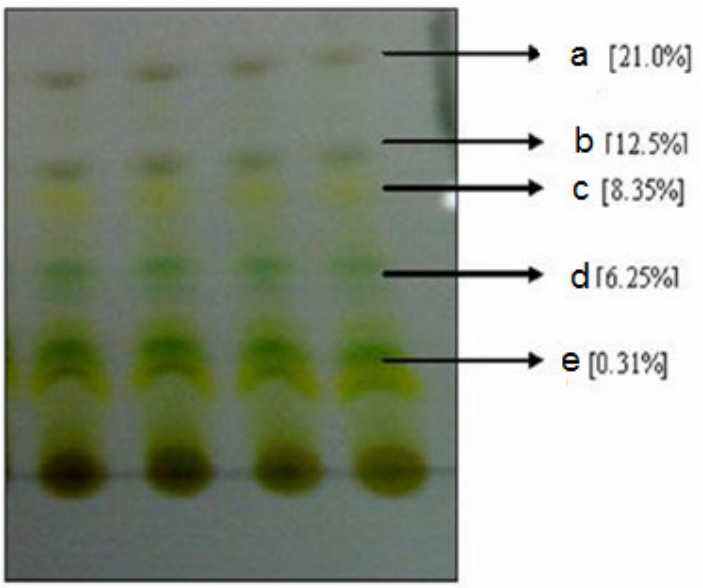

Figure 8. Fractionation of crude methanolic extract of E. crassipes using silica gel TLC and hexane / ethyl acetate (8.5: 1.5, v/v) as mobile phase.

Identification of the active compounds in the fractions (by MS) revealed that (Fig. 9&10) fraction (a) is an alkaloid (M.Wt 352 Da) while fractions (b),(c) ,(d), (e) are phthalate derivatives (of M.Wts 278,390,662 Da). The higher antioxidant activity of the crude methanolic extract (E_3) indicated that synergistic effect occur between the five components of the fractions leading to the higher activity of the crude extract including them and the activity was decreased as these compounds are separated by fractionation . The higher activity may be due to the presence of the great number of double bonds, amine and hydroxyle groups known by their oxygen scavenging and hydrogen donating antioxidant activities. Phthalate derivatives (esters) including dioctyl phthalate , as fractions (c), (d) and (e) in this study, [44,45] ,dibutyl phthalate , phthalic acid bis (iso-octyl) ester,iso-monyl phthalate [46], 1,2 benzene dicarboxylic acid bis (2-ethyl hexyl) ester ,as fraction (b) in this work [47,48] were extracted from different bacteria, marine organisms, seaweed species and exhibited antibacterial as well as antimicrobial activities. The crude methanolic extract (E_3) and its separated fractions (a-e) in addition to their scavenging antioxidant activity, they were recorded to have antibiotic and anticancer activities [49, 50]. Oxygen in the air or water, chemically react with metals leading to its corrosion. The technique of adding inhibitors to the environment of a metal is a well known method of controlling corrosion in many branches of technology. Reduction of oxygen availability in the corroding system (by oxygen scavengers) as well as the presence of chemical barrier of corrosion inhibitor between the electrode surface and the oxygen, retarding or even inhibiting the rate of metal

corrosion [9,10,51]. The obtained results in this investigation showed that ,the methanolic crude extract (E_3) of *E. crassipes* exhibited higher antioxidant activity as well as anticorrosion behavior than other extracts (ethyl acetate E_2 and hexane E_1) with the order $E_3 > E_2 > E_1$.

