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Corrosion and Fungal Growth Inhibiting Effects of *Piper guineense* Extracts

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Abstract Seed extracts of *Piper guineense* were assessed for anticorrosion and antifungal activities. The study was performed on aluminum (Al) coupon with weight percentage composition of Al>95% and 3x1.5x0.1cm in size. Anticorrosion effects of the extracts was studied using gravimetric and potentiodynamic polarization techniques, while the antifungal potency of ethanol, methanol, cold water and hot water extracts respectively against the corrosion-associated *Aspergellius fumigatus* was assessed by agar disc diffusion methods. Results revealed that the corrosion of the aluminum was inhibited by adsorption of the extract organic matter on the surface of the metal. Proximate phytochemical analysis of the *P.guineense* reveals the presence of alkaloids (1.67±0.29%), flavonoids (0.64±0.05%), tannins (0.67±0.01%), saponin (39.24±1.2%) and Phenols (1.92±0.04%). Fungal growth inhibition of *P. guineense* can be attributed to the actions of the phytochemical constituents of the extract.

Keywords: *biocides, corrosion, fungi, inhibition, plant extract, polarization, metals*

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1. Introduction

Fungi are considered the primary colonizers of surfaces in both natural and man- made environments [1]. In aqueous environments, metals are corroded not only by purely chemical or electrochemical reactions but also by metabolic activities of microorganisms [2]. Many fungi adhere to metals and form mat of hyphae or biofilms on material surfaces, with interactions governed strongly by the material surface properties and adhesion mechanisms [3,4]. Microbial growth on the surface of metals exposed to soil, oil fields and environmental contaminants can influence its corrosion in most adverse ways [5]. The possibilities of some fungal species to grow on metal surfaces are determined by their secreted metabolites which enable them to adapt to new environmental and nourishment conditions. Chemicals that control microbial activities are called biocides. Biocides are chemical substance intended to destroy, deter, render harmless, or exert a controlling effect on any harmful organism by chemical or biological means. Biocides can be either oxidizing or non-oxidizing agents [6]. Oxidizing agents such as chlorine, chlorinating compounds, choramines and bromine are commonly used in freshwater systems [7]. Non-oxidizing biocides are more stable than oxidizing biocides [7] and can be used in a variety of different environments. They have been shown to be effective against a broad range of microorganisms such as bacteria, fungi and algae as well as a greater persistence in the environment [6]. The choice of the biocide or inhibitor to use in the control of biocorrion is very important. A study by Rajaskar *et al.*, [8] shows that the widespread use of ester based or toxic biocides in the petroleum industry has led to the growth and dominance of *Bacillus* species, due to their ability to form resistance spores. Therefore the effectiveness of a biocide depends on the nature of the target microorganisms and the service environment.

The known hazardous effects of most synthetic inhibitors and the need to develop cheap, nontoxic and eco-friendly process have made researchers to focus on the use of natural products [9]. Most biocides in use today are inherently toxic and are very difficult to degrade as they persist in the environment. The environmental toxicity of inorganic corrosion inhibitors has promoted the search for green corrosion inhibitors as they are biodegradable, do not contain heavy metals or other toxic compounds. Stringent environmental regulation have restricted the use of toxic inorganic corrosion inhibitors such as chromates, nitrates and oxides leading to their replacement by organic compounds [5], mostly plant extracts. In addition to being environmentally friendly and ecologically acceptable, plant products are inexpensive, readily available and renewable [4,10,11]. Environmental concern has lead to the promulgation of legislations that encourages the replacement of environmentally toxic inorganic biocides with more readily degradable corrosion inhibitors that are environmentally less toxic. The interest in the use of plant extracts in the control and inhibition of biocorrosion can be attributed to the phytochemical constituents of the extract which often bear similar molecular, electronic structure with organic corrosion inhibitors as well as antimicrobial properties [12]. Tannin, organic acids, alkaloids, pigments and proteins from plants are known to inhibit metal corrosion [13,14].

Extracts of *Zenthoxylum alatum* were active on the corrosion of carbon steel in phosphoric acid [10]. Li *et al.,* [4] investigated the inhibitory effect of berberine extracted from *Coptis chinensis* in soft steel which was active against corrosion in 1M sulfuric acid. Molecules present in aqueous extract of Fenugreek leaves were spontaneously adsorbed on mild steel surface and were capable of inhibiting corrosion on steel in a dose-dependent manner in the presence of HCl and H_2SO_4. Aqueous extract of *Rosmarinus officinalis* [15], *Lawsonia inermis* leaves [11], *Allium sativum* [16] and *Phaseolus vulgaris* [17] inhibited metal corrosion.

