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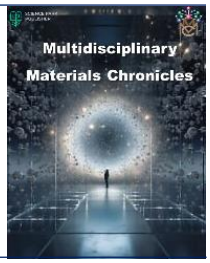

Silybum marianum Extract: A green inhibitor for Iron Metal Corrosion and Bacterial Growth in Fresh and Marine Media

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Abstract

Egyptian *Silybum marianum* ethanolic-hexane crude extract efficiency was investigated as an inhibitor of bacterial growth and iron steel corrosion in fresh water and marine environment media. Iron steel corrosion inhibition rate was quantitatively measured by two methods: one as a concentration of iron released in the medium over time intervals at different inhibitor ratios against the control using Inductive Coupled Plasma Mass Spectrometry (ICP-MS), and the second was relative weight loss method. Meanwhile, the development of the adsorption layer of the inhibitor on the surface of the iron steel specimen was monitored qualitatively using scanning electron microscopy. The adsorption of the extract constituents was found to obey Langmuir adsorption isotherm. Bacterial growth inhibition was examined using a well-diffusion method. After three days, the percentage of corrosion inhibition in fresh and marine media was 95% and 91%, respectively at the ratio of 1.5 mg/mL of the crude extract, and a homogeneous adsorption layer was observed on the surface of the iron specimen. The same dose was enough to inhibit bacterial growth in both media. Natural ecofriendly inhibitor extracted from a wild native Egyptian plant was found to be anticorrosion and at the same time antibacterial in both fresh and marine water media.

Keywords: Egyptian *Silybum Marianum*, ethanolic hexane crude extract, Corrosion inhibitor, Bacterial inhibitor– Marine media, Fresh media –weight loss- Iron released in the medium

1. Introduction

Worldwide distribution of *Silybum marianum* (*S. marianum*) is known to spread around the Mediterranean and much of Europe to Central Asia and India; in Africa it reaches as far as south Ethiopia [1]. The plant grows wildly in various habitats and is also cultivated in Europe, Asia, and North America [2]. This is due to its medicinal importance and sometimes as a food supplement [3-4]. On the other hand, it has been reported that the plant extract inhibits metal corrosion [5-7]. Regarding the Arabian region, only in Morocco and Iraq, the plant was investigated for its anticorrosion efficiency. Using an electrochemical method for the determination of corrosion inhibition efficiency, it reached 89.1% and 81.7 in Morocco and Iraq respectively [6,7]. Also, extract of *S. marianum* growing in south Algeria, Iraq and Egypt was found to inhibit bacterial growth [8-11]. Investigating the Egyptian *S. marianum* ecotypes for anticorrosion efficiency and bacterial growth inhibition could

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help in knowing the extent of diversity of the chemicals responsible for metal corrosion and bacterial growth inhibition; as well as genotype-environment interaction. Silybin and its derivatives were found to be the active compounds responsible for metal corrosion and bacterial growth inhibition [6-7, 12-14]. By investigating the Egyptian *S. marianum* extract for its potential to inhibit metal corrosion and bacterial growth, one could use these two parameters to indicate the stability of the active compounds in this plant species in the Middle East region. In addition, the present work elucidates the effect of fresh and marine water media as a factor in inhibiting metal corrosion and bacterial growth. Two methods for measuring metal corrosion inhibition rate were used to test the sensitivity of the techniques applied in the field of metal corrosion inhibition and different isotherm models were applied to figure out the type of adsorption.

2. Materials and Methods

2.1. Plant identification and floristic characterization

According to Täckholm [15], the plant was identified as *Silybum marianum* (Photo 1). The plant is a thistle with broad, white-mottled leaves, numerous lateral spines, and a very long recurved terminal spine with purple or white flowers. In the western desert of Egypt, the Egyptian ecotype grows, and its flowering season is spring.

2.2. Plant material preparation and extraction

Sampling collection was done at Borg El-Arab district. Then, leaves were cut off, washed, rinsed, air dried, and crushed into small pieces by a grinder to fine powder. Twenty grams (20 g) of Plant material were suspended in a mixture of pure n-hexane and 70 % ethanol (1:1 by volume) in a 500 mL conical flask. The flask was shaken for three days in a shaking incubator at 100 rpm at room temperature of 25± 5. The crude extract was filtered off using Whatman No1 filter paper. The total crude extract yield of the obtained two phases, oily and aqueous (Photo 2) was calculated to be 2.5 g after solvent evaporation.

Photo 1. *Silybum marianum* in the field during the flowering season.

Photo 2. Oily and aqueous two phases of *Silybum marianum* ethanol-hexane extract in a separating funnel.

