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Hostile to Corrosion Studies on Ethanolic Extract of Hibiscus Rosa Sinensis and Azadirachita Indica Leaves

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Abstract: *The current examination was done about the consumption hindrance and adsorption conduct of Neem and Hibiscus, as a green inhibitor of Zinc erosion in acidic intervened erosion causing soil organisms by utilizing weight reduction and thermodynamic investigations. It was even examined both quantitatively and subjectively biochemical substances present in Neem and Hibiscus leaves of ethanol remove which go about as anticorrosive operator of metallic and non-metallic substances in fermented soil. Ethanolic concentrate of Neem and Hibiscus leaves remove indicated the solid antimicrobial action against soil microorganisms, for example, S.aureus, Streptococcus, B. subitilis, Lactobacillus, Proteus, Corynebacterium, Pseudomonas, A.niger, Mucor and Desulpho Vibro sp. Langmuir and Freundlich, Temkin and Florry Huggins models are utilized to examination adsorption happened in the test information of adsorption isotherms. The Freundlich, Langmuir and Temkin models are utilized to investigation adsorption happened in the test*
Keywords*: Corrosion inhibition; Neem and Hibiscus; anticorrosive activity; antimicrobial activity*

I. Introduction

Green chemistry offers an opportunity to propose any research in non-polluting way with minimum production of waste and minimum consumption of energy. It is a way of life which is equally applicable in all fields wherever chemistry involves (Linthorst, 2010; Sharma et al., 2010a; Sharma et al., 2011; Sharma et al., 2009a). "Corrosion" is a phenomenon where chemistry helps to explain its mechanism and role of ions and energy behind it. It is simply a destruction of materials resulting from an exposure and the interaction with the environment. One of the latest and popular approaches is the use of substances called corrosion inhibitor. These inhibitor molecules consist of heterocyclic compounds with polar functional groups (e.g. N, S, O, and P) and conjugated double bonds with different aromatic system. Basically, these substances adsorb on the metal surface to block the destruction reaction with aggressive media. They are both physically and chemically active adsorbate type substances (Buchweishaija, 2009 and Thompson et al., 2007). It is a major problem that must be confronted for safety, environmental, and economic reasons in various chemical, mechanical, metallurgical, biochemical, and medical engineering applications and more specifically, in the design of a much more varied number of mechanical parts which equally vary in size, functionality, and useful lifespan. Corrosion attack can be prevented by various methods such as materials improvement, combination of production fluids, process control, and chemical inhibition. Among these methods, the implementation of corrosion inhibition is the most excellent approach to avoid disastrous destruction of metals and alloys in corrosive media. The use of corrosion inhibitors is the most economical and convenient technique to control corrosive attack on metals. Corrosion inhibitors are chemicals either synthetic or natural which, when added in small amounts to an environment, decrease the rate of attack by the environment on metals. A number of synthetic compounds are known to be applicable as good corrosion inhibitors for metals (Ebenso et al., 2012a; Kabanda et al., 2012a and Quraishi et al., 2012). The importance of a corrosion study depend on the fact that corrosion causes great loses to our economy and is a major threat for human safety. Corrosion costs worldwide are therefore on the order of US$552 billion (Chauhan and Gunasekaran, 2007; Schmitt et al., 2009a). Even countries such as India are suffering badly due to this problem of corrosion (Sharma and Sharma, 2011). Several efforts have been made using corrosion-preventive practices, and the use of green corrosion inhibitors is one of them (Anuradha et al., 2008; Mudhoo and Sharma, 2010; Sharma et al., 2010b; Sharma et al., 2010c; Sharma et al., 2009b; Sharma et al. 2009c). Hence the present study was undertaken in view of the above fact the countries that are now facing in order to reduce corrosion effort in metal pipings by adopting by green chemical technology.

II. Materials And Methods

Plant Materials Collection

Leaves of the Azadirachta indica and Hibiscus rosasinensis leaves were collected in and around the Krishnarajapura area, Bangalore, Karnataka.

Preparation of Ethanol Extract of Plant Samples

10 grams of air dried powder Neem and Hibiscus leaves were placed in 100 ml of organic solvent (ethanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant of different samples was collected and the solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 40 mg/0.1 ml. It was stored at 4 °C in airtight bottles for further studies.

