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Review

Biological activities and anticorrosion efficiency of water hyacinth(*Eichhornia crassipes*)

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***Eichhornia crassipes* (Mart) solms is an invasive macrophyte, causing serious problems to the network of irrigation and drainage canals in the Nile Delta region and different places around the world. 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. Spectroscopic methods of the separated fractions revealed the presence of different compounds of variable anticancer and antioxidant activities which acted synergistically in the crude extract leading to its greatest activities.**

Key words: Water hyacinth, chemical composition, biological activities.

INTRODUCTION

Water hyacinth, *Eichhornia crassipes* (Mart) Solms, originated in the state of Amazon, Brazil, spread to other regions of South America, and was carried by humans throughout the tropics and subtropics. It is now widespread and recognized as one of the top 10 weeds in the world. Water hyacinth has invaded Africa, Asia, North America and occurs in at least 62 countries by 2010. It causes extremely serious ecological, economical and social problems in regions between 40° North and 45° South (Gao and Bo, 2004). *E. crassipes* forms dense monocultures that can threaten local native species diversity and change the physical and chemical aquatic environment, thus altering ecosystem structure and function by disrupting food chains and nutrient cycling. The large, dense monoculture formed by this species covers lakes and rivers, blocking waterways and interfering with the water transport of agriculture products, tourism activities, water power, and irrigation of agricultural fields. Dense mats of water hyacinth can lower dissolved oxygen levels in water bodies leading to reduction of aquatic fish production. Water hyacinth is very efficient in taking up calcium, magnesium, sulfur, ferric, manganese, aluminum, boron, copper, molybdenum, zinc, nitrogen, phosphorus, and potassium favoring its growth over other aquatic species (Dandelot et al., 2008). When this macrophyte (water hyacinth) dies, sinks and decomposes, the water becomes more eutrophic due to the large release of nutrients (Gao and Bo, 2004). Water quality deteriorated, clean drinking water can be threatened and human health impacted. Aggressive growth of *E. crassipes* was correlated with increased temperature, high solar radiation and sunshine duration which may result in an intensive plant growth during summer that may be increased by global warming. High biomass production of water hyacinth corresponded with large amounts of phenolic allelochemicals in the water, which may also help in the process of invasion. High air temperatures in summer (35°C) caused an increase in the rate of evapo-transpiration leading to decrease in water level and consequently a possible increase in allelochemical concentration in the aquatic habitats (Dandelot et al., 2008).

Although, many studies were carried out on water hyacinth concerning either its growth inhibition by coexisting with micro algae in the same ecosystem (Sharma, 1985; Sharma et al., 2005) or its antialgal activity manifested on many algal species becoming two different algal divisions, yet little is known about the active compounds responsible for these effects. Only Jin

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et al., (2003) and Awad (2008) separated allelochemicals from crude *E. crassipes* extract by fractionation which have allelopathic effect on the tested green algae (Jin et al., 2003) or inhibiting germination of seeds and growth of seedlings of some crop plants (Awad, 2008). 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 (Matsukawa et al., 1997). 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 (Grillo and Dulout, 1995). 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 (Abd El-Baky et al., 2008). 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 (Zia-Ul-Haq et al., 2012). Reduction of oxygen availability in the corroding system and presence of a barrier (physical, chemical, and biological) between the electrode surface and oxygen, retarding or even inhibiting the rate of metal (or alloy) corrosion (Mansfeld, 2007). 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.

This work aimed to illustrate the chemical compositions of water hyacinth and its biological activities as antimicrobial (antibacterial, antialgal, antifungal, and antiyeast), antioxidant, anticancer, and anticorrosion activities.

ANTIMICROBIAL ACTIVITY

The crude methanolic extract as well as the thin layer chromatography (TLC) separated fractions were tested for antimicrobial activity using four bacterial species (Gram positive bacteria: *Bacillus subtilis* and *Streptococcus faecalis*; Gram negative bacteria: *Escherichia coli* and Staphylococcus aureus), two fungal species (*Asparagillus flavus* and *Asparagillus niger*) and one yeast (*Candida albicans*). Strains were obtained from Microbiology Department, Microanalytical Center, Faculty of Science, Cairo University, and they will be available on request. Antimicrobial activity was screened by using the paper disc diffusion bioassay, and the diameter of inhibition zones were compared with those obtained by the standard antibacterial agent; tetracycline and (antifungal agent B) amphotericin.

Sterilized filter paper discs (6 mm) saturated with solutions of either tetracycline, crude extract or fraction(s) of at 20 to 250 mg/ml were placed on the surface of Petri dishes (14 cm) containing solid bacterial medium [nutrient agar broth]. Fungal Doxs medium seeded with cell or spore suspensions of the fungal species and standard antifungal amphotericin B were also performed. The inoculated plates were incubated in the favorable conditions for bacterial (35 to 37°C for 24 to 48 h) and fungal growth (25 to 27°C for 3 to 7 days). The diameter of the clear inhibition zones surrounding the paper disc saturated with the crude methanolic extract or hexane/ethyl acetate fractions were taken as a measure of the inhibitory power of the sample against the particular test organisms (Ebrahimzadeh and Niknam, 1998; Nisar et al., 2010a, b, 2011; Zia-Ul-Haq et al., 2011c; Qayum et al., 2012). Standard antibacterial and antifungal agents were used as positive controls (tetracycline and amphotericin B, respectively). All experiments were carried out in triplicates.