2.3. Iron metal nails and their preparation

To conduct corrosion experiments, commonly used iron metal nails were purchased from the local market in Alexandria, Egypt. Analysis of the specimen was done using an Atomic Absorption Spectrometer (Varian 240FS Atomic Absorption System – AAS) and it was found to contain 97% iron. To determine the most common weight (the mode) and how near it is to the mean weight, thirty iron metal nails were randomly selected weighed, variance, and standard deviation were calculated to verify the sample's homogeneity. Nails were reshaped to be cylindrical by cutting their heads and tips, then abraded to be smooth at each edge with almost equal length of 10 ± 2 mm and a radius of 1 ± 0.1 mm; as for weight, the mean was 0.1740 g ± 0.0048 and a variance of 0.00002 g. This indicated a more or less homogeneity in the experimental material which minimized experimental error. The cylinder surface area was calculated and found to be 69.08 ± 1.32 mm^2.

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2.4. Fresh and marine water

Domestic tap water was used as fresh water medium, and marine water was sampled from El-Shatby beach of the Mediterranean Sea, Alexandria, Egypt as a marine water medium.

2.5. Treatment

2.5.1. Iron corrosion inhibition experiment

Glass vials with caps of 25 mL volume were strictly cleaned. *S. marianum* crude extract Stock media at ratios of 0.1, 0.5, 1, and 1.5 mg/mL fresh or marine water were prepared. Three vials each containing 10 mL of each ratio and three for control were prepared as replicates. In each vial, one iron metal cylinder was immersed, and vials were incubated at 25 Ċ in an incubator for different time intervals. Measurements were recorded daily over 3 days.

2.5.2. Bacterial growth inhibition experiment

Nutrient broth liquid medium was used to supplement the bacterial microflora in both fresh and marine water (N.B). To enrich the water, 3 mL of sterilized N.B. was added to 2 mL of fresh tap water; the same procedure was used for marine water in two different sterilized test tubes. Overnight, test tubes were incubated at 37 Ċ. These test tubes were used as stock cultures for the bacterial microflora of fresh and saltwater. The bacterial growth inhibition of *S. marianum* hexane-ethanol crude extract was tested using both bacterial cultures.

2.6. Measurements

2.6.1. Corrosion inhibition efficiency

2.6.1.1. Relative weight loss measurements

The American Society of Testing and Materials [16] provided the methodology. Using equations (1) and (2), the relative weight loss of control and treatment for each iron metal cylinder was determined.

Weight inequality of the iron metal cylinders employed in the experiment is the basis for justifying the calculation of relative weight loss instead of absolute weight loss.

$$\textbf{Relative weight loss of control cylinder (R.W.L.C)} = \frac{\text{I.W} - \text{C.C.W}}{\text{I.W}} \quad (1)$$

$$\textbf{Relative weight loss of treated cylinder (R.W.L.T)} = \frac{\text{I.W} - \text{T.C.W}}{\text{I.W}} \quad (2)$$

Where I.W is the initial weight of the iron metal cylinder and C.C.W is the control cylinder weight and T.C.W is the treated cylinder weight. The corrosion inhibition efficiency (I.E %) was calculated using equation (3).

$$\text{I. E \%} = \frac{R.W.L.C - R.W.L.T}{R.W.L.C} * 100 \quad (3)$$

2.6.1.2. Iron released in the medium measurement

Using Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) from Agilent Technologies, 5100 VDV, Australia, the corrosion rate was measured over 3 days in addition to the relative weight loss method. Iron released in the medium was also measured. According to [17], the amount of iron released in the test medium (fresh or sea water) in the form of ferric hydroxides is measured to determine the corrosion rate inhibition efficiency. The amount discharged into the medium is meant to correspond with the weight loss of iron metal due to corrosion. The corrosion inhibition efficiency (I.E %) was calculated using equation (4).

$$\text{I. E \%} = \frac{\text{C.A.R.M} - \text{T.A.R.M}}{\text{C.A.R.M}} * 100 \quad (4)$$

Where C.A.R.M is the control amount of iron released in the test medium while T.A.R.M is the treated amount of iron released in the test medium.

2.6.1.3. Metal surface corrosion and its inhibition using microscopic examination

Changes in the morphology of the iron metal surface which might occur for the corrosion process were examined for three successive days at a ratio of 1.5 mg/ mL medium. Optical (light microscope ZEISS MC63A, West Germany) and Scanning electron microscopy (JEOL, JSM – IT200 Series, JAPAN) were used.

2.6.2. Bacterial growth inhibition

Well diffusion method [18] was used for testing bacterial growth inhibition of *S. marianum* extract where three wells were cut into nutrient agar plates; sterilized distilled water was added

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to one well as a control and the extract was added to the other two wells at ratios of 1 and 1.5 mg/mL medium. Plates were three times replicated and incubated at 37 ċ overnight. Bacterial growth inhibition zone around wells was observed.