Isolation and purification of bioactive compounds from Neem and Hibiscus extracts

The isolation and purification of bioactive compounds were carried out using repeated silica gel column chromatography and thin layer chromatography (TLC). The purified bioactive compounds were characterized by subjected to UV, IR, LC-MS and NMR spectroscopy

Isolation of Flavonoids

Ethanol extract of Neem and Hibiscus leaves were subjected to fractionate by column chromatography over silica gel column 100 – 200 mesh using solvents with increasing polarity. The length and diameter of the column was 72 cm and 5 cm. The admixture was packed on a silica gel column (Merck, India) and elute with various solvent such as benzene, chloroform, chloroform: Acetone (1:1), ethanol, methanol and distilled water in the order of increasing polarity. All the collected fractions were run for TLC. Based on TLC profile fractions with similar Rf values were pooled into some fractions.

The methanol fraction kept in a refrigerator resulted in the precipitation of yellow powder which was further purified. Qualitative analysis of isolated compound was done. The isolated compound was subjected to TLC to ascertain its nature and its melting point was determined. The isolated compound was subjected to UV, IR, Mass and NMR spectroscopy and structure of isolated compound was determined.

UV-Visible Spectroscopy

UV-Visible spectrum of the isolated compound was recorded on a UV-Visible spectrometer (Shimadzu UV-160A, Singapore) at room temperature. About 1 mg of compound dissolved in 20 mL of acetone/chloroform and was used to record the spectrum in the wavelength range of (l) 200 to 800 nm.

Infrared (IR) Spectroscopy

IR spectrum of isolated compound was recorded on a Perkin-Elmer FT-IR Spectrometer (Spectrum 2000) at room temperature. About 1 mg of isolated compound was mixed with spectroscopic grade KBr and well ground before preparing the pellet. The IR spectrum was taken in the frequency range (n) of 4000 cm-1 - 400 cm-1.

Liquid chromatography-Mass spectrometry (LC-MS)

Mass spectrum of the compound was recorded on instrument HP 1100 MSD series (Palo Alto, CA) by electro spray ionization (ESI) technique with a flow rate of 0.2 mL min-1 on C-18 column and total run time of 40 min. The sample used for recording the mass spectrum was prepared by dissolving 0.1 mg of compound in 10 mL of methanol/acetonitrile.

NMR Spectroscopy

The 1H NMR was recorded for the isolated compound (5 mg in 600µL DMSO-D6) at 500MHz on BRUKER AQS NMR spectrometer, Bruker biospin AG, Switzerland. The J-modulated spin-echo for 13C - nuclei coupled to proton to determine number of attached protons (SEFT) was recorded at 125 MHz. The spectral width for 1H NMR was 0-12 ppm and 0-220 ppm for ^{13}C NMR.

Column Chromatography

Ethanol extract (10 g) was subjected to column chromatography on silica gel (100-200 mesh) (Merck) eluted with mixtures of chloroform, ethyl acetate, ethanol and methanol of increasing polarity, to obtain fractions for yellow amorphous powder. About fractions were eluted with different solvents with increasing polarity. Column fractions with ethyl acetate: ethanol (80:20) in the TLC mobile phase solvent ratio of chloroform: methanol (1:1) showed Rf value of 0.46 equal to that of standard Quercetin. The fractions were then

combined and crystallized and the final yield approximately 100 mg. This process was repeated several times by using bulk quantity of samples until the desired amount of Quercetin has been obtained.

Estimation by TLC Technique

The samples were spotted in the form of bands with Camag Microlitre Syringe on a pre-coated silica gel plates F254 (10 cm X 10 cm with 0.2 mm thickness, E. Merck) using Camag linomat at V automatic sample spotter of band width 7 mm. The plates were developed in a solvent system in Camag glass twin through chamber previously saturated with the solvent for 30 min. The distance was 8 cm subsequent to the scanning. TLC scanners were air dried and scanning was performed on a Camag TLC scanner in absorbance at 254 nm and operated by Wincats Software 4.03 version (Sethi, 1996).