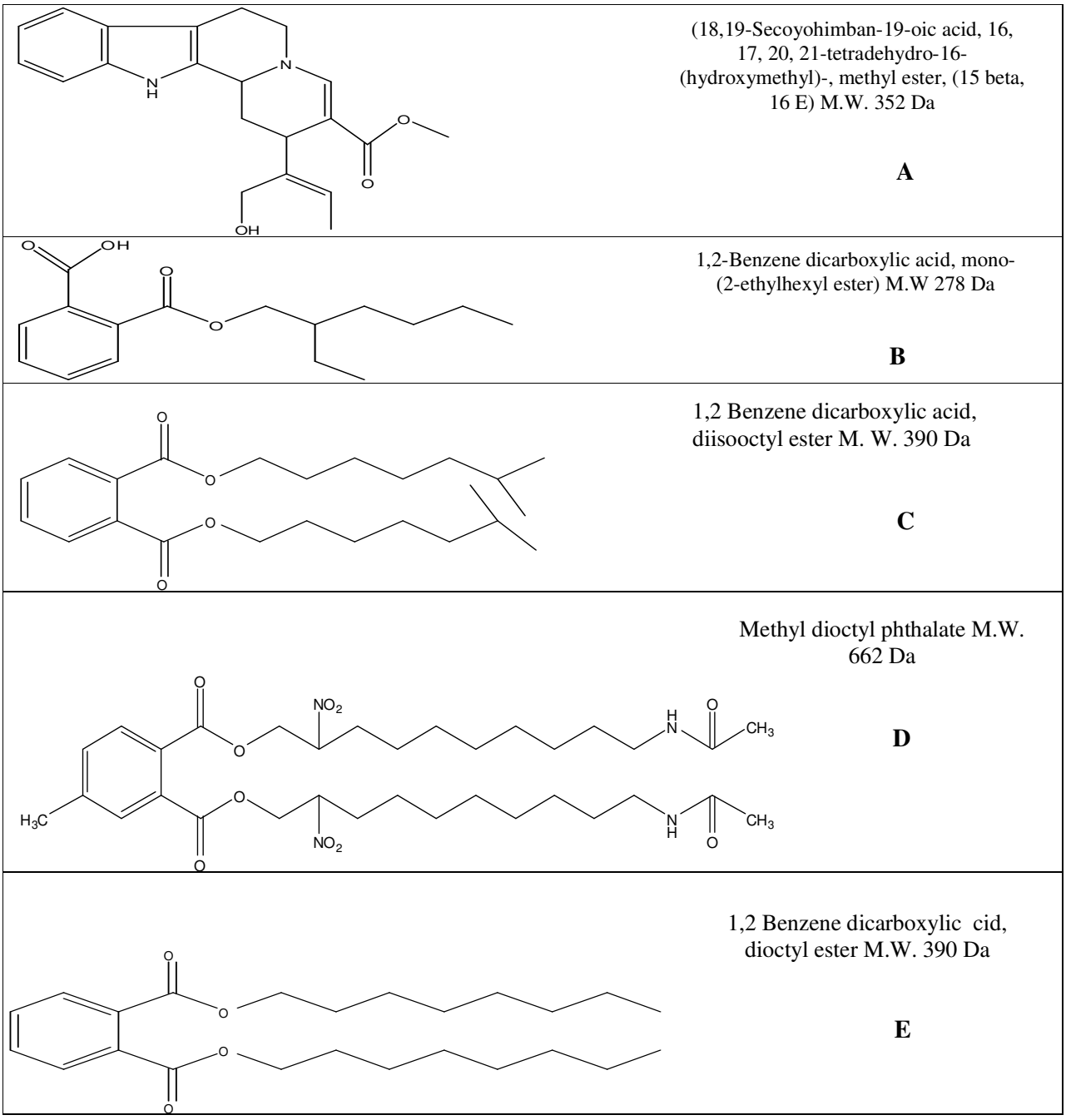

Figure 9. The identified five fractions (A-E) of the crude methanolic extract (by Mass Spectra)

The five fractions of the crude methanole extract demonstrated comparable free radical scavenging activity (antioxidant) as well as inhibiting the corrosion of AZ31E magnesium alloy. Fraction (a) which is an alkaloid, recorded the greatest rate of inhibition followed in descending order by phthalate derivatives of the other fractions(b,c,d,e) in the order a > d > b > c > e .

