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Biocorrosion Inhibitory Potential of Aqueous Extract of *Phyllanthus amarus* against Acid Producing Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. Author WFB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HOS and GCO managed the analyses of the study. Authors OMI and CJU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Biocorrosion is a form of corrosion of metallic and concrete materials mediated by microorganisms. Acid producing bacteria are major culprits in the corrosion of materials in the environment. This study focused on the inhibition of biocorrosion by acid producing bacteria using aqueous extract of *Phyllanthus amarus* (PAAE). Acid producing bacteria were isolated from produced water samples collected from oilfields located in Niger Delta, Nigeria. Multiple fermentation tube technique was adopted for the isolation of the acid producing bacteria using phenol red dextrose broth as culture

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broth medium. The gravimetric analysis was performed with different concentrations of the plant extract incorporated in universal culture bottles containing broth, sample and carbon steel coupon. The setup was incubated at 20ºC, 30ºC and 40ºC for 7 days and for 14 days. The least corrosion rate (CR) at 20ºC, 30ºC and 40ºC for the 7 days test were 1.446mp/y (80mg/ml PAAE); 27.558mp/y (5 mg/mlLeu+20 mg/ml PAAE) and 5.134 mp/y (80 mg/ml PAAE) with corresponding inhibition efficiency (IE) of 81.92, 87.750 and 80.91 respectively. For the 14 days, the CR values at 20ºC, 30ºC and 40ºC were: 3.192mp/y (5mg/mlLeu+20mg/ml PAAE); 1.458 mp/y (5 mg/mlLeu + 20 mg/ml PAAE) and 117.345mp/y (5 mg/mlLeu + 20 mg/ml PAAE) with corresponding IE of 86.09, 83.87 and 98.89 respectively. The results obtained show that the extract could be considered as a good inhibitor for the biocorrosion of carbon steel mediated by acid producing bacteria.

Keywords: Biocorrosion; inhibition; acid producing bacteria; Phyllanthus amarus.

1. INTRODUCTION

Biocorrosion of metals or industrial facilities is a major concern in different industries due to the application of water in the industrial processes which leads to corrosion of materials by microorganisms causing reduction of the quality and economic value of industrial materials. Common corrosive bacteria include the iron oxidizing bacteria, manganese oxidizing bacteria, sulphate reducing bacteria, acid producing bacteria and nitrate reducing bacteria. Acid producing bacteria also known as fermentative bacteria are among the group of well-known corrosive bacteria [1].

Virtually all corrosive bacteria can be found in oilfield environment but the possibility of being able to cultivate these bacteria of great economic importance depends on the sample type and laboratory conditions optimized for their cultivation. Examples of such samples include soil beneath buried oil and gas pipeline, oily sludge, injection water, produced water and slurry from drilling operations. The laboratory conditions include the temperature, presence of oxygen and type of culture medium. Currently, due to the problem of corrosion caused by bacteria in the oil industry, there had been the need for the production of corrosion inhibitors to combat the corrosion and souring menace. Synthetic biocides such as chlorine, bromine, ozone, carbamates, glutaraldehyde, guanide and isothiazoline are known to have corrosion problems; hence, current biotechnological research focuses on green chemicals which can be used as biocorrosion inhibitors [2].

Plants have been the major source of different medicines, food supplements and industrial chemicals for decades. Plants are considered colossally beneficial to man, warranting their use in biotechnology [3]. Plants are rich in secondary metabolites with bioactive properties. *Phyllanthus amarus* is a common shade loving plant widely distributed in Nigeria and West Africa at large. The plant belongs to the plant family Euphobiaceae, genus Phyllanthus and species *amarus.* This herb which grows in the rainy season as a small erect annual weed is now considered a very useful plant with a number of bioactive properties [4,5]. Much attention is yet to be given to it as a plant of great economic value.

Most scientific studies on biocorrosion have been centred on sulphate reducing bacteria, although there are pockets of information on other corrosive bacteria today. However, the species diversity and richness in terms of ecological function and activities of bacteria warranted the study of the acid producing bacteria as one major oilfield bacteria of economic importance in the hydrocarbon industry. This study aimed to evaluate the biocorrosion inhibition potential of the aqueous extract of *Phyllanthus amarus* against acid producing bacteria isolated from oilfields located in Niger Delta, Nigeria.