Analysis of Quercetin and Rutin

The chromatographic analysis were performed on a 250 mm x 4.6 mm i.d. CIS (ODS), Shimaden, Japan with 0.5 % aqueous solution of Orthophosphoric acid and Alcohol (HPLC grade) as mobile phase at a flow rate of 1mL/min. A Waters 486 tunable absorbance detector was operated at 254 nm; detector sensitivity was 0.05 AUFs and the column oven temperature was 30^{0} C. Determinations were performed after three separate extractions of each sample and each extract was injected in triplicate (n=3).

Preparation of Stock Solution

Each sample was subjected to Petroleum ether so as to get rid of fats and waxes then the mark was percolated with ethanol during 24 hrs for 3 times. The ethanol extracts were collected and concentrated under vacuum before being eluted with boiling water and filtered. The filtrate was subjected again to extraction using ethyl acetate. Finally the ethyl acetate extract is subjected and concentrated to be used in the inhibition test.

Electrolyte

Analytical reagent grade HCl was used for preparing solution. Appropriate concentrations of acids were prepared by using double distilled water. The stock solution of the extract thus obtained was used in preparing different concentration of the extract by dissolving in Hydrochloric acid (HCl) solution such as 0.5N, 1.0N and 2.0N, prepared in double-distilled water.

Preparation of Zinc Specimen

The zinc strips of rectangular in shape, having dimensions 2.5 x 5.0 x 0.05 cm with a small hole of 2 mm diameter near the upper edge of the strip was taken as a test material for handling. The specimen was cleaned by polishing to produce a smooth finish with the help of emery paper and then clean-washed with absolute alcohol (100 % ethanol) and dried in natural air. Each specimen was suspended by a plastic thread tied in the hole and immersed in a beaker containing 50 ml of the test solution of Azardirachta Indica (Neem) and Hibiscus rosasinensis extract in Hydrochloric acid (HCl) solution at 30˚C and 60˚C and left exposed to air for about half an hour for the determination of the corrosion rate (CR).

Weight Loss Method (Gravimetric Method)

The Zinc coupons were weighed and placed vertically in 60 ml of aerated, unstirred 2M HCl with and without the inhibitor for four hours. The coupons were removed from the solution and they were cleaned by brushing under running tap water to remove the corrosion products, dried and reweighed to determine the weight loss. The weight loss method was employed for the two temperatures 30˚C and 60˚C. In this procedure, the mass loss of the metal in without Azardirachta indica and Hibiscus rosasinensis extract (uninhibited solution) and with Azardirachta indica and Hibiscus rosasinensis extract (inhibited solution) were measured and recorded.

From the data, the percentage of inhibition efficiency (% I) and degree of surface coverage (Θ) were calculated using the following equations,

Percentage of inhibition efficiency IE = $(1 – W_2 /W_{1)}$ x 100
Where W_1 and W_2 are the weight of the metal in uninhibited and inhibited solutions
Degree of surface coverage (Θ)
Corrosion rate (CR)
= **$(1 – W_2 /W_1)$**
= **mm /d = 87.6 x (W / DX A X T)**

W = weight loss in milligrams, D = metal density in g/cm^3 A = area of sample in cm^2

T = time of exposure of the metal sample in hours, 87.6 is a conversion factor.

III. Results And Discussion

Test for Zinc in Soil Corroded Metal Solution

To 10 drops of solution, 6 MNH_3(aq) was added to give a neutral pH. Then the solution was made slightly acidic to litmus paper with 6 M HCl. One or 2 drops of 0.5 M $K_4Fe(CN)_6$ was added and stirred. A gray-white precipitate of K_2 $Zn[Fe(CN)_6]_2$ was formed.

Effect of Hydrochloric Acid Concentration on Zinc Corrosion

Zinc corrodes in different concentrations of HCl solutions, since there was a decrease in the original weight of zinc. The corrosion is attributed to the presence of water, air and H^+, which accelerate the corrosion process. The corrosion of the zinc in HCl increases with the concentration of the acid and time. The corrosion of the zinc in HCl increases with the concentration of the HCl acid. Similar results were obtained at 303K (30˚C) and 333K (60˚C). This observation is attributed to the fact that the rate of chemical reaction increases with increasing concentration. This observation has been reported by several authors (Ita and Edem, 2000; James et al., 2007).

