BIOCHEMICAL CHARACTERISATION OF THE LEAF OF *MORINDA LUCIDA*: PROSPECTS FOR ENVIRONMENTALLY-FRIENDLY STEEL-REBAR CORROSION-PROTECTION IN AGGRESSIVE MEDIUM

Joshua Olusegun OKENIYI[1], Olubanke Olujoke OGUNLANA[2], Oluseyi Ebenezer OGUNLANA[3], Taiwo Felicia OWOEYE[4], Elizabeth Toyin OKENIYI[5]

[1]Mechanical Engineering Department, Covenant University, Ota 112001, Nigeria
[2]Biochemistry Programme, Department of Biological Sciences, Covenant University, Ota 112001, Nigeria
[3]Biochemistry Programme, Department of Biological Sciences, Crawford University, Igbesa 112001, Nigeria
[4]Department of Chemistry, Covenant University, Ota 112001, Nigeria
[5]Petroleum Engineering Department, Covenant University, Ota 112001, Nigeria

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Abstract

This paper employs atomic absorption spectroscopy (AAS), Fourier transform infrared spectroscopy (FTIR) and phytochemical screening methods for biochemical characterisation of the inorganic and organic constituents of the leaf of *Morinda lucida*. AAS results showed that this well-known medicinal-plant is high in iron (Fe = 5143.54 μg/g), low in cadmium (Cd = 2.9506 μg/g) and does not contain chromium (Cr). Also, Euclidean hit-list from the FT-IR instrument suggests *Morinda lucida* leaf-extract contains S–, N–, Br– and O– containing hetero-atoms. The phytochemical analyses indicated presence of tannins, phlobatannins, saponins, flavonoids and terpenoids. These results bare prospects on suitability of leaf-extract from *Morinda lucida* for environmentally-friendly steel-rebar corrosion-protection in aggressive medium. Preliminary tests based on this showed that use of 0.083 wt% cement of *Morinda lucida* retarded steel-rebar total-corrosion and eventually reduced corrosion rate as admixture in duplicated 3.5% NaCl-immersed concretes, relative to control samples in the same medium.

Introduction

Mitigating concrete steel-rebar corrosion by the use of corrosion inhibitors is generally accepted as an effective method for addressing this deterioration mechanism affecting durability of the most widely used construction material [1–2]. Corrosion inhibitor usage is considered attractive because it combines excellent inhibition performance on concrete steel-reinforcement corrosion, in aggressive environment, with simplicity of application, relatively low cost and labour saving compared to other corrosion protection methods [1,3]. However, many factors have been identified in studies that could improve, or on the other hand, militate against the effectiveness of corrosion inhibiting substance on concrete steel-reinforcement corrosion as well as its acceptability of use for inhibiting steel-rebar corrosion. Among these factors are the mechanism of inhibition on steel-rebar corrosion, e.g. anodic, cathodic or mixed, relative cost of inhibitor types compared to other types, as well as the toxicity and hazardousness of the inhibitor substance to the environmental ecosystem. For instance, mixed inhibitors are preferred to both anodic and cathodic inhibitors because of the tendencies, for instances, of corrosion aggravation

if anodic inhibitors are present in insufficient dosage and of essentially lesser corrosion-inhibition effectiveness of the cathodic than the anodic inhibitors [3,4]. Also, organic types of inhibitors are generally known to be less costly and highly effective inhibitors of steel-rebar corrosion compared to inorganic types of inhibitors. Specifically, organic compounds containing nitrogen (N), oxygen (O) and sulphur (S) atoms, multiple bonds and hetero-atom are known to be highly effective inhibitors because they can combine mixed anodic and cathodic inhibition mechanisms with corrosion-protective adsorption on rebar surface [3,5]. Furthermore, compounds of nitrites and chromates, for example, that are well-known as inhibitors of concrete steel-rebar corrosion in aggressive environments, are having their usage being restricted in many countries due to their carcinogenic tendencies on biological organs [1,3,5]. Because of this, current research trends are shifting towards search for eco-friendly alternatives without compromising the form of high effectiveness attainable from the use of the toxic inhibitors.