The results in Table 1 and Figure 1 showed that both crude methanolic extract (K) and the separated five TLC fractions (A to E) exhibited antibacterial activities of different percentages (21.0, 12.5, 8.35, 6.25, and 0.31%, respectively). Crude extract and fractions showed moderate activities against the Gram positive and Gram negative bacterial species. The activity represents nearly 50% of that recorded by the standard antibacterial agent tetracycline. The reported data suggests that the crude extract contained different antibacterial substances with variable efficiencies and mode of actions which may act antagonistically leading to a decrease in the diameter of inhibition zone (as in the case of fraction C (8.35%) with *E. coli*) or synergistically causing an increase in the diameter of inhibition zone as in the case of fractions A (21.0%) and B (12.5%) with *S. faecalis*, compared with the crude extract (K). Alternately, the greater purity of fractions may show the opposite effects of higher concentrations of active compounds offset by fewer bioactive compounds present. Antifungal activities of extracts [crude and fractions] were manifested only against *C. albicans* (yeast). Both *A. flavus* and *A. niger* were shown to be highly resistant to all extracts and gave no sign of growth inhibition (Table 1). Fractions B and C, exhibited higher activities against *C. albicans* than those of fractions A (21.0%), D (6.25%), and E (0.31%).

ANTIALGAL ACTIVITY

The crude methanolic extract and the TLC separated

Table 1. Diameter of inhibition zones (mm) of crude methanolic extract and fractions of *Eichhornia crassipes* against tested microorganisms [bacteria after 24 h and fungi after 72h].

Microorganism	Gram reaction	Diameter of inhibition zone [mm]								LSD at 0.01
		Stander antibiotic		Fractions						
		tetracycline [antibacterial agent]	Amphotericin B [antifungal agent]	Crude (K)	A	B	C	D	E	
Bacillus subtilis	Gram positive bacteria	33±0.0	--	12±0.5	13±0.0	14±0.3	12±0.0	14±0.5	13±0.2	0.25
Escherichia coli	Gram negative bacteria	33±1.5	--	12±0.0	14±1.0	14±0.0	11±0.5	13±0.3	12±0.4	0.25
Staphylococcus aureus	Gram negative bacteria	32±0.6	--	12±0.2	15±0.2	13±0.0	12±0.6	15±0.0	15±1.2	0.25
Streptococcus faecalis	Gram positive bacteria	30±0.9	--	14±0.2	16±0.7	15±1.0	12±0.0	14±0.8	13±0.5	0.25
Aspergillus flavus	Fungus	--	16±0.8	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0.09
Aspergillus niger	Fungus	--	15±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0.09
Candida albicans	Yeast	--	18±0.0	13±0.5	12±1.1	15±0.4	14±0.0	11±0.3	12±0.0	0.25
LSD at 0.01		0.326	0.413	0.21	0.21	0.21	0.21	0.21	0.21	

--:not tested; * Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.01 according to Duncan's multiple range test.

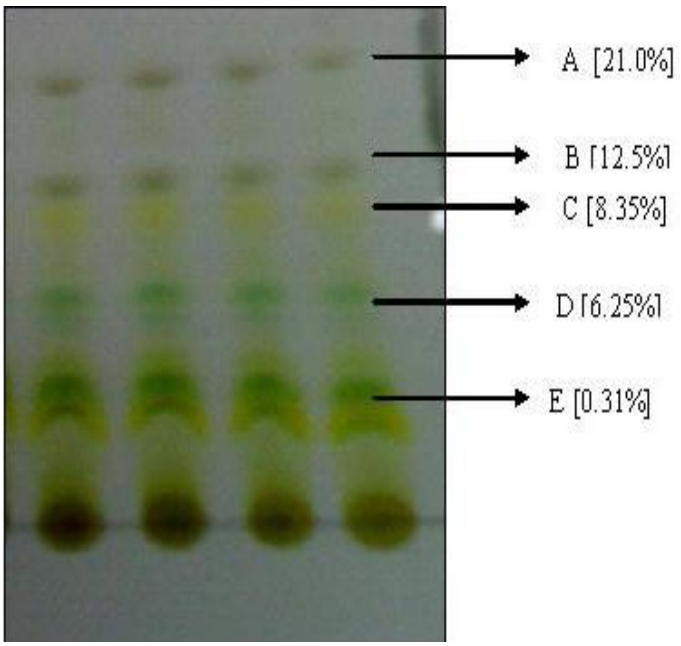

Figure 1. Fractionation of crude methanolic extract of *E. crassipes* using silica gel TLC and hexane/ethyl acetate (8.5:1.5, v/v) as mobile phase (R_f values: A=0.47; B= 0.37; C= 0.24; D=0.15; E=0.12). The data between brackets represented the percentage of each fraction in crude methanolic extract.

fractions were tested against two green microalgae (*Chlorella vulgaris* Beijer. and *Dictyochloropsis splendida* Geitler.) and two cyanobacterial species (*Spirulina platensis* (Nordist.) Geitler. and *Nostoc piscinale* Kutz.) Both were isolated (from River Nile), identified (33 to 36) and cultured by Sanaa M. M. Shanab. They are available on request. The filter papers were loaded with 20 to 250 µg/ml fractions or crude extract. The inoculated plates were overlaid with the dried filter papers at their center and incubated at the optimal conditions for algal growth (20 ± 1°C, light intensity of 30 µE/m^2/s and photoperiod of 16/8 light, dark cycles for 10 days). The diameter of the clear inhibition zones surrounding the paper discs saturated with crude extract or fractions after 24 or 72 h were taken as a measure of the inhibitory power of the extracts against each of the tested algal species.

From all the tested algal species (2 green microalgae and 2 cyanobacteria), water hyacinth crude extract and fractions exhibit potent antialgal activity against only the green microalga *C. vulgaris* (Table 2). The growth of other algal species demonstrated no sign of inhibition caused by all extracts.

Data from Tables 1 and 2 illustrates clearly that the crude methanolic extract (K) showed a moderate zone of inhibition (13 mm) which was greatly enlarged by the five fractions (18 to 33 mm) indicating increase in activity.