Effect of Temperature on the Corrosion of Zinc

There was a progressive increase in weight loss as the temperature is increased from 30˚C and 60˚C. This signifies that the dissolution of the metal coupons increased at higher temperatures. This observation was attributed to the general rule guiding the rate of chemical reaction, which says that chemical reaction increases with increasing temperatures. Also an increased temperature favors the formation of activated molecules, which may be twice in number, with rise in temperature, thereby increasing the reaction rate. This is because the reactant molecules gain more energy and are able to overcome the energy barrier more rapidly. An increase in temperature may also increase the solubility of the protective films on the metals, thus increasing the susceptibility of the metal to corrosion. This observation has been reported by several authors (James et al., 2007 and James and Etela, 2008).

Table 1 Corrosion rate (CR) for the corrosion of zinc in all concentrations of HCl

Concentration of HCL (N)	**Corrosion Rate (CR) mg/h/cm2)at 30˚C**	**Corrosion Rate (CR) mg/h/cm2)at 60˚C**
0.5	0.5	0.5
1.0	1.0	2.2
2.0	6.3	7.6

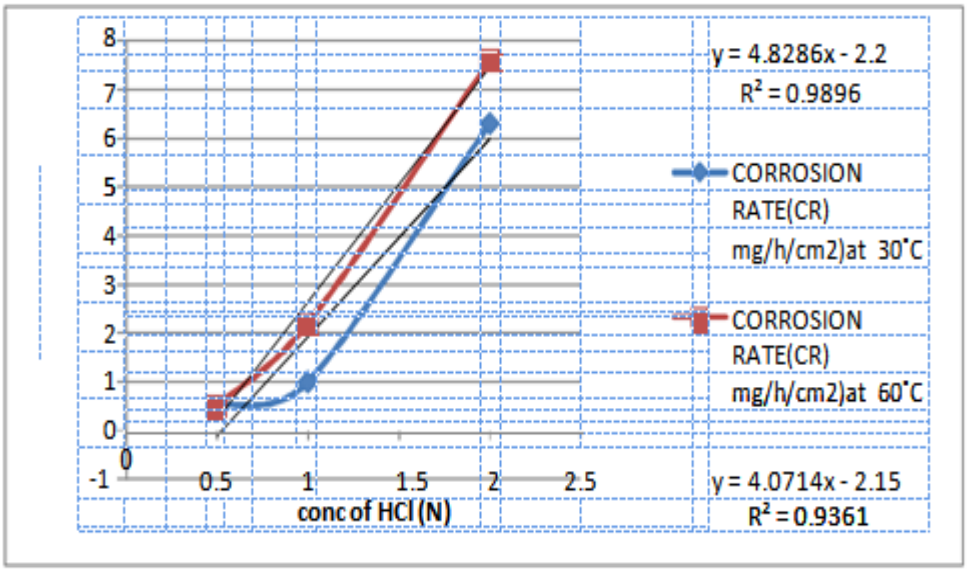

Figure 1 Corrosion rate (CR) for the corrosion of zinc in all concentrations of HCl

Effects of Ethanol Extract of Azardirachta Indica and Hibiscus Rosa-Sinensis on the Corrosion of Zinc in Acid Media

Variation of weight loss change with increase in concentration of AZI and HR leaves extract for HCl at two different temperatures was investigated. The variation of weight loss of Zinc for the corrosion of Zinc in 2 N HCl in the absence and presence of various concentration of ethanol extract of Azardirachta indica and Hibiscus rosa-sinensis at 303 K (30˚C) and 333K (60˚C) is shown in Table 2 and Fig.2.

Ethanol extract of Neem and Hibiscus leaves are indeed a corrosion inhibitor for zinc in hydrochloric acid solution. Since there was a general decrease in weight loss at the end of the corrosion-monitoring process at the temperatures studied.