Extracts from natural plants are potent at neither being toxic nor hazardous to the environmental ecosystems. They are also natural and highly economical sources of organic hetero-atoms that could combine the advantages of mixed inhibition mechanism on metallic corrosion with the corrosion-protective adsorption of their electron sharing atoms with the reinforcing steel metal [3,6]. Although, inhibition of concrete steel-reinforcement corrosion in aggressive media by many plants has been studied in research works [7], there is dearth of study on the biochemical characterisation of plants for assessing their prospects as environmentally-friendly inhibitors of rebar corrosion. Biochemical study could be useful for understanding the organic constituents, and thus the hetero-atoms that could find usefulness for inhibiting metallic corrosion. In addition, biochemical study of inorganic constituents will also find usefulness for ascertaining presence of heavy metals, which plants could accumulate from soil through phytoremediation [8], but some of which could be toxic, while some could aggravate or inhibit corrosion [9-10].

Morinda lucida is a natural, well-known medicinal plant habitat in West Africa, for which toxicology study has shown that its leaf-extract is non-toxic to living organs [11-12]. However, there is paucity of study in which leaf of *Morinda lucida* have been characterised biochemically for gaining insights on its prospect as an inhibitor of steel-reinforcement corrosion in corrosive medium. This study therefore deliberates on the biochemical characterisation of the leaf of *Morinda lucida* and using this information for assessing prospects on the usage of extract from this plant for environmentally-friendly corrosion-protection of steel-rebar in aggressive medium.

Experimental Methods

Experimental Materials

Fresh leaves of *Morinda lucida* (*M. lucida*) *Rubiaceae* were collected from the Forestry Research Institute, Ibadan, Nigeria, and were identified at the Forestry Herbarium Ibadan (FHI), Nigeria, with a sample deposited there with the voucher FHI. No. 109500. The leaves were dried in a well aerated room maintained at 20 °C.

Experimental Test-Procedures

For the AAS (Atomic Absorption Spectrometer) study of inorganic (heavy metal) constituent, ashing of some dried and pulverised leaves of *M. lucida* was done in a muffle furnace maintained at 500°C for 2 hrs. The ash content obtained by this = 6.07%. 0.25 g of the ashed material was then soaked overnight in 5 ml of 1:1 nitric-perchloric acid mixture. Then it was heated in a reflux condenser maintained at 150 °C for 1 hr before gradually raising the temperature to 235 °C, at which heating was continued for another 2 hr when dense fume occurs. Thereafter, this was removed from the heating block and cooled to 100 °C before adding 1:1 HCl, then heated to white fumes until colourless solution was obtained. The resulting colourless solution was poured into 100 ml flask, washed with water five times, each time adding the washing to the flask before

making up the volume to the 100 ml mark with water. The solution was then filtered and the filtrate taken to the S Series, AA Spectrometer that employs Hollow Cathode Lamps by Thermo Electron Corporation.

M. lucida leaf-extract was obtained, for the study of organic constituents, by using procedure described in reported work [13]. Two methods were employed for the study of organic constituents. The first method includes use of KBr pellets of the plant extract mounted on the FT-IR (Fourier Transform Infra Red Spectrophotometer) instrument of the Perkin-Elmer FT-IR System Spectrum BX. The obtained spectra were subjected to the Euclidean Search of Fluka library by the Perkin-Elmer instrument.

