

EXTRACT OF HIBISCUS ROSA SINENSIS AND AZADIRACHITA INDICA LEAVES

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Abstract - The present investigation was carried out about the corrosion inhibition and adsorption behavior of Neem and Hibiscus, as a green inhibitor of Zinc corrosion in acidic mediated corrosion causing soil microbes by using weight loss and thermodynamic studies. It was even analyzed both quantitatively and qualitatively biochemical substances present in Neem and Hibiscus leaves of ethanol extract which act as anticorrosive agent of metallic and non-metallic substances in acidified soil. Ethanolic extract of Neem and Hibiscus leaves extract showed the strong antimicrobial activity against soil microbes such as *S.aureus*, *Streptococcus*, *B. subtilis*, *Lactobacillus*, *Proteus*, *Corynebacterium*, *Pseudomonas*, *A.niger*, *Mucor* and *Desulpho Vibro* sp. Langmuir and Freundlich, Temkin and Florry Huggins models are employed to analysis adsorption occurred in the experimental data of adsorption isotherms. The Freundlich, Langmuir and Temkin models are employed to analysis adsorption occurred in the experiment.

Index Terms - 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

A. Figures and Tables

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 R_f 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
(1) 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 ¹³C 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 R_f 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° 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,

$$\text{Percentage of inhibition efficiency IE} = (1 - W_2 / W_1) \times 100$$

Where W_1 and W_2 are the weight of the metal in uninhibited and inhibited solutions

$$\text{Degree of surface coverage } (\Theta) = (1 - W_2 / W_1)$$

$$\text{Corrosion rate (CR)} = \text{mm/d} = 87.6 \times (W / DX A \times T)$$

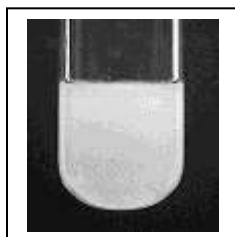
W = weight loss in milligrams, D = metal density in g/cm^3 A = area of sample in cm^2

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 $\text{MNH}_3(\text{aq})$ 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 $\text{K}_4\text{Fe}(\text{CN})_6$ was added and stirred. A gray-white precipitate of $\text{K}_2\text{Zn}[\text{Fe}(\text{CN})_6]_2$ was formed.



Effect of Hydrochloric Acid Concentration on Zinc Corrosion

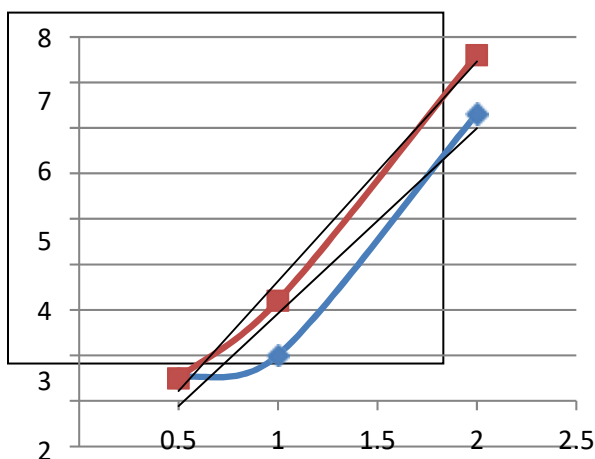
Zinc corrodes in different concentrations of HCl solutions, since there was a decrease in the original weight of zinc. The corrosion is attributed to the presence of water, air and H^+ , which accelerate the corrosion process. The corrosion of the zinc in HCl increases with the concentration of the acid 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 H

Concentration of HCL (N)	Corrosion Rate (CR) mg/h/cm^2 at 30°C	Corrosion Rate (CR) mg/h/cm^2 at 60°C
0.5	0.5	0.5
1.0	1.0	2.2
2.0	6.3	7.6



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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