From the variation of weight loss with time of exposure of zinc in 2M hydrochloric acid (blank) at 30^{o}C and 60°C compared with those containing the additives, there is a remarkable decrease in weight loss signifying corrosion inhibition. At 60^{o}C, as the concentration of Ethanol extract of Neem and Hibiscus leaves increases from 0.01g/dm^{3} to 0.10g/dm^{3}, the weight losses of the zinc coupons reduce. This shows us that Ethanol extract of Neem and Hibiscus leaves is still effective in inhibiting the corrosion of zinc at 60^{o}C. The weight loss of the zinc coupons still reduced with increasing Ethanolic extract of Neem and Hibiscus leaves concentration. This depicts that, even at 60^{o}C, Ethanolic extract of Neem and Hibiscus leaves inhibits the corrosion of zinc in hydrochloric acid solution. It is shown in Table 2 and Fg.2.

Table 2 Showing the Corrosion Rate (from weight loss method) for the corrosion of Zinc in 2N Hydrochloric acid (HCl) of AZI leaves extract at two different temperatures

Concentration of Azardirachta Indica leaves extract (mg/ml)	**Corrosion Rate (CR) at 30˚C (mg/h/cm^{2})**	**Corrosion Rate (CR) at 60˚C (mg/h/cm^{2})**
0	6.22	6.90
9	1.83	4.63
16	1.79	2.35
23	1.06	2.33

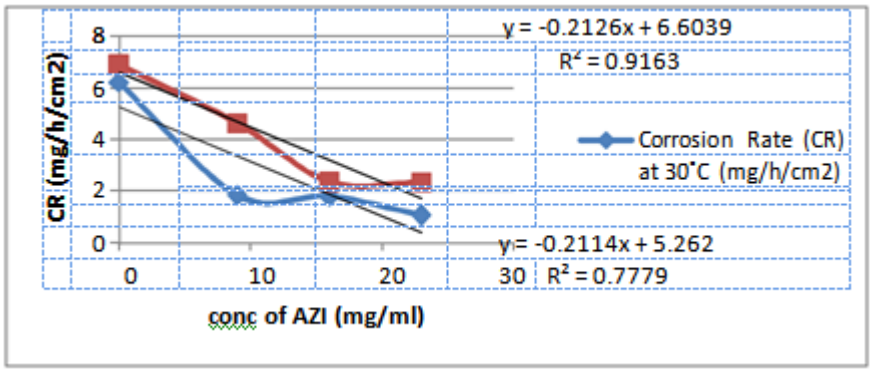

Figure 2 Corrosion of Zinc in 2N Hydrochloric acid (HCl) of AZI leaves extract at two different temperatures

Table 3 Corrosion Rate of Zinc in 2N Hydrochloric acid (HCl) of HR leaves extract at two different temperatures

Concentration of Hibiscus rosasinensis leaves extract (mg/ml)	Corrosion Rate (CR) at 60°C (mg/h/cm^2)	Corrosion Rate (CR) at 30°C (mg/h/cm^2)
0	140.2	99.7
20	127.4	90.2
40	110.2	86.7
60	101.6	63.3

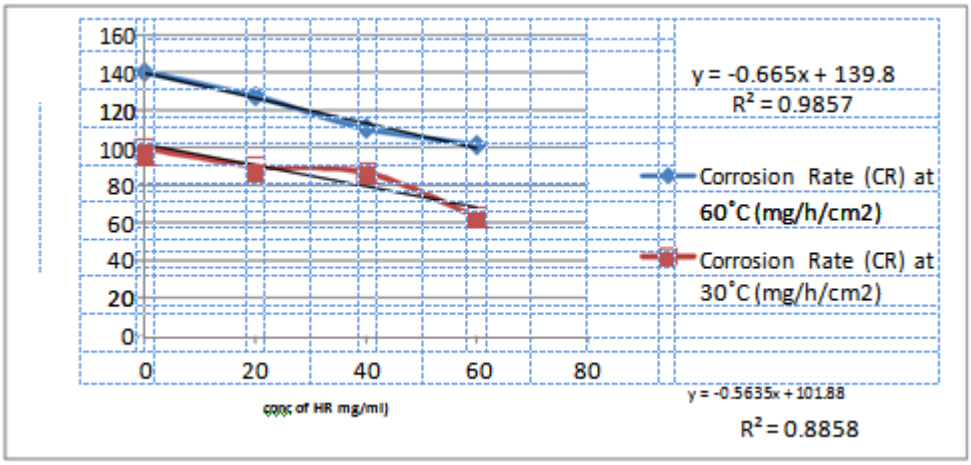

Figure 3 Corrosion Rate of Zinc in 2N Hydrochloric acid (HCl) of HR leaves extract at two different temperatures