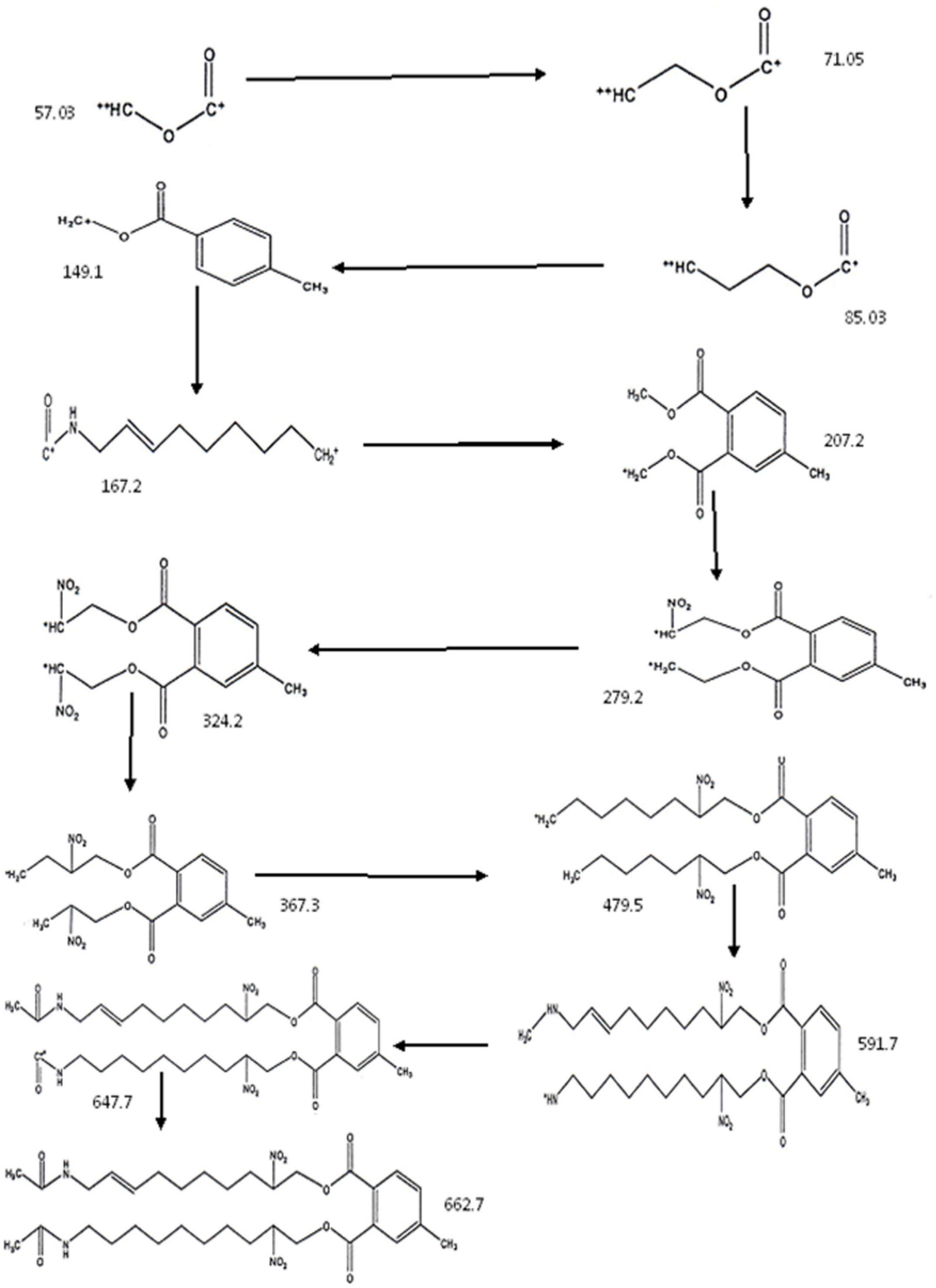

Figure 10. Fragmentation patterns of active compounds separated from *E. crassipes* crude Methanolic extract (by MS).

The antioxidant activity of the five fractions (a-e) that separated from the promising methanol extract (E_3) were tested for their anticorrosion efficiency of the AZ31E Mg alloy.

3.4. Electrochemical measurements on the fractions (a-e pure compounds) of the promising methanol extract (E_3)

The bode blots of 20 ppm of E_3 and the five fractions shown in Table 4 and Figure 11 indicated that some of these pure compounds (a,d,b) demonstrated higher resistance of alloy corrosion than the crude extract E_3 , While others (c,e) showed lower resistance.

Table 4. Impedance parameters for AZ31E alloy in 3.5% NaCl containing 20 ppm of E_3 and its fractions at 298 K

	R_S / Ω cm^2	R_1 / Ω cm^2	C_1 / μF cm^{-2}	α_1	R_2 / Ω cm^2	C_2 / μF cm^{-2}	α_2
E3	20.30	31.2	25.96	0.8	530	3.8	0.9
a	34.00	98.7	14.66	0.7	930	2.8	0.9
d	30.38	61.1	21.19	0.7	800	3.2	0.9
c	22.47	30.1	26.72	0.9	120	6.1	0.9
b	18.30	34.8	23.19	0.8	590	3.7	0.9
e	23.77	25.5	28.94	0.9	80	6.8	0.9

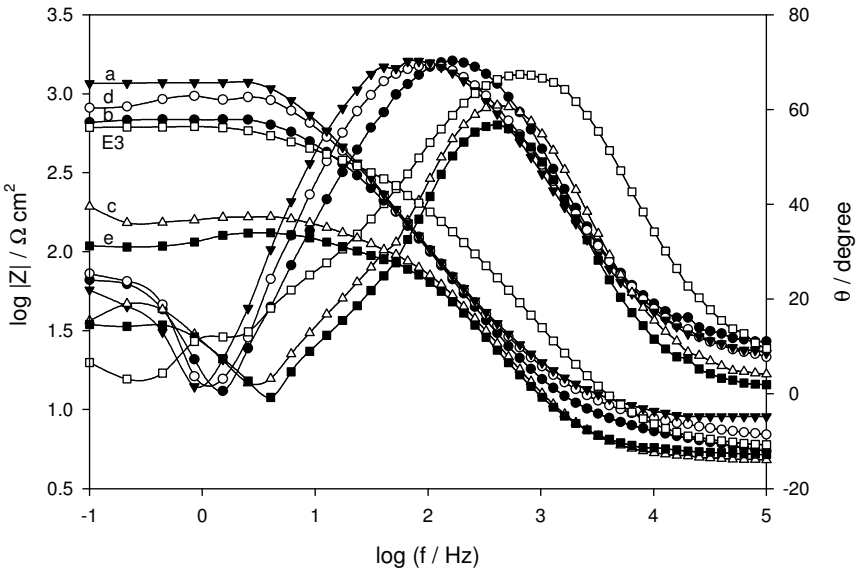

Figure 11. Bode plots of AZ31E in 0.15 M NaCl without and with 20 ppm of E3 and its fractions.