2. MATERIALS AND METHODS

2.1 Isolation of Acid Producing Bacteria from Produced Water Samples

The acid producing bacteria were isolated from produced water samples collected from oilfield environments (2 flow stations and 8 injection wells) located in Imo River (Abia State), Caw-thorn Channel (Rivers State), Oyigbo (Rivers State) and Benisede (Bayelsa State) within the Niger Delta, using Phenol red dextrose culture broth. The broth medium was prepared by mixing 10 g of peptone, 5 g of dextrose, 5 g of sodium chloride and 18mg of phenol red powder with 1litre of distilled water. The medium was autoclaved at 121ºC for 15 minutes before use. The multiple tube fermentation technique was

adopted for the isolation of acid producing bacteria [6]. The inoculated broth was incubated at 37ºC for 7 days under aerobic condition [7]. The isolates were purified by sub-culturing in MacConkey agar as a differential/selective medium for isolation [7].

2.2 Harvesting and Preparation of Plant

The whole plant of *Phyllanthus amarus* was harvested from Mgbuoba town and dried for six (6) weeks. The dried plant was cut for proper drying before blending using electric blender to obtain a powdered granular texture of the plant. While fresh leaves of lemon grass was collected from a garden in Ogoni land, Bori, Rivers State, Nigeria. The leaves were dried for duration of twelve (12) weeks for proper drying. The whole dried plant was macerated to particulate form and ground to powder before preparation of extract [8-10].

2.3 Phytochemical Screening of Plant Extracts

Procedures for the detection of some phytochemicals: alkaloid, protein, glycosides, flavonoids, tannins, phenol, saponins, steroids and terpenoids in the plant extract are as described in Ugochukwu et al. [11] and Pandey and Tripathi [12].

2.4 Determination of Functional Groups Present in the Plant Extracts

The functional groups in the aqueous plant extract were determined using the Agilent Cary 630 FTIR spectroscopy in transmittance mode. The powdered form of potassium bromide (KBr) was mixed with the extract sample to form small pellets and placed on the FTIR analysers to measure the peak of absorption at a range of 400 - 4000 wave number per centimetres. The FTIR spectra interpretation was done according to Adina et al. [13]. The spectra of absorption produced bands which indicated the functional group of some chemical compounds present in the aqueous extract of the test plant [14].

2.5 Preparation and Conditioning of Carbon Steel Coupon

The dimension of the carbon steel coupons used was 40 cm x 3 cm x 1.7 cm. The coupons were sourced and prepared from the Science and Engineering workshop, University of Port Harcourt. The coupons used were cleaned using grits paper then rinsed in 20% HCl, before rinsing in 1 g of $NaHCO_3$/50 ml distilled water and 50ml of acetone to remove every particulates from the surface of the coupons before conditioning. The weights of the coupons were determined gravimetrically before conditioning in the aqueous extract [14,15].

2.6 Biocorrosion Analyses

The multiple tube fermentation technique was adopted for the biocorrosion assay [6]. Fifteen millilitres (15 ml) of phenol red dextrose broth medium was dispensed into universal culture bottle using sterile syringe. The broth medium was sterilized at 121ºC for 15minutes using an autoclave. Produced water samples (10ml, 1ml and 0.1 ml) were dispensed into the tubes containing the culture broth. Coupons were conditioned by immersion for 24hours in extract, extract + the amino acid leucine as a biocide enhancer or glutaraldehyde [14]. Coupons treated with the plant extract (conditioned) and the untreated coupons were added to the universal culture bottles containing the broth medium and the produced water sample. Different concentrations (20mg/ml PAAE, 40mg/ml PAAE, 60 mg/ml PAAE, 80 mg/ml PAAE, 100 mg/ml PAAE and 5 mg/ml Leucine +20 mg/ml PAAE) of the extract were incorporated into the setup to determine the rate of inhibition of corrosion caused by acid producing bacteria. The blank control bottle contained only the broth and untreated coupon while the cell control contained acid producing bacteria, broth and untreated coupon. The setup was incubated at 20ºC, 30ºC and 40ºC for 7days and for 14days. The corrosion rate was determined by gravimetric analysis of the coupons before and after incubation, using the formula:

CR= KW/DAT, where K= rate constant 22,300; W= weight loss in grammes; D= density in gcm^{-3}, A= exposed area in in^2 and T = time of exposure in days. While the inhibition efficiency of the extract was determined by the formula:

$$IE = 100[1- (W_2/W_1)]$$

Where W_1 is the corrosion rate in the absence of the inhibitor, W_2 is the corrosion rate in the presence of the inhibitor.