Effect of Inhibitor Concentration on Inhibition Efficiency

Inhibition efficiency of AZI and HR leaves extract for different concentrations and at different temperatures shown in Table 4, 5 and fig. 4 and 5. From the inhibition efficiencies obtained from the weight loss experiments for 2 N HCl, it was found that the Inhibition efficiency increases with increase in inhibitor concentration of both the extracts for acidic media. The increase in efficiency of the inhibitor with increase in both the extracts concentration may be attributed to increase in number of molecules occupied by the inhibitor on the Zinc–acid solution interface. As the number of molecules increases, the corrosion reactions are prevented from occurring over the active sites of the Zinc surface covered by adsorbed inhibitor species, whereas the corrosion takes place on the surface not covered by the inhibitor molecules. Thereby, one may conclude that the greater the surface coverage the greater the inhibition efficiency. This assumption has been applied to deduce the effect of concentration on the adsorption of inhibitors.

The effect of increase in temperature on the inhibition efficiency of Ethanol extract of Neem and Hibiscus leaves is displayed graphically. We can observe from the graph that, as the reaction temperature is increased from 30^{o}C and 60^{o}C, the inhibition efficiency increases. Thus it is appropriate to say that increase in

temperature favours the inhibition efficiency of Ethanol extract of Neem and Hibiscus leaves on zinc in hydrochloric acid. The results of the present study coincide with the studies of James and Etela (2008).

It portrays an increase in inhibition efficiency of Ethanol extract of Neem and Hibiscus leaves as the concentration of the extract increases in the acid solution. This can be observed from the upward progression of all three temperatures. The active components are responsible for the inhibitory action of Neem and Hibiscus leaves. The inhibitory action of Neem and Hibiscus leaves was due to the presence of Quercetin. Quercetin is one of the flavonoid compounds present in Neem and Hibiscus leaves. It is a compound with conjugated system and contains hetero atoms and carbonyl groups that are electron rich which can serve as a good adsorption site onto the metal surface thereby inhibiting the corrosion of the zinc.

Table 4 Inhibition efficiency (% IE) for weight loss method of Azardirachta indica leaves extract for the corrosion of Zinc in 2N HCl at two different temperatures

Concentration of Azardirachta indica leaves extract (mg/ml)	**weight loss method Inhibition efficiency (% IE) at 30˚C**	**weight loss method Inhibition efficiency (% IE) at 60˚C**
9	62.1	36.7
16	71.7	60.8
23	78.3	68.9

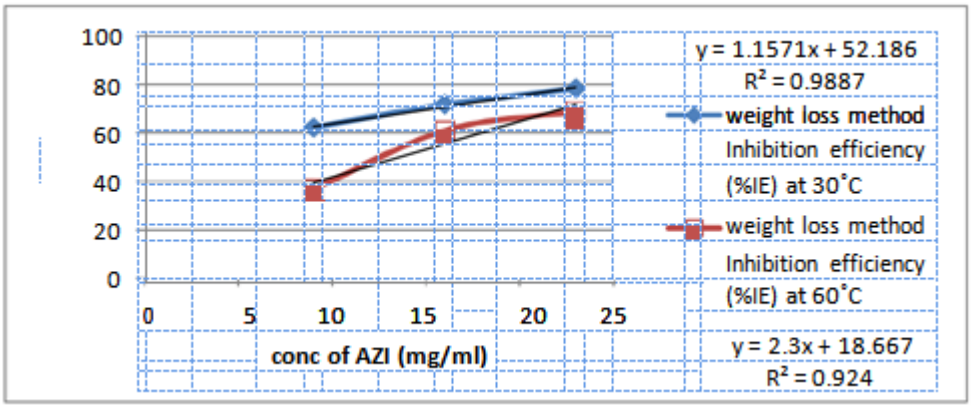

Figure 4 Inhibition efficiency of AZI leaves extract for different concentrations and at different temperatures