3. Results and Discussion

3.1. Iron corrosion inhibition

3.1.1. Comparison between corrosion inhibition efficiency in fresh and marine water media

S. marianum ethanol-hexane crude extract inhibition efficiency data were illustrated in Table (1) and Figure (1) in the two media. The results showed clearly that *S. marianum* ethanol-hexane crude extract is strongly active in both media as inhibition efficiency increased with increasing extract dose. Multifactorial analysis using Graph Pad Prism 7 software was applied to the data for testing three factors, namely: ratio of the inhibitor, time interval (1 and 3 days), and type of medium (fresh tap water and marine water). Table 2 showed that inhibitor ratio and time interval are highly significant in affecting corrosion inhibition efficiency while fresh or marine water media as a factor as well as interactions were insignificant.

Table 1. *S. marianum* ethanol-hexane crude extract inhibition efficiency in fresh and marine water media, at different ratios and time intervals applying relative weight loss method

Ratio (mg/mL medium)	Inhibition efficiency % ± S. D after one and three days			
	Fresh water		Marine water	
	1	3	1	3
0.1	40±3	62±1	58±1	48±7
0.5	49±13	80±2	53±21	68±9
1	71±16	77±5	65±20	75±6
1.5	93±3	95±4	78±20	91±6

S.D: Standard Deviation

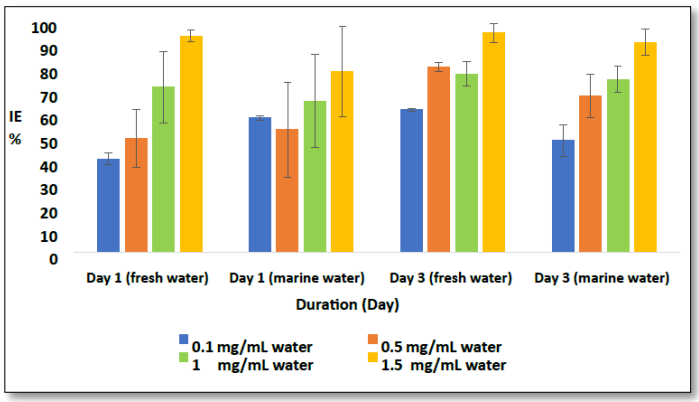

Figure 1. Corrosion inhibition efficiency (IE%) of *S. marianum* ethanol-hexane crude extract in fresh and marine water media, at different inhibitor ratios and time intervals.

Table 2. Multifactorial analysis of the effect of extract ratio, time interval, type of medium (fresh - marine water) on corrosion inhibition efficiency of iron metal

Three-way ANOVA				
Alpha	**0.05**			
Source of Variation	% of total variation	P value	P value summary	Significant?
Ratio	55.68	<0.0001	***	Yes
Fresh vs Marine	1.447	0.1625	ns	No
Time	9.252	0.0010	**	Yes
Ratio x Fresh vs Marine	1.353	0.5968	ns	No
Ratio x Time	3.377	0.2110	ns	No
Fresh vs Marine x Time	1.447	0.1625	ns	No
Ratio x Fresh vs Marine x Time	4.793	0.1008	ns	No

ns= Not significant **= Significant

*****= Highly significant**

3.1.2. Comparison between relative weight loss and iron released in the medium methods in measuring corrosion inhibition efficiency in fresh water medium

Table 3 shows the comparison of corrosion inhibition efficiency (% IE) after three days using two different methods

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and its correlation. Results illustrated that the two methods are applicable. Choosing the best method always depends on the type and objective of the work and which method is more suitable and applicable.

Table 3. Comparison between applying relative weight loss and iron released in the medium methods for measuring iron metal corrosion inhibition efficiency (IE %) of *S. marianum* ethanol-hexane crude extract in fresh water medium after three days

Extract ratio (mg/mL medium)	Mean ± S.D of 3 replicates of the amount of relative weight loss(g)	IE %	Mean ± S.D of 3 replicatesof the amount of iron released in themedium (µg/10 ml)	IE %
0.0	0.037±0.0097		539.55± 50.33	
0.1	0.014±0.0041	62	492.46± 142.88	9
0.5	0.007± 0.0013	81	259.67±37.18	52
1	0.007±0.0020	81	175.42± 52.77	68
1.5	0.0024±0.0020	95	143.27±9.42	74

Correlation coefficient $R^2 = 0.94$

3.1.3. Adsorption isotherm

Different isotherm models [19] were applied to describe different adsorption processes, mechanisms and study how the adsorbed inhibitor molecules interact with the metal surface. The Surface coverage values θ as a function of inhibitor concentration obtained from weight loss data. Results indicated that the Langmuir isotherm model (Figure 2) gives the best fit with $R^2 > 0.95$ which indicates the adsorbed molecules occupy only one site and there are no interactions with other adsorbed species. The adsorption equilibrium constant (K_{ads}) value was calculated according to equation (5).