Table 2. Diameter of inhibition zones (mm) around paper disc loaded with crude methanolic extract and fractions of *E. crassipes* against tested microalgae after 72 to 168 h.

Sample	Inhibition zone diameter (mm)			
	Dictyo.	Nos.	Chl.	Spir.
Crude[K]	0 ± 0.0	0 ± 0.0	13 ± 0.2	0 ± 0.0
A	0 ± 0.0	0 ± 0.0	33 ± 1.5	0 ± 0.0
B	0 ± 0.0	0 ± 0.0	22 ± 0.8	0 ± 0.0
C	0 ± 0.0	0 ± 0.0	18 ± 0.0	0 ± 0.0
D	0 ± 0.0	0 ± 0.0	26 ± 0.6	0 ± 0.0
E	0 ± 0.0	0 ± 0.0	31 ± 1.8	0 ± 0.0
LSD at 0.01	-	-	0.26	-

Dictyo. = *Dictyochloropsis splendid;* Nos. = *Nostoc piscinale;* Chl. = *Chlorella vulgaris;* Spir. = *Spirulina platensis*. Each value is presented as mean of triplicate treatments, LSD: Least different significantly at P ≤ 0.01 according to Du Duncan's multiple range test.

Fractions A manifested the greatest antialgal activity *against C. vulgaris* (33 mm) followed in descending order by fractions E, D, B, and C. The lowest antialgal activity was exhibited by the crude methanolic extract and the greatest activity of the five fractions indicated that different antialgal substances may be present in the crude extract. Compounds in the crude extract might act antagonistically leading to a marked decrease in activity (inhibition zone of 13 mm) whereas, the separated and highly purified fractions showed greater activity manifested by an enlargement of inhibition zone (18 to 33 mm). Fractionation of this crude extract and the separation of these antialgal compounds in the form of the five fractions may have alleviated the antagonism between compounds in crude extract or raised the purity of these active compounds leading to increase in diameter of inhibition zone (18 to 33 mm).

Chromatographic and spectroscopic analysis of the crude extract and fractions (Using gas chromatographic/mass spectrometry (GC/MS) (Table 3) and mass spectrometry (MS), respectively) suggested that, 1, 2-Benzenedicarboxylic acid bis (2-ethylhexyl) ester was present in both crude extract and fraction C. This compound has potent antibacterial, antifungal as well as moderate antialgal activities and as recorded by many investigators from different sources (El-Mehalawy et al., 2008).

Fractions B, D, and E contained compounds that were identified by spectroscopic methods (Figures 1 and 2) as phthalate derivatives (ethylhexyl (B), methyl-dioctyl (D), and dioctyl phthalate (E) with molecular weights of 278, 662 and 390, respectively) which manifested moderate antimicrobial and antialgal activities (Tables 1 and 2) (Shanab et al., 2010). These findings agreed with those reported for phthalate derivatives isolated from seaweeds (El-Naggar, 1997); marine sponges; and from bacteria (Al-Bari et al., 2006). These phthalate derivatives exhibited moderate activities against different pathogenic bacteria, unicellular and filamentous fungi. Fraction A (with R_f 0.47, molecular weight 352 Da) of the crude extract was identified by spectroscopic methods as an alkaloid (18, 19-Seco-15 beta-yohimb) and this is the first record for the separation of this compound from water hyacinth and was shown to exert potent antibacterial, antialgal, and moderate antifungal activities (Tables 1 and 2) (Shanab et al., 2010). This fraction was not detected in GC/MS of the crude extract K (Table 3). The fractionation of this compound may be explained by a possible interaction between certain phthalate backbone [skeleton] and the acetamide derivatives or other nitrogenous compounds, recorded in GC/MS of the crude extract (Table 3). The fraction may be formed during the extraction and fractionation processes (Shanab et al., 2010). The mass spectrum of active compounds separated from methanolic extract of *E. crassipes* showed that all compounds share the common fragment ion as the following: 57, 71, 85, 149, 167, 207, and 279 Da and these results were confirmed by H-NMR. The H-NMR data indicated that all fractions had the following types of protons; a multiplex signal at d 6.89 to 7.47 ppm was characteristic of aromatic protons; the singlet signal at d 5.320 ppm was characteristic of the two protons of olifinic (CH= CH); the singlet signal at 3.342 ppm was characteristic of four protons of two O-CH_2 group; the singlet signal at d 1.26, 1.6, and 2.5 ppm was characteristic of the protons of methylene (CH_2) group; and the singlet signal at d 1.9 ppm was characteristic of protons of methyl (CH_3) group. However, it was recorded from the phytochemical analysis of the crude extract which contained 0.98% alkaloids, 4.35% phenolic compounds, and 1.53% terpenoids as illustrated in Figure 2. The minimum inhibitory concentration (MIC) of fraction A (Table 4) which inhibited the growth of different bacterial, fungal, and algal species ranged between 20 (in case of *C. vulgaris*) and 95 mg/ml (in case of *C. albicans*) (Shanab et al., 2010).

Table 3. GC/MS of crude methanolic extract of *Eichhornia crassipes*.