3. RESULTS

3.1 Phytochemical Analysis

The results of the phytochemical analysis of the extract are presented in Table 1. The extract has

high amount of phenol, moderate amount of tannins and glycosides, low amount of alkaloid, while protein, saponins and steroids were not detected. The FTIR absorption spectrum of the extract contained functional groups of compounds such as alcohols- O-H (3300-3600cm^{-1}); amines-N-H (3300-3500cm^{-1}); alkynes C-C-(2100-2140 cm^{-1}); amides-C=O (1630-1695 cm^{-1}) and alkenes- C=C (1630-1670 cm^{-1}) (Fig. 1).

3.2 Corrosion Rate and Inhibition Efficiency

Results for the corrosion rates at 7 days and 14 days of incubation are presented in Figs. 2 and 3 respectively. For the 7 days test, the corrosion rate was higher at 30ºC, followed by 40ºC and least at 20^{o}, while for the 14 days test, the corrosion rate was higher at 40ºC followed by 30ºC and least at 20ºC.

Table 1. Aqueous extract of *Phyllanthus amarus*

S/no	Biochemical component	Observation
1.	Alkaloid	+
2.	Protein	-
3.	Glycosides	++
4.	Flavonoids	-
5.	Tannins	++
6.	Phenol	+++
7.	Saponins	-
8.	Steroids	-

Keys: - = absent, + = low, ++ = moderate, +++ = high

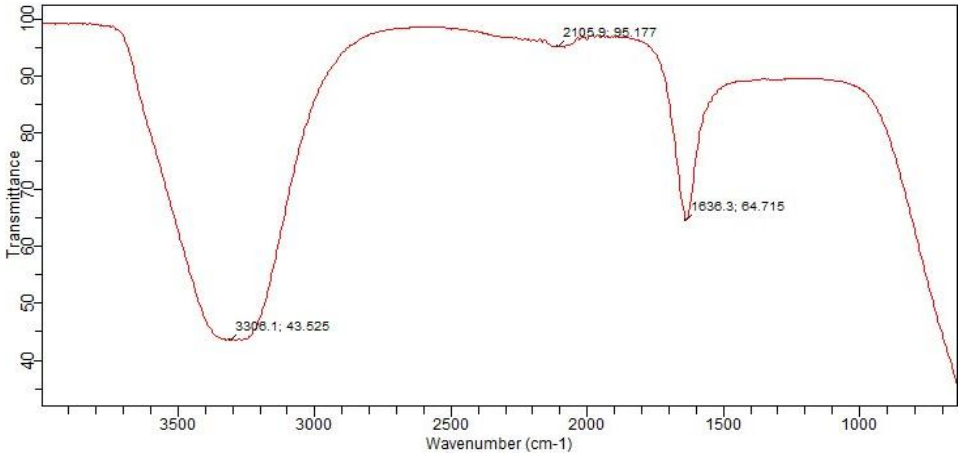

Fig. 1. FTIR absorption spectra showing the functional groups in the aqueous plant extract