$$\frac{C}{\theta} = \frac{1}{K_{ads}} + C \qquad (5)$$

where C is the concentration of the inhibitor, K_{ads} is the adsorption equilibrium constant and θ is the surface coverage of the inhibitor. The standard free energy of adsorption, ΔG_{ads}, is related to the equilibrium constant of adsorption K_{ads} and can be calculated using equation (6) where 55.5 mol/L was the molar concentration of water, R is the gas constant and T is the absolute temperature in Kelvin [19].

$$\Delta G_{ads} = -RT\ln(55.5\ K_{ads}) \qquad (6)$$

ΔG_{ads} was calculated (Table 4) for both fresh and marine media and the negative values obtained indicated the spontaneity of the adsorption process and stability of the adsorbed layer on the metal surface. Generally, values of ΔG_{ads} up to -20 kJ mol^{-1} are compatible with the electrostatic interactions between the charged metal (physisorption) while those around -40 kJ mol^{-1} or higher are associated with chemisorption as a result of sharing or transfer of unshared electron pairs or pie-electrons of organic molecules to the metal surface to form a coordinate type of bond (chemisorption) [19, 20-21]. Hence, it is clear that the crude extract is physically adsorbed onto the metal surface.

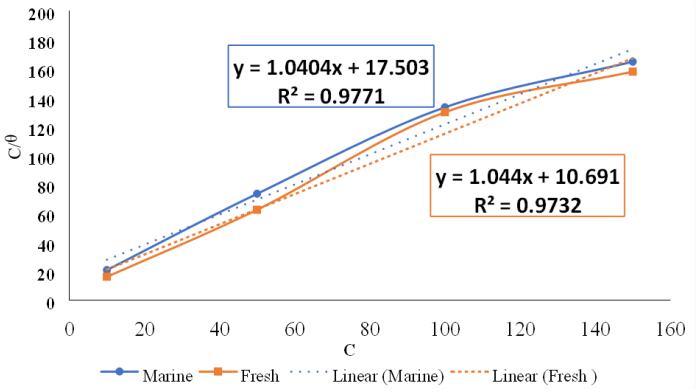

Figure 2. Langmuir adsorption isotherm plot for the adsorption of *S. marianum* ethanol- hexane crude extract on the iron metal surface.

Table 4. The values of K_{ads} and ΔG_{ads} of *S. marianum* ethanol-hexane crude extract at 25 Ċ

Medium	Adsorption equilibrium constant K_{ads}	Free energy of adsorption (ΔG_{ads})KJ/mol
Fresh	0.093	-4.066
Marine	0.057	-2.853

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3.1.4. Qualitative measurements of corrosion rate and its inhibition

3.1.4.1. Optical microscopy

Optical microscopy revealed clear daily successive changes of control and treated iron metal surface (Photo 3 A, B, C for control and A`, B`, C` for treated). Adsorption of inhibitor on the metal surface was all around the cylinder (Photo 4). More details of the adsorbed layer on the metal surface are shown in Photo 5 A, B, C, and D at higher magnifications. The plant extract was found to contain oil and other substances some of which are in crystalized form (Photo 6 A, B), this oil fraction probably played a role in the adsorption process. Microscopic examination of the corrosion inhibition medium showed a wide and dense dispersion of oil droplets of diverse sizes (Photo 7 A, B, C, and D). It is clear from examining photos of adsorption and plant extract constituents, that a certain fraction of the extract is adsorbed on the metal surface, which forms a homogenous layer on the metal surface.

3.1.4.2. Scanning electron microscopy

There were noticeable distinctions between the treated and control iron metal surfaces. Plant extract adsorption on the metal surface progressively increased day by day until it covered the whole surface in both fresh and marine water media (Photos 8 and 9). Simultaneously, the control showed gradual deterioration of the iron metal surface. There was no difference between fresh water and marine water media for such changes, which confirms the effectiveness of the extract to inhibit corrosion in both media.