Peak number	Rt	Compound	Relative percentage
1	3.15	1-Deuterio-trans-1,3 dihydroxy	8.03
2	5.09	(E)-4-acetoxy-2-pentenenitrile	2.13
3	8.59	Trans-2-Tridecenal	2.21
4	8.90	3(2H)-Isothiazolone, 2-methyl	2.97
5	9.97	9-Hexadecenoic acid	2.49
6	10.51	Urox	2.83
7	11.73	Silane	6.84
8	11.77	Isopropylimino dibenzyl	4.61
9	12.01	13-oxabicyclo (10.1.0)tridecane	2.76
10	12.23	2-dodecenoic acid	2.26
11	12.89	Androsta-3, 5-dien-3-ol, 3-O-dimethyl	10.84
12	13.86	1,2-Benzenedicarboxylic acid	3.63
13	14.15	Eicosamethylcyclodecasiloxane	3.87
14	15.67	Benzeneacetic acid	3.58
15	16.43	Sotalol	2.62
16	16.66	Phosphonic acid	3.25
17	16.89	Fenuron	2.16
18	17.17	10-Undecenoic acid	2.13
19	17.43	Hydantoin, 1-N-Formyl-5-hydroxy	3.72
20	17.54	Cyclohexanone	5.27
21	17.64	Butanoic acid	4.27
22	17.68	N, N-diacetyl-1,7-diaminoheptane	3.92
23	17.72	N-(3-methylbutyl)-acetamide	3.20
24	19.66	Urea	2.27
25	20.07	Alpha-D-Xylofuranoside	2.48
26	20.15	9-Octadecenoic acid (Z)	3.46
27	20.66	3-Tidecanone	2.19

ANTIOXIDANT ACTIVITY

Free radical scavengers protect both human body and food from the harmful effect of ROS. The screening of alternative antioxidants of natural origin, replacing the carcinogenic synthetic ones, is of great importance. Water hyacinth was tested for its antioxidant activity as well as other biological activities.

Preparation of *E. crassipes* extracts

E. crassipes (Mart) Solms. was collected from El-Zomor canal, Giza, in spring 2009, and was 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 (10 g) 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 to 45°C) were followed by weighting the extract residues and preparing different concentrations from each extract.

Determination of active compounds with antioxidant activity

Chlorophylls and carotenoids

The amounts of chlorophylls and carotenoids were extracted with acetone and determined according to Holden method (Holden and Goodwin, 1965). The absorbance of the combined extract and washings was measured at 663, 645, and 452 nm. The following equations were used for the calculation of chlorophylls and carotenoids (as mg/g).

Chlorophyll a (mg/g) = 12.3 × A663 – 0.8 × A645 × V/A × 100 × W

Chlorophyll b (mg/g) = 19.3 × A645 – 3.6 × A663 × V/A × 100 × W

Total carotenoids (mg/g) = 4.57 × A452 – 0.22 × total chlorophylls

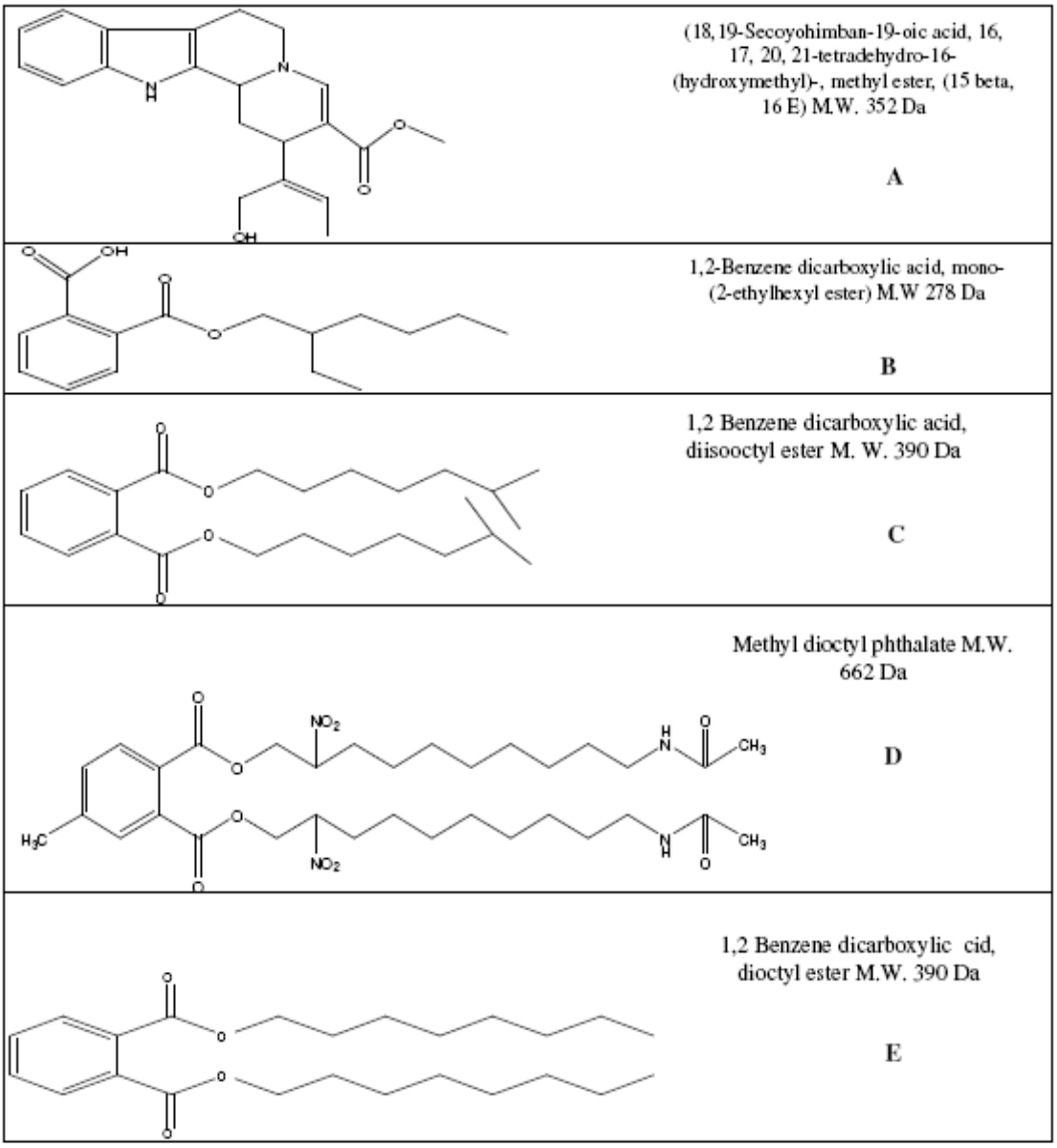

Figure 2. Predicted chemical structure of different active ingredients which separated from *E. crassipes.*

where A452, A645, and A663 are the absorbances at 452, 645, and 663 nm; V is the volume in milliliter; A is the length of light path in the cell; and W is the fresh weight of sample in grams.