The second method used for organic constituent characterisation includes phytochemical screening methods using the procedures detailed in literature [14]. These methods include use of aqueous solution obtained from adding 2 g of the plant extract to 20 ml of distilled water as sample and with this sample the following test-procedure were conducted.

i. Test for tannins: 0.4 ml of sample boiled in 5 ml water, this was filtered and a few drops of 0.1% $FeCl_3$ was added for observing brownish-green or blue-black colouration.
ii. Test for phlobatannins: sample was boiled in 1% HCl for observing deposit of red colour.
iii. Test for saponins: 0.4 ml of sample was boiled in 4 ml distilled water in a water bath and filtered, filtrate was shaken vigorously for stable persistent froth, which was mixed with 3 drops olive oil and shaken vigorously for observing formation of emulsion.
iv. Test for flavonoids: 0.4 ml sample was heated with 2 ml ethyl acetate over steam bath for 3 min after which the mixture was steeped and 0.4 ml of the filtrate was shaken with 0.1 ml dilute ammonia solution for observing yellow colouration
v. Test for steroids: 0.4 ml of acetic anhydride was added to 0.1 g extract + 0.4 ml H_2SO_4 for observing colour change from violet to blue or green.
vi. Terpenoids (Salkowski test): 1 ml of sample was mixed with 0.4 ml chloroform + 0.6 ml concentrated H_2SO_4 carefully to form a layer for observing reddish-brown colouration.
vii. Glycosides (Keller-Killani test): 0.5 ml of sample was treated with 0.2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution and that had also been underlaid with 1 ml concentrated H_2SO_4 for observing brown ring and/or violet ring below the brown ring or green ring through the thin layer.
viii. Test for alkaloids: 10 ml of water was added to the sample and to 1 ml of this, 2 drops of Wagner's reagent (Iodo-potassium iodide, obtained by dissolving 0.2 g of iodine and 0.6 g of KI in 1 ml of water) for observing brown or reddish-brown precipitate.

Results and Discussion

AAS Test-Results of Heavy Metals

The test-results of inorganic constituents, the heavy metals, from *M. lucida* by the AAS are presented in Table 1. The table shows that this studied *M. lucida* was richer in inorganic constituent that exhibited positive influence on its micronutrients than the constituent that could exhibit toxicity to the environmental ecosystem. For example, it could be observed from the table that the tested *M. lucida* is low in Cadmium (Cd = 2.9506 µg/g), Nickel (Ni = 15.6299 µg/g), Lead (Pb = 48.4057 µg/g) and does not contain Chromium (Cr). The concentration of these constituents of heavy metals in *M. lucida* are below the Ecological Soil Screening Levels (Eco-SSLs), the concentrations of contaminants in soil that are protective of ecological receptors that commonly come into contact with and/or consume biota that live in or on soil, for plants [15]. According to USEPA regulations, Eco-SSLs for plants for Cd = 32 µg/g [16], for Ni = 38 µg/g [17] and for Pb = 120 µg/g [15]. Although plant toxicity data for Cr concentration ranged from 3

µg/g to 138 µg/g in [18], Eco-SSLs value was still not derived by USEPA for Cr due to inadequacy of acceptable data for that regulation document in [18].

Table 1. AAS Test-results of inorganic constituents from *M. lucida*

Inorganic Constituent	Nickel (Ni)	Cadmium (Cd)	Lead (Pb)	Chromium (Cr)	Iron (Fe)
Concentration (µg/g)	15.6299	2.9506	48.4057	-	5143.54

The high concentration of Fe in the studied *M. lucida* bare indication that the plant was able to absorb iron as micronutrients that is needed for its growth, for the formation of chlorophyll and for the functioning of enzymes of its respiratory (photosynthesis) mechanism. This is an implication that followed the fact that the environment of the studied plant's growth was not toxic with other heavy metals which could have resulted in the deficiency of Fe in the studied plant [19].

FT-IR Characterisation of *M. lucida* Leaf-Extract

The FT-IR spectra indicating characterisation of *M. lucida* leaf-extract is shown in Figure 1. This indicated overlaps of many organic hetero-atoms in *M. lucida*. The 3723.00 cm^{-1} and 3411.42 cm^{-1} absorptions suggest asymmetric and symmetric N–H stretching of aromatic amines group also having C–N stretching at 1259.06 and 1163.67. These have fingerprints of NH_2 scissoring at 1562.26 cm^{-1} and NH_2 and N–H wagging at 897.00 cm^{-1}, 851.55 cm^{-1} and 731.39 cm^{-1} [20].