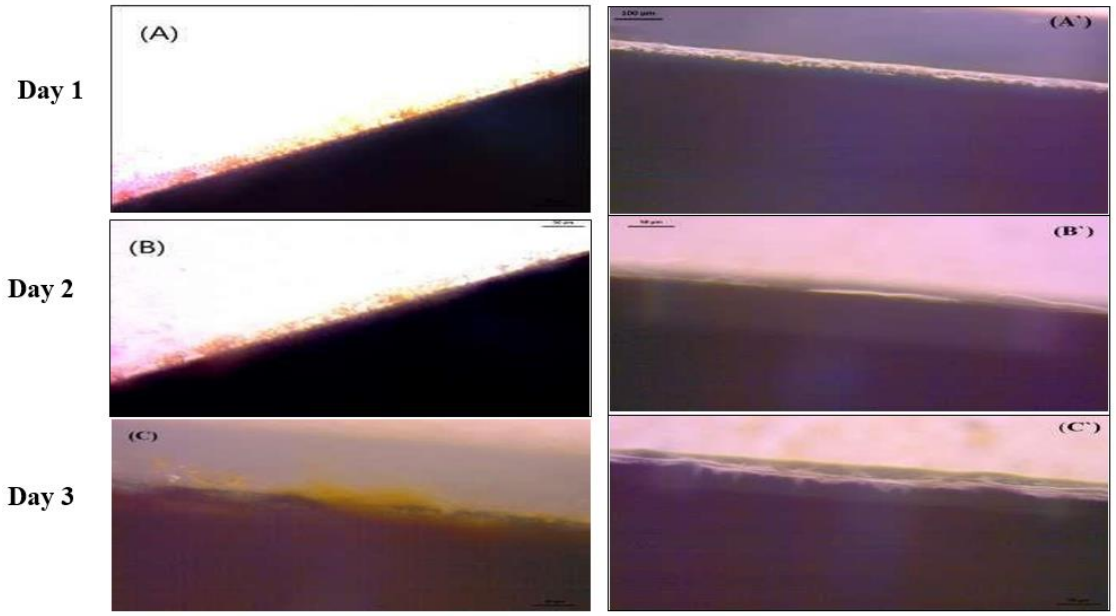

Photo 3. Optical microscopic images of the iron metal surface over three successive days A, B, C immersed in fresh water as control and A`, B`, C` immersed in *S. marianum* ethanol-hexane crude extract as treated.

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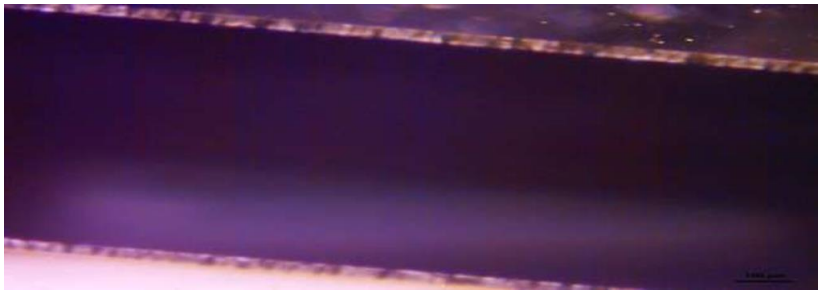

Photo 4. Optical microscopic image of the all-around inhibitor adsorption layer on the metal surface.

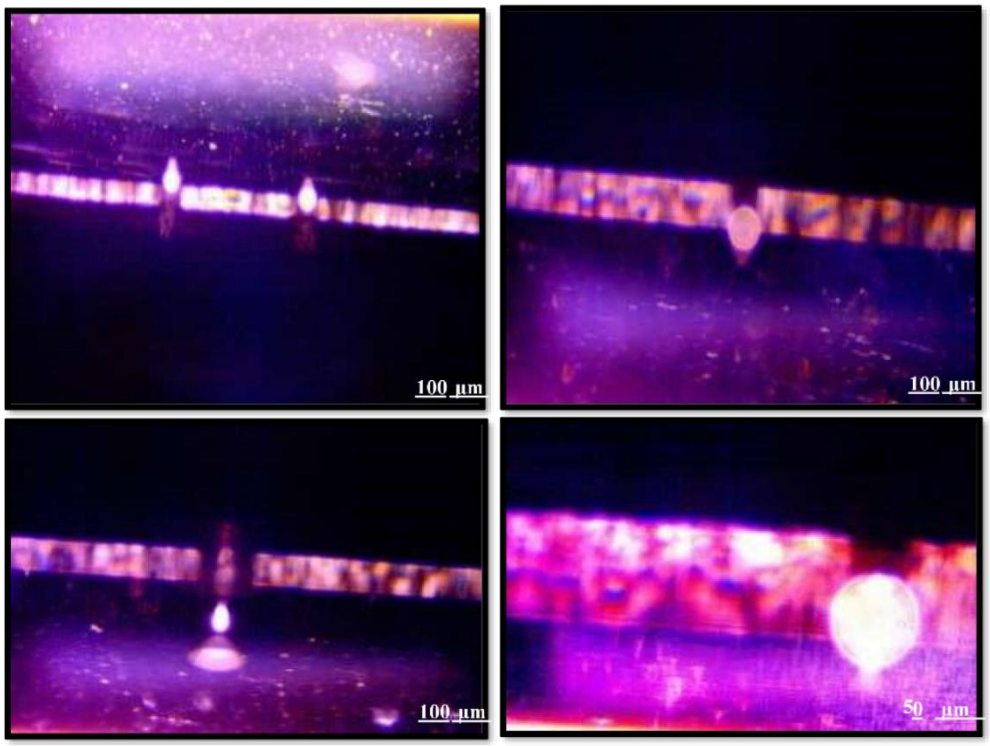

Photo 5. Optical microscopic images of higher magnification showing the adsorbed inhibitor layer on the metal surface.