Determination of total phenolic content

Total phenol contents of different extracts were determined by Folin-Ciocalteu method (Meda et al., 2005). 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.

Phytochemical analysis of methanol extracts (E_3)

The major secondary metabolites (total phenolic compounds, alkaloids, and terpenoids) were determined spectrophotometrically (Meda et al., 2005).

ANTIOXIDANT ACTIVITY

DPPH method

The antioxidant activity of water hyacinth extracts was evaluated by using the 2,2'diphenyl-1-picryl hydrazyl (DPPH) assay (Burits and Bucar, 2000). The extracts (50

Table 4. Minimum inhibition concentration (MIC) of active fraction (D) from *E. crassipes*

Test organisms	MIC (µg/ml)
B. subtilis	92
E. coli	78
S. aureus	76
S. faecalis	55
C. albicans	95
C. vulgaris	20

Table 5. Determination of pigments (Chlorophyll a, b and total chlorophylls and caretemoids) and total phenolic contents (as mg/g and gallic acid equivalents (GAEs)).

Extract	Total phenol (as GAEs)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Total 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^a	0.32^b	0.08^b	0.40^b	1.43^a	0.41
Methanol (E_3)	38^c	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

to 100 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 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.

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, 100 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 to 16 h 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 (in MeOH solution) and was 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) × 100, where At and Ac are the respective absorbances of tested samples and $ABTS^+$ was expressed as µmol (Re et al., 1999).

Weight of methanol extract (Table 5) was nearly double those of hexane and ethyl acetate (0.89, 0.44, and 0.41 mg, 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 5. 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 5), methanol extract showed the highest level (38 gallic acid equivalent (GAE)) followed in descending order by that of hexane (28 GAE) and ethyl acetate (27 GAE).

These results were confirmed by the great content of phenolic compounds in methanol extract produced by its phytochemical analyses (Figure 3). The antioxidant activity of the three extracts E_1, E_2, and E_3 by both DPPH and ABTS methods (Table 6) revealed that, the activity is concentration dependant and it was increased with doubling the extract concentration (50 and 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, respectively 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

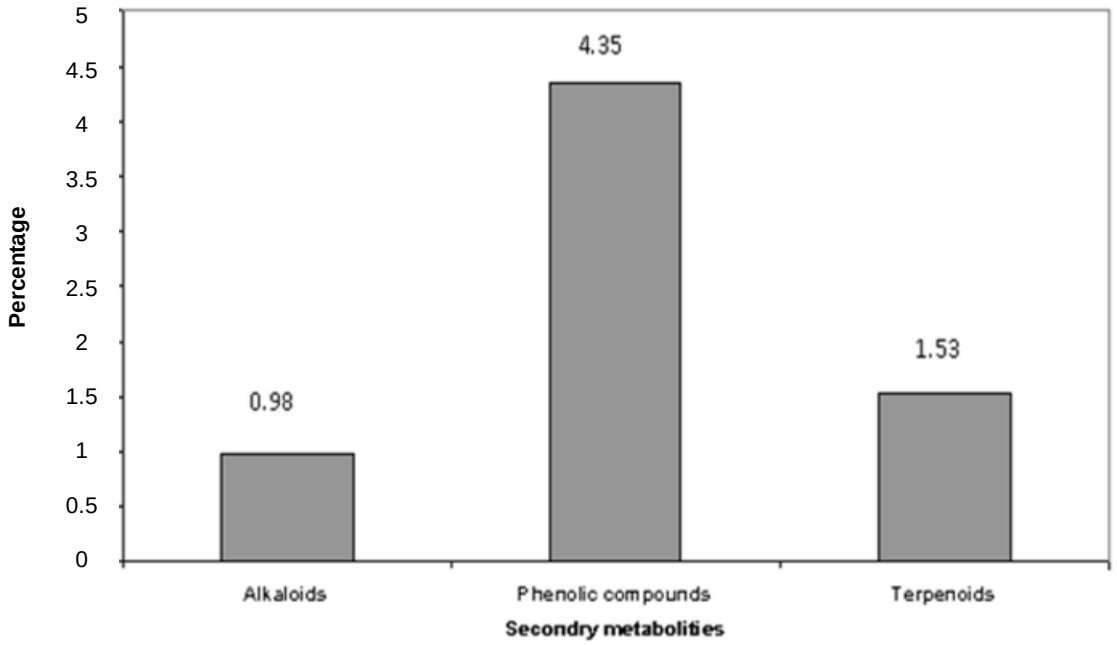

Figure 3. Phytochemical analysis of major secondary metabolites present in methanolic extract of *E. crassipes* doi:10.1371/journal.pone.0013200.g004

Table 6. Antioxidant activity (%) of successive extracts from *E. crassipes* against DPPH and ABTS radicals.