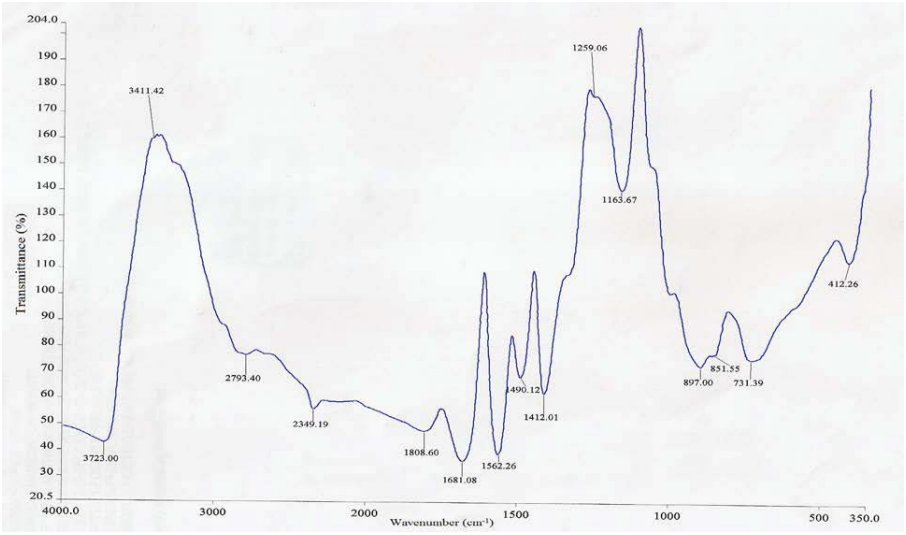


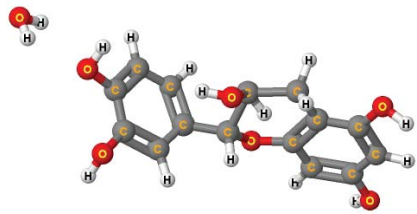
Figure `1. FT-IR spectra of *M. lucida* leaf-extract.

The absorption at 2793.40 cm^{-1}, in Figure 1, exhibited the higher frequency of carbonyl compound group associated the terminal aldehydic C–H stretch with suggested fingerprint of α–CH_2 bending at 1412.01 cm^{-1}. However, this 1412.01 cm^{-1} exhibited potency of overlap, for this frequency could also indicate fingerprint of C–O–H bending [13,20], for the C=O stretching vibration of acyl halide (RCOX), a carboxylic acid derivative group, at 1808.60 cm^{-1}, where X = Br for the absorption that ranged 1800 ± 15 cm^{-1}. This further suggests that the 897.00 cm^{-1}

could also be an overlap of O–H out of plane bending vibration fingerprint of carboxylic acid derivative. The absorption at 1681.08 cm^{-1} bare indication of C=O stretching of amide I band with suggested fingerprint overlap at 1562.26 cm^{-1} and another fingerprint at 1490.12 cm^{-1} for making up the two bands required for bending vibration of the suspected amide group frequency. By this, the absorbed frequencies at 2349.60 cm^{-1} and at 412.26 cm^{-1} remained to be assigned. These bare suggestions of reduced absorption frequencies that could be due to heavier element or groups of elements substitution/conjugation in the C–H stretch and fingerprints of the amines or the carbonyl aldehyde or the carboxylic acid derivative groups that have been identified thus far.