The adhesive protein from the marine mussel *Mytilus edulis* and the bovine serum albumin (BSA) were both absorbed on carbon steel and were able to inhibit corrosion [18]. Plant products have also shown antimicrobial activity and studies in the use of extracts or isolated compounds to combat human-pathogens and phytopathogenic bacteria and microorganisms involved in corrosion are well documented [15,19,20]. The activity of an aqueous extract of *Brassica nigra* on planktonic and sessile *Pseudomonas* sp., the fungus *Aspergillus fumigatus* and a mixture of sulfate-reducing bacteria (SRB) revealed a promissory biocidal action against microorganisms frequently found in industrial biofilms [21].

In this study, the inhibiting effect of cold water (CW) extracts of *Piper guineense* on the fungal influenced corrosion of aluminum including evaluation of the phytochemical constituents of the extract was investigated. Anticorrosion effects of the extract on the corrosion associated *Aspergillius fumigatus* was assessed using gravimetric and electrochemical techniques. Antifungal screening to determine the growth inhibition of the extract and the minimum inhibitory concentration were assessed using agar disc diffusion method.

2. Materials and Methods

2.1. Material Preparation

Metal coupons: Corrosion experiments were performed on aluminum metal with weight percentage composition of Al>95%.

2.2. Plant Extracts

The dried plant seeds were washed with sterile distilled water. The seeds were then pulverized with a blender. The stock solution was prepared using standard procedure outlined by [22]. Hundred grams (100g) of each powdered seed was soaked in 500mL of 95% ethanol (ET), methanol (MT), cold water (CW) and hot water (HW) for 48 hrs to allow for maximum extraction of components. The filtrates were then evaporated to eliminate the solvents using a rotary evaporator. The residue (crude extracts) were then stored in sterile reagent bottles at 21°C until analysis. The amount of plant material extracted was quantified by comparing the weight of the dried residue with the initial weight of the dried plant material before extraction. From the individual stock inhibitor test solutions were prepared in the desired concentration range by diluting with distilled water. Quantitative and qualitative phytochemical screening of the extract was done using standard laboratory procedures [5].

The test fungus was isolated from aluminum roofing sheet. The biofilm covered area of the aluminum sheet was aseptically scrapped. One gram (1g) of corroded metal samples was serially diluted into 9mL of sterile distilled water in sterile 20mL test tube. About 0.1mL of dilutions 10^{-5} to 10^{-7} was plated in triplicate on potato dextrose agar PDA (Oxoid) plates supplemented with antibiotics streptomycin (5µg/mL). Each morphological discrete fungal colony was then sub-cultured and purified by repeated streaking on PDA plates. Pure cultures were then preserved on PDA slants in Bijou bottles and stored at 4°C in a refrigerator for further studies. Each fungal isolate was characterized and identified based on their morphological characteristics and microscopic analysis using taxonomic guides and standard procedures as outlined by [22].

2.3. Gravimetric Experiments

Gravimetric experiments were conducted on test coupon of dimension 3x1.5x0.1cm. The coupons were wet- polished with abrasive paper (from grad no. 400-1000), rinsed with distilled water, dried weighed and stored in moisture free desiccators prior to use. The metal coupons were placed in Petri dishes filled with malt extract poor in nutritive materials and supplied with streptomycin (5µg/mL). The medium with the metal coupons treated with the *P.guineense* extract (25mg/mL conc.) was then inoculated with fungi isolated from corroded aluminum metal. For the control C1, metal was exposed to common conditions (room temperature and humidity) and not contaminated with fungi. Medium with metals was incubated at 27°C. The entire experiments were uniformly prepared in triplicates, labeled accordingly and inserted on the same day. The experiment was observed for a period of 60days. To determine weight loss with respect to time, the coupons were retrieved after 10 days intervals progressively, scrubbed with bristle brush, washed with distilled water, dried and weighed. The weight loss was taken to be the difference between the weight of coupons at a given time (day) and its initial weight. All tests were in triplicate and the data showed good reproducibility.

2.4. Antifungal Screening

The plant extracts were assayed for antifungal activity by the disc diffusion method. About 100g of the dried and powered seed was extracted with 500mL each of methanol, ethanol, hot water (90°C), and cold water (20°C) respectively to yield four distinct extracts. The Petri dishes were filled with malt extract poor in nutritive materials and supplied with streptomycin (5µg/mL). The medium was then inoculated with fungi and filter discs impregnated with the extracts (100mg/L and 50mg/L concentrations) subsequently placed aseptically on the seeded plates which were incubated at 27°C for 7 days. The radius of the zone of inhibition was measured from the edge of the disc to the edge of the zone. To determine the minimum inhibitory concentrations of the extract, the stock was diluted to yield four different concentrations (12.5, 25, 50

and 100mg/mL). The medium was prepared as above but at this time the filter discs were impregnated with the different concentrations of the extract. The lowest concentration of the extracts that showed clear visible growth inhibition was recorded as the minimum inhibitory concentration.