Extract	Scavenging activity (%)			
	DPPH		ABTS	
	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 ($p < 0.01$).

components of chlorophylls, carotenoids and phenolic compounds. The crude methanolic extract (E_3) contains higher contents of pigments (Chl a, b, and carotenoids), phenolic compounds (Table 5 and Figure 4) as well as alkaloids and polysaccharides (not determined here). Each of these components manifests certain and specific free radical scavenging activity as recorded in many literatures (Prescott, 1978). Natural antioxidants as carotenoids, chlorophylls, and phenolic compounds exhibit potent scavenging activity against ROS (Zia-Ul-Haq et al., 2008, 2011a, b, 2012). The phenolic compounds extracted from different sources were known to exert potent antimicrobial and antioxidant activities (El-Mehalawy et al., 2008).

So, fractionation of the methanolic extract by precoated TLC (Figure 1) revealed the separation of five fractions (A to 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% compared with the standard BHA (86.4 to 88.9%), while the crude methanolic extract (E_3) using both methods recorded higher activities (62.6 to 80.0%) than its five fractions depending on the extract concentration (100 to 200 µg/ml).

Identification of the active compounds in the fractions (by MS) revealed that (Figure 2) fraction A is an alkaloid (molecular weight 352 Da), while fractions B, C, D, and E are phthalate derivatives (molecular weights 278, 390, and 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

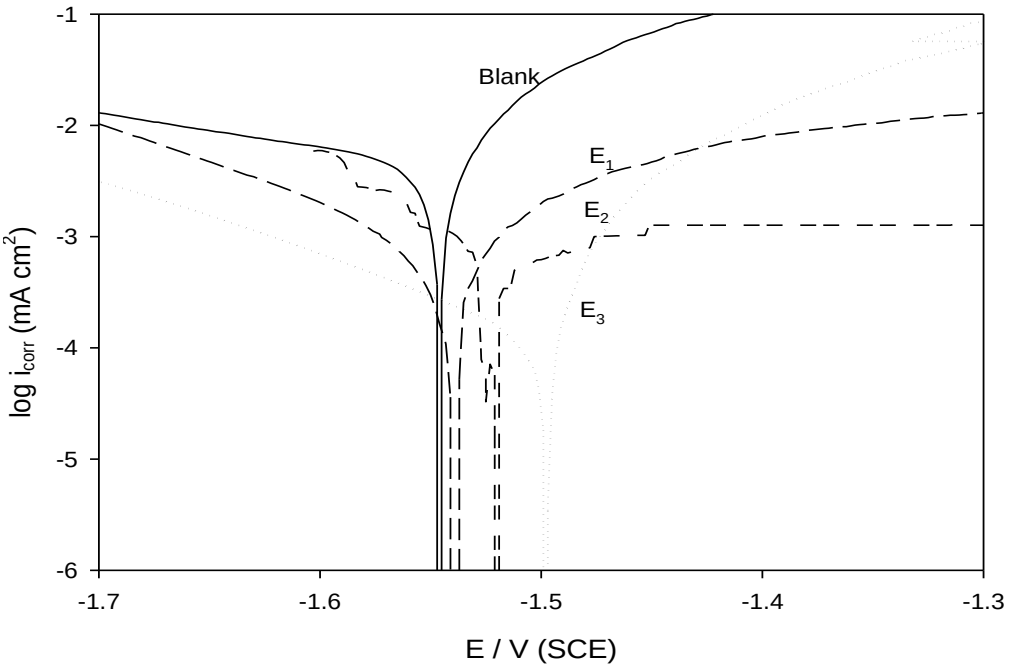

Figure 4. Equivalent circuit model representing two time constants for an electrode/electrolyte solution interface.

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; dibutyl phthalate, phthalic acid bis (iso-octyl) ester, iso-monyl phthalate, 1,2 benzene dicarboxylic acid bis (2-ethyl hexyl) ester, as fraction B in this work 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 to E) in addition to their scavenging antioxidant activity were recorded to have antibiotic and anticancer activities. 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 (Wang and Feng, 2001); so, *E. crassipes* extracts were tested for their anticorrosion efficiencies.

ANTICORROSION ACTIVITY

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 *E. crassipes* (E_1, E2, and E3) where shown to exhibit antioxidant activity of the order E3 > E_2 > E_1, it was interesting to test electrochemically the anticorrosion behavior of these extracts on the widely applied AZ31E magnesium alloys.

An electrode of magnesium alloy (AZ31E) was donated from department of Mining, Metallurgy and Materials Engineering, laval University, Canada, with chemical composition (weight, %): 2.8 Al, 0.96 Zn, 0.28 Mn, 0.0017 Cu, 0.0111 Fe, 0.0007 Ni, 0.0001 Be 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 (0.196 cm^2). The surface of the test electrode was mechanically polished by emery papers with 400 to 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 15 × 20 × 2 mm as a counter electrode (CE), saturated calomel (SCE) as a reference electrode (RE) and the alloy as the working electrode (WE). 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 (E1, E2, and E3) were prepared and the promising concentration of the pronounced corrosion inhibiting extract was used for the electrochemical studies of fractions (A to E).