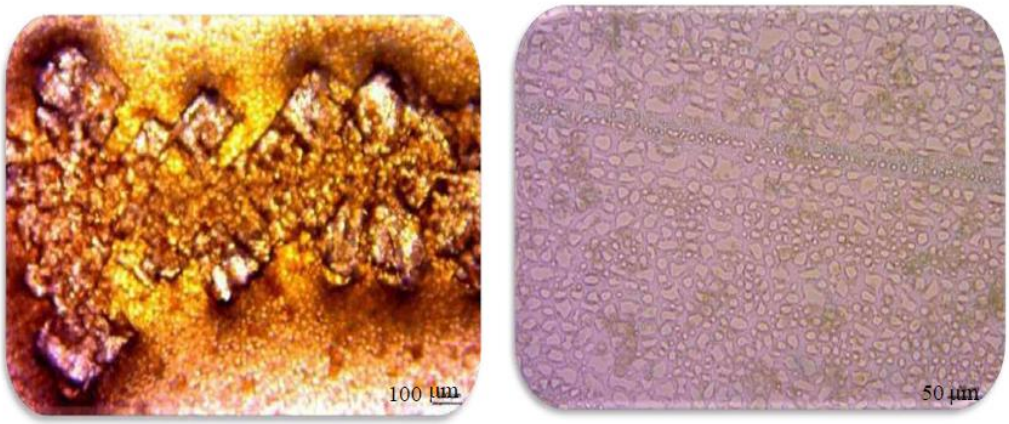

Photo 6. Optical microscopic images of the crystalized forms of substances and oil in *S. marianum* ethanol-hexane crude extract.

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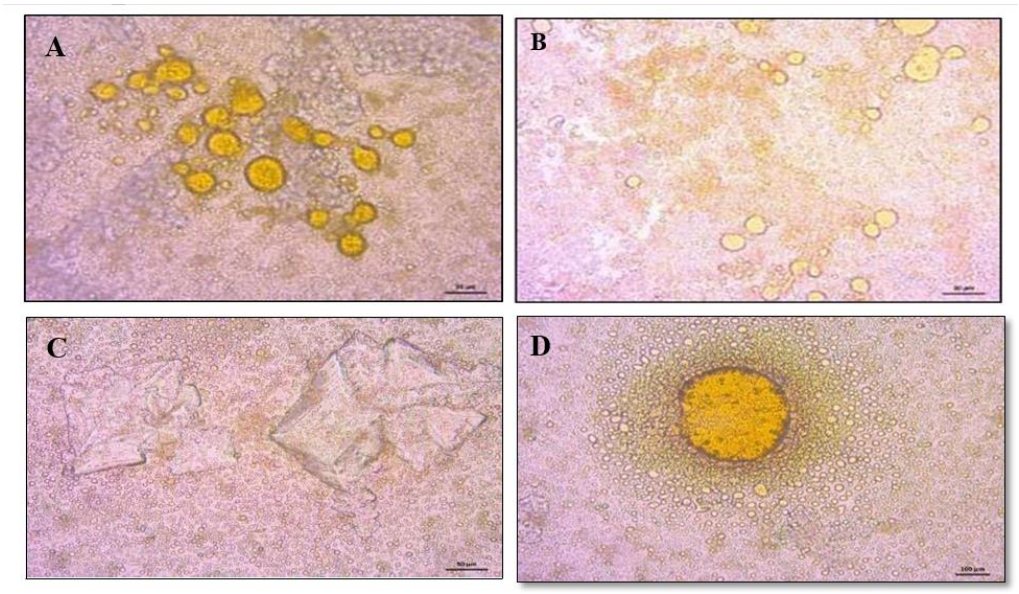

Photo 7. Optical microscopic images of oil droplets of various sizes and substances of crystallized forms in corrosion inhibition medium, A, B, C 50 µm, and D 100 µm.