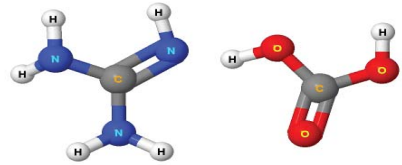
The Euclidean search hit-list by the Perkin-Elmer instrument, of suggested organic compounds from the *M. lucida* FT-IR spectra, based on Fluka library, are presented as 3-D optimised structure of the compounds in Figure 2. Out of these ten compounds, 8 contain aromatic rings while 3 are S– (sulphur), 7 are N– (nitrogen), 2 are Br– (bromine) and 8 are O– (oxygen) containing hetero-atoms. Hetero-atom compounds, similarly rich in lone pair electrons as the ones obtained in this study have been identified, in literature, with good inhibition of steel-rebar corrosion in aggressive environments.

a. (+–)-Catechin

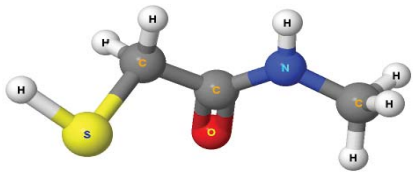


b. (+)-Catechin Hydrate

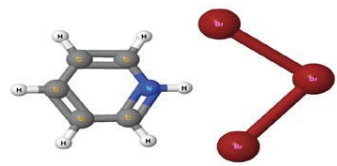
c. (–)-Epicatechin



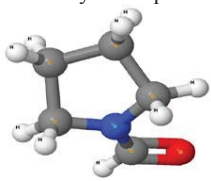
d. Guanidine Carbonate



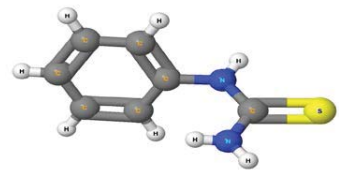
e. N-Methylmercaptoacetamide



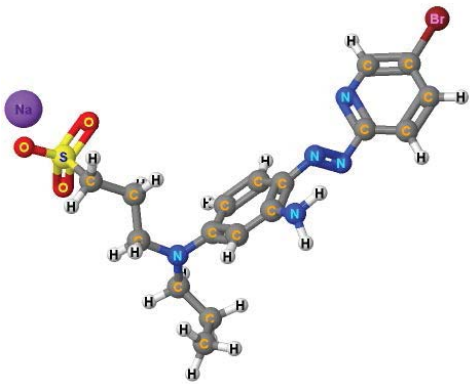
f. Pyridine Hydrobromide Perbromide



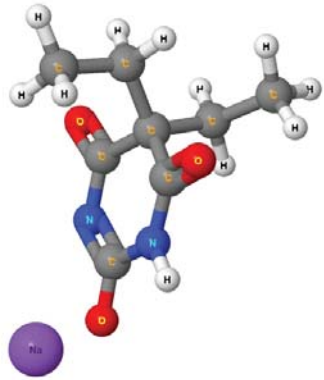
g. 1-Formylpyrrolidine



h. N-Phenylthiourea



i. 2-(5-Bromo-2-Pyradzo)-5-(Propyl-3-Sulfopropylamino) Aniline Na Salt



j. 5,5-Diethylbarbituric Acid Sodium Salt

Figure 2. Euclidean Search hit-list of FT-IR spectra of *M. lucida* leaf-extract.

Phytochemical Characterisation of *M. lucida* Leaf-Extract

The results of phytochemical characterisation of *M. lucida* leaf-extract are presented in Table 2. These showed that *M. lucida* leaf-extract contains five, including tannins, phlobatannins, saponins, flavonoids and terpenoids, out of the eight constituents tested in the study. These type of phytochemical containing plants have been found useful in studies for inhibiting concrete steel-rebar corrosion.