The obtained data showed that, the addition of the

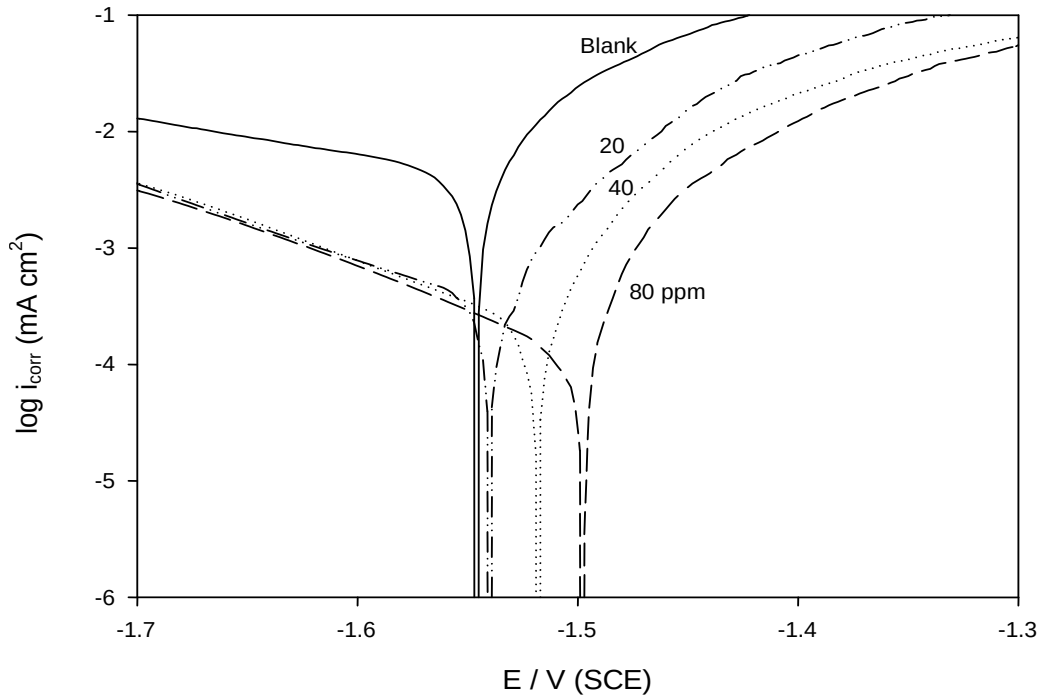

Figure 5. Dependence of R_T on the concentration for AZ31E in 0.15 M NaCl containing E1, E2, and E3.

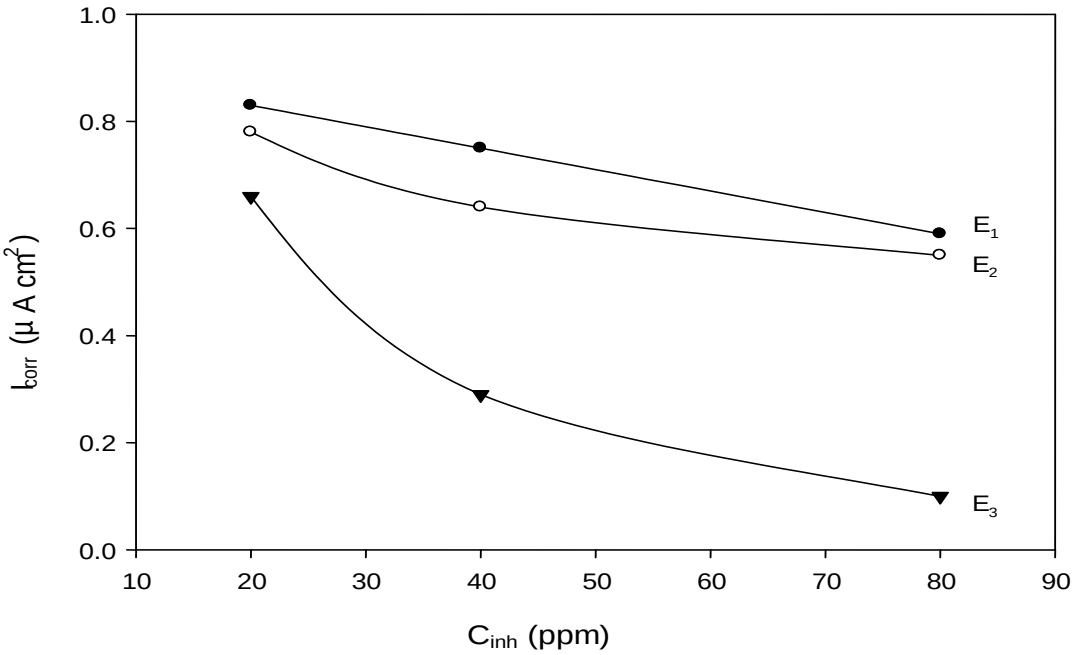

Figure 6. Polarization curves of AZ31E in 0.15 M NaCl without and with 80 ppm of E1, E2, and E3.

three *E. crassipes* extracts (E1, E2, E3) shifted the corrosion potential (E_{corr}) initially slightly in the positive direction. Figures 4 and 5 represented the polarization curves of AZ31E in blank solution without and with 80 ppm of E1, E2, E3 and different concentration of E3, respectively. Figure 6 illustrated the relation between i_{corr} and the concentration of E1, E2, and E3. It is clear from this figure that in all the three components, the icorr decreases with increasing the concentration. Also, the order of icorr is: icorr (E3) < icorr (E2) < icorr (E1).