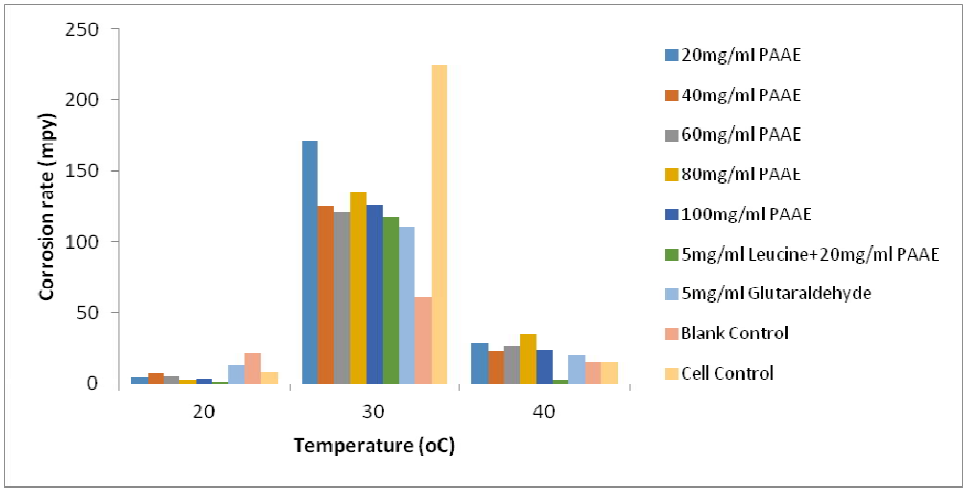

Fig. 2. Corrosion rate at different temperatures and concentration of aqueous plant extract after 7 days of incubation

Results for the corrosion rates at 7 days and 14 days of incubation are presented in Figs. 2 and 3 respectively. The extract had higher inhibition efficiency at all temperatures when used with leucine.

Fig. 4 shows the most probable number (MPN) of acid producing bacteria present during the biocorrosion analyses at various temperatures for the 7 days studies while Fig. 5 shows the MPN values for 14 days.

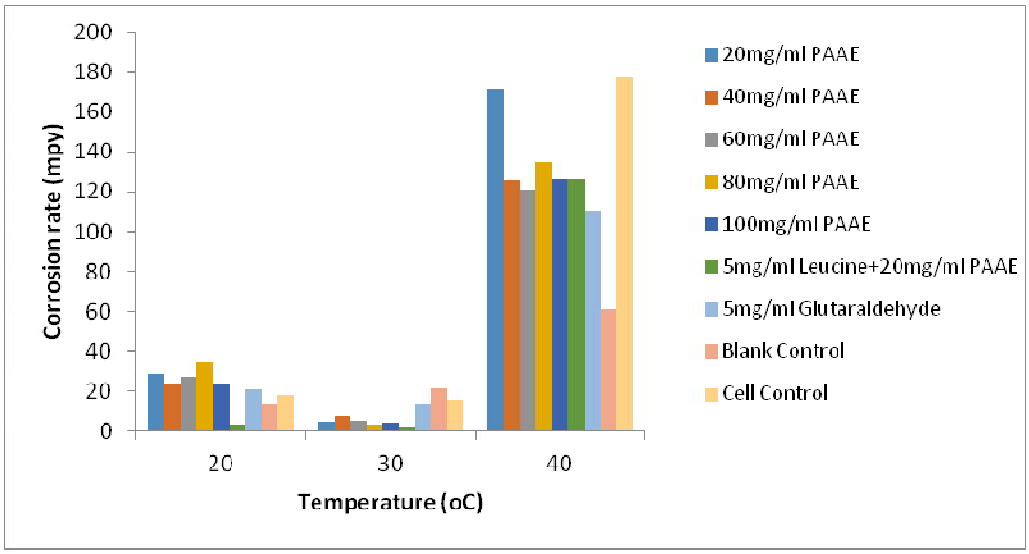

Fig. 3. Corrosion rate at different temperatures and concentration of aqueous plant extract after 14 days of incubation