The variable anticorrosion behaviors of the crude methanolic extract (E_3) and fractions (a-e) were ranked as a > d > b > E_3> c > e .This means that fractions (a) ,(d) , (b) recorded higher rate of inhibition of AZ31 E magnesium alloy corrosion than both the crude extract E_3 and the other fractions

(c) & (e). Fractions (a), (d) have a chemical structure with many double bonds, hydroxyl , and amine groups which have high oxygen scavenging activity leading to oxygen reduction in the corroding system and consequently reduces the rate of metal corrosion.. It may also form a monolayer of chemical barrier between the alloy electrode and the corroding system, increasing the rate of corrosion inhibition [51]. The obtained results were confirmed by the studies reported by many authors [52-54] who found that certain organic substances with polar fractions containing nitrogen, sulfur and/or oxygen in the conjugated system severely inhibit the corrosion of steel in acidic and alkaline environments. The inhibitory behavior of the organic compounds is that it forms a protective layer between the metal (or alloy) surface and the corrodent thereby reducing the corrosion rate of that metal. Fraction (b) have a low molecular weight (278 Da) with few number of double bonds, hydroxyl group, so moderate oxygen scavenging activity as well as moderate rate of inhibition of corrosion were recorded. Fractions (c) and (e) inspite of the fact that both have the same molecular weight (390 Da) and number of double bonds in their chemical structures , but little difference in the side chain is present which may affect their anticorrosion behavior (diisooctyl ester in (c) and dioctyl ester in (e) .These corrosion inhibitors may reduce the anodic or cathodic process by sample blocking the active sites of the metal surface or may act by increasing the potential of the metal surface causing its entrance to the passivation region with a natural oxide film formation. Some authors [55-57] examined the adsorption conformation and orientation of protective films formed by corrosion inhibitors using a combination of reflectance fourier transform infra red (FTIR) spectroscopy, ellipsometry and electron spectroscopy chemical analysis (=X-ray photoelectron spectroscopy, XPS) they found that some corrosion inhibitors posses long chain hydrocarbon (tail) and a more polar head. At higher concentration of the inhibitor, the hydrocarbon chain will become increasingly vertically arranged relative to the metal surface; promoting greater packing of the inhibitor with self-assembled multiple monolayers at the interface making it more impenetrable to the corroding environment. Methanolic extract (E_3) have recorded higher antioxidant activity than all the five fractions (a-e) which may be attributed to the synergistic effects of all the compounds in the extract, but concerning anticorrosion behavior of this crude extract, it demonstrated slightly moderate anticorrosion affinity. This may be due to the formation of somewhat porous chemical barrier from the five compounds due to their different chemical structural features, the poor packing of these molecules on the electrode surface, leading to partial corrosion resistance [57]. In some cases the aromatic functional groups of the hydrocarbon chain that produces antioxidant behavior (commonly OH^-, NH^-, $^-S^-$… etc) since the polar functional groups that produce antioxidant activity is typically sterically hindered during the monolayer formation of the compounds on the surface of the alloy, causing a decrease in the rate of corrosion inhabitation.

4. CONCLUSIONS

1. The obtained results in this investigation recorded that, water hyacinth crude extracts (E_1, E_2, E_3) and pure compounds (a-e) exhibited antioxidant as well as anticorrosion behaviours of AZ31E Mg alloys.

2. $I_{corr.}$ Increases with increasing extract conc. In the order $I_{corr.}$ $(E_3) < I_{corr.}$ $(E_2) < I_{corr.}$ (E_1).

3. Total resistance R_T^{-1} increased rapidly with the increase of the extract conc. And depend on the type of extract.

4. C_T^{-1} and R_T values are in the order of $E_3 > E_2 > E_1$.

5. The problem of navigation, drinking water deterioration induced by the invaded hydrophyte (*E. crassipes*) to the River Nile and its canals can be resolved by collecting and using this weed in different fields.

6. *E. Crassipes* can be used for the manufacture of moderate antibiotic, antioxidant and anticancer against different cell lines. Its extracts and pure compounds can be used as corrosion inhibitor of the worldwide applications of AZ31E Mg alloy.

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