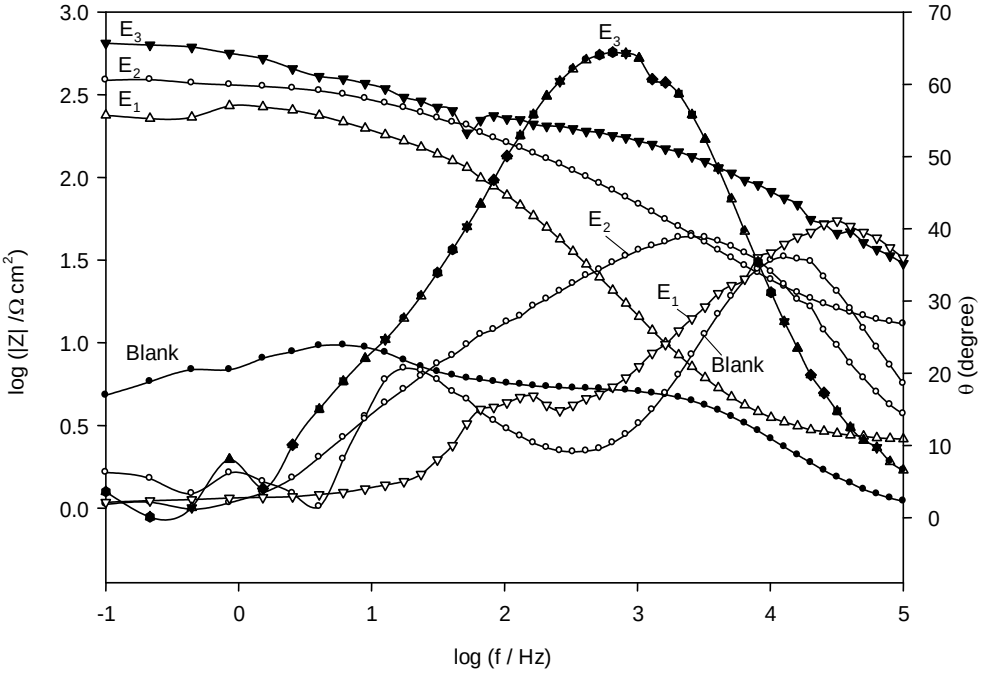

Figure 7. Polarization curves of AZ31E in 0.15 M NaCl without and with different concentrations of E3.

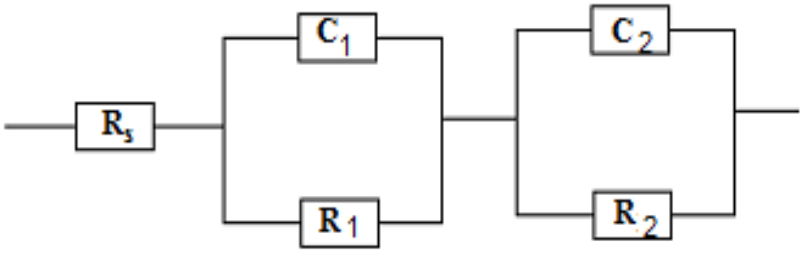

Figure 8. Relation between corrosion current and concentrations of E1, E2 and E3.

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: E1, E2, and E3. It can be seen from Figure 7, 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 for the alloy is clearly found to depend on both the three types of extracts and their concentrations.

The Bode format of Figure 7 confirms the presence of two time constants as there are two maximum phase lags that 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, as shown in Figure 8 consists of two circuits in series from R1C1 and R2C2 parallel combination and the two circuits are in series with resistance. In this way, C1 is related to contribution from the capacitance of the outer layer and the Faradaic reaction therein and C2 pertains to the inner layer, while R1 and R2 are the respective resistances of the outer and inner layers constituting the surface film, respectively (El-Mehalawy et al., 2008). 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 (El-Mehalawy et al., 2008). In this complex formula, an empirical exponent 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 (Freshney, 2002). 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 (El-Shoubary, 2010).

ANTICANCER ACTIVITY

The cytotoxicity of crude methanolic extract and the fractionated compounds (5 fractions) of *Eichhornia* species were tested against HepG-2 and MCF-7 cells by sulforhodamine B (SRB) assay as previously described (Freshney, 2002). Exponentially growing cells were collected using 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) and plated in 96-well plates at 1000 to 2000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with trichloroacetic acid (TCA, 10%) for 1 h at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm.

SRB-U assay was used to assess the cytotoxicity of the crude extract and its derived fractions against three different solid tumor cell lines and one ascitis tumor cell line. The cytotoxicity parameter, IC_{50} was calculated using E_{max} model as described in the methods section. The obtained results of the crude extract showed acceptable potency against HeLa and MCF-7 cell lines with IC_{50} of 1.6 ± 0.5 and 1.2 ± 0.2 µg/ml, respectively. However, HepG2 and EACC cell lines showed relatively higher resistance against the crude extract with IC_{50} of 7.6 ± 1.5 and 6.04 ± 0.5 µg/ml, respectively. This means that the cytotoxicity pattern of the crude extract on both MCF-7 and HeLa cell lines was similar, while it differs on HepG2 and EACC. Upon fractionation, fraction D showed the most potent cytotoxicity against HeLa cervix cancer cell line (IC_{50} = 4.3 ± 2.3 µg/ml) and the other fractions showed moderate cytotoxic effect with IC_{50}'s ranging from 7.7 to 14.1 µg/ml. With respect to MCF-7 breast cancer cell line, fractions B and A showed the best cytotoxic profile with IC_{50}'s of 13.4 ± 1.9 and 13.6 ± 5.3 µg/ml, respectively. The other fractions showed milder cytotoxic effects with IC_{50}'s ranging from 17.5 to 69.1 µg/ml. In HepG2 liver cancer cell line, other fractions showed much humble cytotoxic profile against HepG2 cell line with IC_{50}'s ranging from 14.9 to 74.2 µg/ml. Concerning EACC cancer cell line, fractions D, C, E, and A showed high potency with IC_{50} of 6.42 ± 0.8, 7.29 ± 1.6, 8.19 ± 1.2, and 8.61 ± 2.1 µg/ml, respectively. The effect of plant extract and its fractions on EACC is very similar to their effects on HeLa cells.

It is worth mentioning, that the higher potency of the crude extract against cancer cell lines in special HeLa and MCF-7 relative to all fractions from the same extract, might be attributed to auto-synergistic effect of these fractions within the same extract (Aboul-Enein et al., 2012).

CONCLUSION

The obtained results could be concluding that, water hyacinth was shown to be an abundant source of new and useful antibiotics active against some pathogenic strains of bacteria, fungi and algae. The active compounds were complex in structure and so would be difficult and expensive to synthesize chemically. Controlling the wide spread of the water hyacinth in the different Egyptian bodies (River Nile and its canals) may be achieved by harvesting it for pharmaceutical uses. Extracts and fractions used pharmaceutically could require the harvest of millions of tones/year.

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