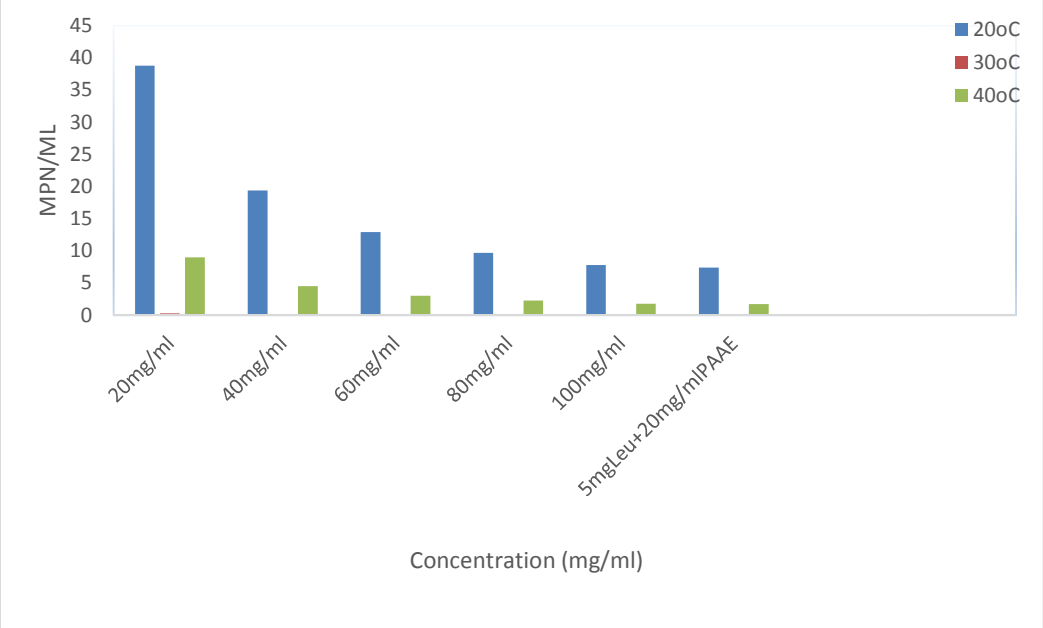

Fig. 4. Acid producing bacteria growth inhibition at different temperature and concentrations of the aqueous plant extract (7days)

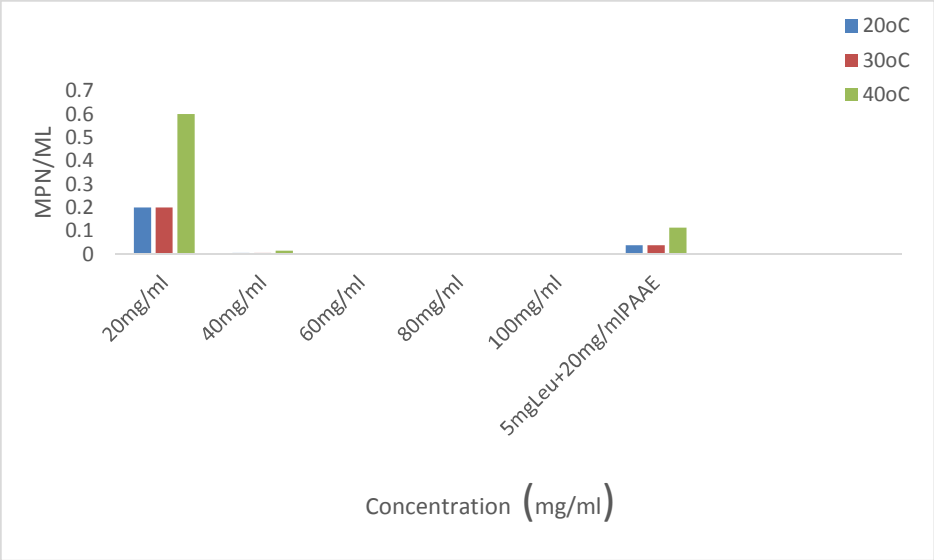

Fig. 5. Acid producing bacteria growth inhibition at different temperature and concentrations of the aqueous plant extract (14days)

Table 2. The Inhibition Efficiency (IE) of the crude aqueous extract at different temperatures (7days)

Concentration (mg/ml)	20°C	30°C	40°C
20	70.03	46.529	78.532
40	79.16	64.945	74.97
60	61.38	84.9	80.271
80	81.9	48.785	80.914
100	73.41	74.501	80.264
5 mg/ml Leucine + 20 mg/ml PAAE	75.93	87.745	79.628
5 mg/ml Glutaraldehyde	63.10	89.04	18.43

Table 3. The Inhibition Efficiency (IE) of the crude aqueous extract at different temperatures (14days)

Concentration (mg/ml)	20°C	30°C	40°C
20	80.07	46.32	44.06
40	83.72	14.98	60.53
60	81.27	39.82	59.09
80	75.82	65.02	60.59
100	83.43	58.89	56.11
5 mg/ml Leucine + 20 mg/ml PAAE	86.09	83.87	98.89
5 mg/ml Glutaraldehyde	65.45	61.22	33.02