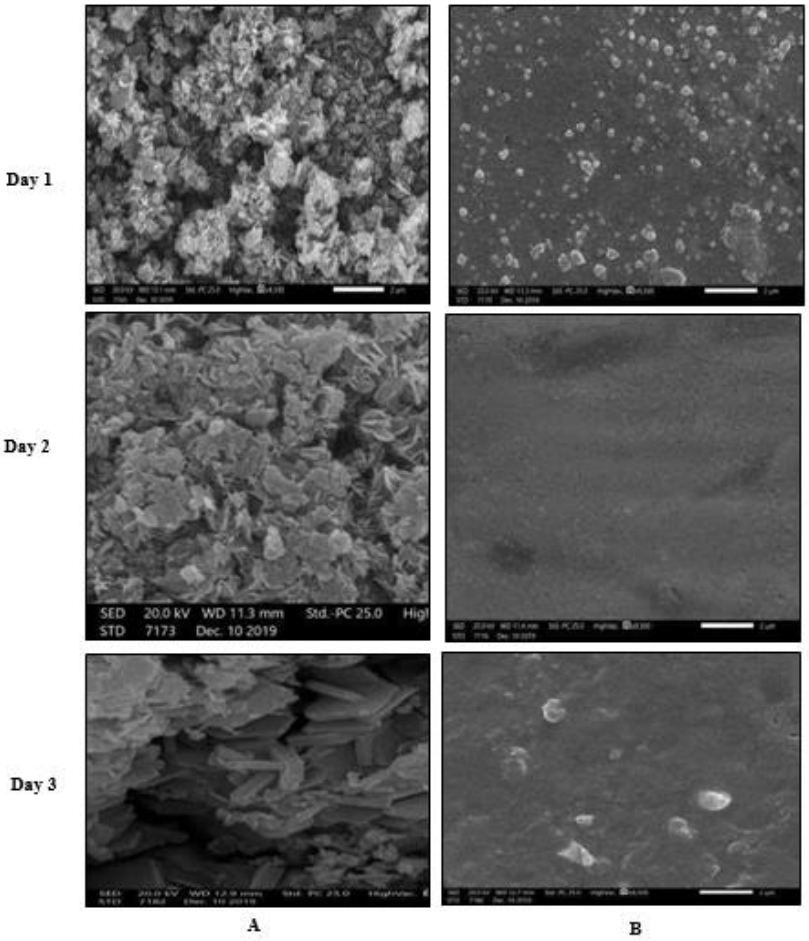

Photo 8. SEM images of iron metal surface 9500 X over three successive days A immersed in fresh water as control and B immersed in *S. marianum* ethanol-hexane crude extract as treated.

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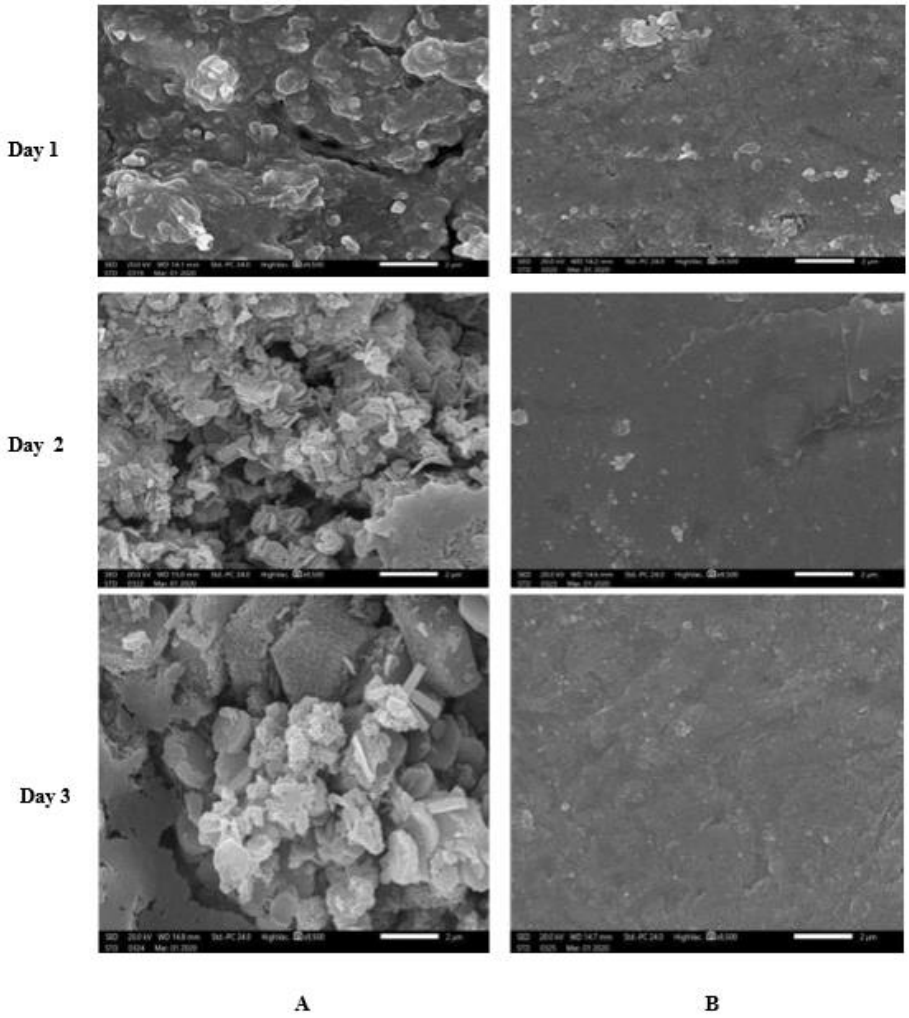

Photo 9. SEM images of iron metal surface 9500 X over three successive days A immersed in marine water as control and B immersed in *S. marianum* ethanol-hexane crude extract as treated.