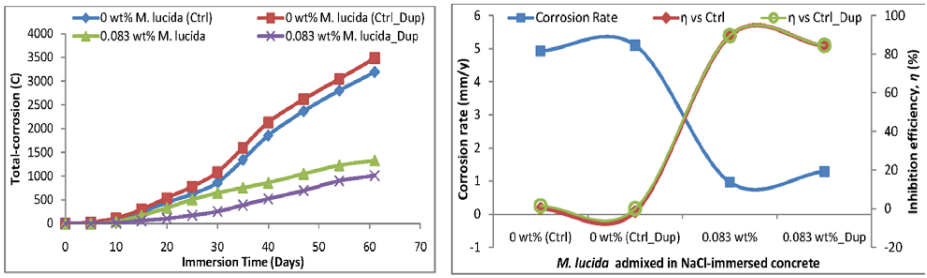
Table 2. Phytochemical constituent of *M. lucida*[*]

Tannins	Phlobatannins	Saponins	Glycosides	Flavonoids	Stenoids	Terpenoids	Alkaloids
+	+	+	–	+	–	+	–

* Present = +; Absent = –

Effect of *M. lucida* Leaf-Extract Admixture in Concrete on Concrete Steel-Rebar Corrosion

The biochemical characterisation of the leaf of *M. lucida* indicated prospects that the plant contains micronutrients, organic compounds and phytochemicals, which not only portray the plant as non-toxic but that could also find usefulness as environmentally-friendly corrosion inhibitor. For this 0.083 wt% cement *M. lucida* leaf-extract was admixed in duplicates "Dup" of steel-reinforced concrete slabs immersed in bowls containing 3.5% NaCl test-solution, used for simulating aggressive marine/saline medium. Corrosion of each steel-rebar was then monitored, along with the rebar of duplicates of controls "Ctrl" (steel-reinforced concretes with 0 wt% admixtures), for sixty-one days, using two different techniques. The first technique includes the total-corrosion (C) model from macrocell current method that employs the zero resistance ampere (ZRA) instrument as per ASTM G109-99a [21]. The second technique includes Weibull mean model of corrosion rate (mm/y) test-data that were measured using linear polarisation resistance (LPR) instrument over the experimental period [2,13]. The results from these are presented in Figure 3, which include plots of total-corrosion in Figure 3a and plots of corrosion rate overlaid with inhibition efficiency, η (%), in Figure 3b.



a. b.

Figure 3. Corrosion test-results from *M. lucida* leaf-extract admixed in NaCl-immersed steel-reinforced concretes. a. Total-corrosion model; b. Weibull mean model of corrosion rate

The results of the corrosion test-results showed agreements in the modelled test-responses between the duplicates of steel-reinforced concretes, the control and its duplicate as well as the duplicates of samples admixed with 0.083 wt% *M. lucida*. The total-corrosion, modelled from the macrocell technique, in both duplicates of 0.083 wt% *M. lucida* admixed samples were retarded compared to the total-corrosion models obtained from the duplicates of control samples, Figure 3a. In similar manner the corrosion rate models from both duplicates of 0.083 wt% *M. lucida* admixed samples were highly reduced compared to the corrosion rate models obtained from the duplicates of control samples, Figure 3b. For the 0.083 wt% *M. lucida* leaf-extract admixed sample, obtained inhibition efficiency, η = 89.45% vs Ctrl or η = 84.30% vs Ctrl_Dup which gives averaged inhibition efficiency η_{ave} = 86.88%. Also, for the 0.083 wt%_Dup *M. lucida* leaf-extract admixed sample, obtained inhibition efficiency, η = 89.61% vs Ctrl or η = 84.54% vs Ctrl_Dup which gives averaged inhibition efficiency η_{ave} = 87.08%. These bare implications of positive prospects of the suitability of *M. lucida* for environmentally-friendly corrosion-protection of steel-rebar in concrete designed for the aggressive saline/marine service-environment.

Conclusions

It could be concluded from this study that the leaf of *M. lucida* contains micronutrients required for the plant's growth at non-toxic levels of heavy metals. The leaf-extract is rich with aromatic, S–, N–, Br–, and O– containing hetero-atoms and phytochemical constituents that are potent at enhancing steel-rebar corrosion-protection in aggressive medium. This was confirmed in the study by 0.083 wt% of *M. lucida* admixture that exhibited very good effectiveness at inhibiting steel-rebar corrosion, relative to the controls, in 3.5% NaCl-immersed steel-reinforced concretes.

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