4. DISCUSSION

The corrosion of carbon steel by acid producing bacteria in the absence and presence of aqueous crude extract of *Phyllanthus amarus* as inhibitor was studied using the weight loss technique at 20ºC, 30ºC and 40ºC temperatures. The phytochemicals detected in the aqueous extract included alkaloid, glycosides, tannins and phenol. It was observed that *Phyllanthus amarus* is a plant that is very rich phenolic compounds which proves its bio-potency and antimicrobial properties. According to Dhandapani et al. [10], *Phyllanthus amarus* is rich in phytochemicals such as phenolic compounds, tannins and alkaloids. This indeed, confirms its antimicrobial properties and bio-potency. The transmittance method was used to determine the presence of

specific functional groups of chemical compounds which might be present in the crude extract formulation. The aqueous crude extract of *Phyllanthus amarus* contained functional groups of compounds such as alcohols- O-H (3300-3600cm^{-1}); amines-N-H (3300-3500cm^{-1}) and amides-C=O (1630-1695 cm^{-1}); alkenes- C=C (1630-1670 cm^{-1}) and alkynes C-C-(2100-2140 cm^{-1}). These functional groups confer enormous biochemical activity on this weed which makes it a very useful plant.

The corrosion rate was determined at 20^{o}C, 30^{o}C and 40ºC and at different concentrations of the extract for 7 and 14 days. During the 7 days incubation, the least corrosion rates were observed with 80mg/ml PAAE at 20ºC and 40ºC; while at 30ºC the treatment 5mg/mlLeu+20mg/ml PAAE, a combination of lowest concentration of the extract plus the amino acid leucine, gave the least corrosion rate. Similarly, the treatment 5 mg/mlLeu + 20 mg/ml PAAE, also gave the least corrosion rates at 20ºC, 30ºC and 40ºC for the 14 days test. It was observed that corrosion rate was very high at 30ºC during the 7 days treatment. This temperature favours the growth of mesophilic acid producing bacteria, hence the high corrosion rate observed. The amino acid Leucine was used as a biocide enhancer in this study. Microorganisms utilize amino acids as a source of nitrogen, during the process the biocide is also incorporated into the bacterial cell. Immanuel et al. [14] also used amino acid biocide enhancer in their study and reported lesser corrosion rate with extract plus amino acid treatment.

The corrosion rate at the different incubation time showed some variations. The corrosion process might have been affected by factors such as fluctuation in temperature, number of acid producing bacterial cell load in the bacterial biofilm, the nature/source of the sample, the extract preparation techniques and the duration of extract from the time of preparation. Thus, there was drastic change in the corrosion rate at the various incubation temperatures for 14 days treatment. At 30ºC the corrosion rate was low due to lesser number of mesophilic acid producing bacteria in the growth medium. The high corrosion rate at 40^{o}C was possibly due to the higher population of coliform bacteria which can grow within 40ºC – 44ºC. Though there was a correlation between the corrosion rate and the microbial population, but it was confirmed according to Little et al. [2] that microbial growth rate is highly affected by temperature and the level of available nutrients. According to Wang and Zhong [16] mesophilic bacteria grow at an optimum temperature range of 20ºC to 50ºC, which supports the observation in this study.

At 20ºC the most probable number (MPN) value indicated a higher number of the corrosive bacteria which also decreased as the extract concentration increased. However, the Acid producing bacteria get depleted as the concentration of extract increases as well as with increase in temperature. The acid producing bacteria decreased at all temperature and concentration except for the 20mg/ml PAAE which showed less inhibition at 40ºC.

The extract was able to inhibit the corrosion of the metal because it was able to adhere to metallic surface in contact such that the planktonic cells of the biofilm bacteria cannot easily gain direct contact with the surface. This study confirms the reports of Immanuel et al. [14] that phytochemicals are more active and the adherence properties of plant extracts are usually more effective at lower temperatures than at higher temperatures. However, there is usually a temperature denaturation problems associated with the properties of plant including the method of extract preparation, the polarity, nature and concentration of the solvent and the method of storage [17,18].

5. CONCLUSION

The biocorrosion inhibition potential of *Phyllanthus amarus* was demonstrated in this study. Much attention is yet to be given to this weed as a plant of great economic value in Nigeria though this plant is widely distributed within the city of Port Harcourt. The active compounds in the plant can be extracted for commercialization and industrial application if the biotechnological processes involved are duly considered and exploited as possible.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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