3.2. Bacterial growth inhibition

The inhibitory effect of *S. marianum* ethanol-hexane crude extract on the growth of bacterial microflora in fresh and marine water is shown in photos 10 & 11. It was clear that the extract inhibited bacterial growth in both fresh and marine water media at a ratio of 1.5 mg/mL.

Photo 10. Crude extract inhibited the bacterial growth of fresh water: one well was a control (distilled sterilized fresh water) two wells were inoculated with the extract at ratios 1 and 1.5 mg/mL; only the ratio 1.5 mg/mL inhibited the growth.

Photo 11. Crude extract inhibited the bacterial growth of marine water: one well was a control (sterilized marine water: two wells were inoculated with the extract at ratios 1 and 1.5 mg/mL; only the ratio 1.5.

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3.3. Comparing the anticorrosion and antibacterial activities of the Egyptian *Silybum Marianum* and other ecotypes in the Middle East region

Tables 5 and 6 illustrate the comparison between the present work results and those of other works of the same plant species growing in the Middle East region. The comparison showed that some factors such as habitat (ecotype), extraction solvent, tested material, and plant part used were found not to affect the inhibition of metal corrosion and bacterial growth activities of the plant extract.

Table 5. Factors affecting the activity of *S. marianum* extract in inhibiting metal corrosion

Ecotype (Habitat)	Part used	Extraction solvent	Type ofmetal	Corrosion medium	Plant extract concentration (mg/mL)	Highest inhibition efficiency %		Reference
						Weight loss method	Electrochemical method	
Iran	leaves	petroleum ether then methanol	304 stainless steel	1.0 M HCl	1.00	95.7	96.0	[5]
Morocco	-------	n-hexane for oil extraction	carbon steel	1 M HCl	3.00	---------	89.1	[6]
Iraq	leaves	aqueous	aluminum alloys	1 M HCl	0.5	---------	81.71	[7]
Egypt	leaves	ethanol-hexane mixture	commercialnails with 97% Iron	Fresh water	1.5	95	---------	Present Work

Table 6. Factors affecting the activity of *S. marianum* extract in inhibiting bacterial growth

Ecotype (Habitat)	Part used	Extraction solvent	Plant extract concentration	Tested bacteria	Reference
South Algeria	-------	aqueous methanol	8 mg/mL	• Staphylococcus aureus, • Escherichia coli, • Klebsiella Pneumoniae, • Pseudomonas aeruginosa, • Enterobacter aerogenes.	[8]
Iraq	seeds	ethanol	1500-2900 µg/mL	• Staphylococcus saprophyticus, • Escherichia coli, • Staphylococcus aureus, • Klebsiella pneumonia.	[9]
	fruit	• ethanol, • methanol, • dichloromethane	25-400 µg/mL	• Staphylococcus aureus ATCC 29213, • Staphylococcus aureus MRSA ATCC 43300, • Enterococcus faecalis ATCC 29212, • Escherichia coli ATCC 25922, • Pseudomonas aeruginosa ATCC 27853, • Acinetobacter baumannii ATCC 19606.	[14]
Turkey	seeds	• n-hexane for fatty oil extraction • ethanol	----------	• Pseudomonas aeruginosa ATCC 27853, • Escherichia coli ATCC 25922, • Staphylococcus aureus ATCC 25923.	[10]
Egypt	seeds	ethanol	0.5-1.0 µg/mL	• Staphylococcus aures, • Listeria monocytog, • Salmonella entirica, • Escherichia coli.	[11]
Egypt	leaves	ethanol-hexane mixture	1.5 mg/mL	Whole bacteria of fresh and marine water	Present work

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4. Conclusions

Six findings are concluded from the present work; they are as follows:

1. On the third day of treatment, Egyptian *Silybum marianum* hexane-ethanol extract showed significant efficiency in preventing iron metal corrosion in both fresh and marine media, with efficiency reaching 95% and 91% in both fresh and marine media, respectively.

2. Iron released in the medium and relative weight loss methods for measuring metal corrosion inhibition efficiency were found comparable.

3. Scanning electron microscopy demonstrated that adsorption on the metal surface, specifically the oilcomponent of the extract, was the mechanism causing the corrosion inhibition.

4. The adsorption of the inhibitor on the iron metal cylinder surface was found to obey Langmuir isotherm and physically adsorbed onto the metal surface.

5. In addition to the metal anticorrosion activity, the extract was found to have antibacterial growth activity in both fresh and marine water media, indicating a dual-function effect of the crude extract.

6. No matter the habitat in which *S. marianum* grows in the Middle East region, no diversity in its extracted active compounds responsible for metal corrosion and bacterial growth inhibition was found.

Declaration of Competing of Interests

The authors declare that they have no known competing of interests.

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