Table 5 Inhibition efficiency (% IE) for weight loss method of Hibiscus rosasinensis leaves extract for the corrosion of Zinc in 2N HCl at two different temperatures

Concentration of Hibiscus rosasinensis extract (mg/ml)	**weight loss method Inhibition efficiency (% IE) at 30˚C**	**weight loss method Inhibition efficiency (% IE) at 60˚C**
20	-100	23
40	-88	26
60	-75	46

Inhibition efficiency of HR leaves extract for different concentrations and at different temperatures

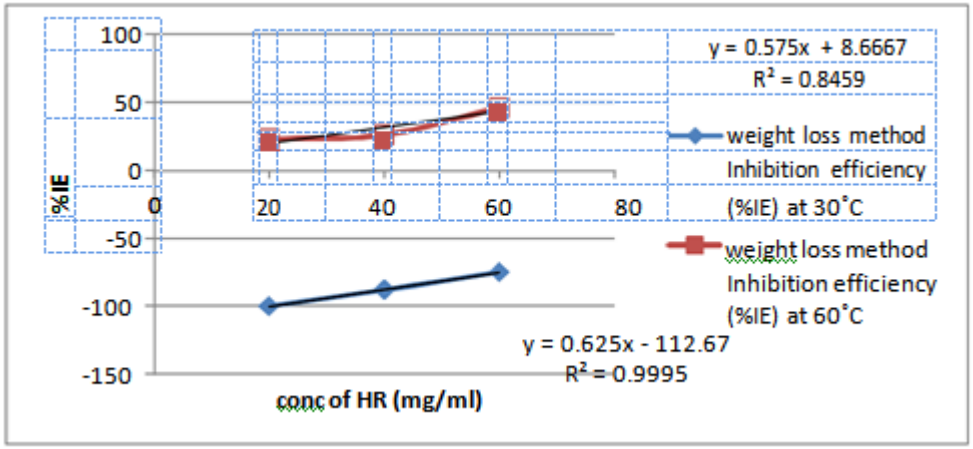

Figure 5 Inhibition efficiency of AZI leaves extract for different concentrations and at different temperatures

IV. Conclusion

Zinc corrodes in different concentrations of HCl solutions, since there was a decrease in the original weight of zinc. The corrosion is attributed to the presence of water, air and H+, which accelerate the corrosion process. The corrosion of the zinc in HCl increases with the concentration of the HCl acid. Similar results were obtained at 303 K and 333 K. The graph shows values of corrosion rate (CR) of zinc in all the concentrations of HCl studied and it shows that corrosion rate increases with an increase in HCl acid concentration. This observation is attributed to the fact that the rate of chemical reaction increases with increasing concentration. These extracts have to be proved that the Neem has higher antioxidant potential than HR. The antioxidant such as Quercetin and Rutin in Neem and Isoquercetin and Rutin in Hibiscus leaves extract have proved to be present which is more efficient for antimicrobial and anticorrosion activity. The percentage of inhibition in the presence of these inhibitors was decreased with temperature which indicates the physical adsorption was the predominant inhibition mechanism because the quantity of adsorbed inhibitor decreased with increasing temperature.

From the molecular structures, it can be observed that these compounds possess lot of hetero atoms and aromatic rings which are responsible for the adsorption of these compounds on to the metal surface. Organic compounds containing π electrons hetero atoms and multiple bonds have been reported to function as effective inhibitor for the corrosion of many metals in various media. Since the Neem and Hibiscus leaves extract contains Quercetin, Rutin and Isoquercetin. The inhibitive activity of the extract is attributed to the combined action of all the compounds present in the extract. The IE of the plant extracts increased with increasing in extract concentration and with temperature moisture and atmosphere. These results suggested that IE of secondary metabolites depends upon the factors such as their charge distribution, no. of adsorption sites, heat of adsorption, mode of interaction with metal surface, mode of inhibition of microbial growth in moist condition and formation of metallic complex.

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