2.5. Electrochemical Measurement

The potentiodynamic polarization test was carried out in a standard three-electrode glass cell of 500 ml capacity using Electrochemical System workstation (PAR 263). A graphite rod served as counter electrode and, a saturated calomel electrode (SCE) was used as reference electrodes. A mild steel and aluminum specimen of 1 cm^2 dimension were used as working electrode. Electrochemical measurements were carried out at 30±1°C, using standard procedures as outlined by [5], in aerated solutions at the end of 1800s of immersion, which allowed the open circuit potential (OCP) values to attain steady state. The potentiodynamic polarization (PDP) experiments were then conducted at a scan rate of 0.333 mV/s. The potential range employed was -250 mV to + 300 mV versus corrosion potential. Powersuite software was used in analyzing the polarization data.

3. Results

3.1. Phytochemical Analysis

Table 1 shows the results of the phytochemical screening and the percentage amounts of key phytochemical constituents present in *P.guineense* seed extract. They include saponins, alkaloids, tannins, flavonoids and phenol. According to [12], the alkaloids present in *P.guineense* extract are essentially piperidyl amide alkaloids, including piperine which is responsible for the pungency. They also observed that tannin moieties present in the extract with their protein –binding abilities could interact with basic constituents of protein present in cell membranes thereby inhibiting some key metabolic functions of the cells [12].

Table 1. Qualitative and Quantitative phytochemical constituents of *P. guineense*

Parameters	Intensity of key phytochemical constituents	Relative amount (%) of key phytochemical constituent
Alkaloids	++	1.67±0.29
Flavonoids	++	0.64±0.05
Tannins	++	0.67±0.010
Saponin	+	39.24±1.2
Phenols	++	1.92±0.04

++= moderately present
+ = slightly present.

3.2. Antifungal Activity of the Extract

The results of the fungicidal activity of the extract of *P. guineense* against *A.fumigatus* are shown in Table 2. The highest growth inhibitory activity was obtained from the stock solution of the cold water extract. Table 3 shows the summary of the minimum inhibitory concentration (MIC) of the extracts. The MIC closely followed the same trend as the growth inhibition effect of the extracts [12] with only the cold water extract exhibiting significant inhibition of the mycelia growth of *A.fumigatus* at the 25mg/mL concentration.

Table 2. Antifungal activity of the seed extract of *P. guineense* against *A. fumigatus*

Solvent	Mean zone of inhibition (mm)	
	100%	50%
Cold water	65.0±2.0	25.6±1.0
Hot water	41.6±1.1	28.1±2.6
Ethanol	18.6±1.3	5.1±1.3
Methanol	34.5±1.6	20.7±1.1

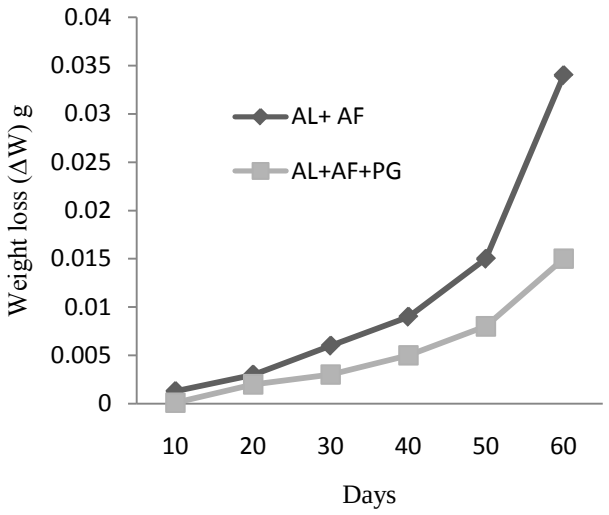
Table 3. Minimum inhibitory concentration (MIC) of different extracts of *Piper guineense* against *A.fumigatus*

Solvents	MIC (mg/mL)		
	100%	50%	25%
Cold water	10.0	9.5	9.0
Hot water	19.5	13.5	-
Ethanol	16.0	10.0	-
Methanol	10.5	8.5	-

3.3. Corrosion Inhibition Results

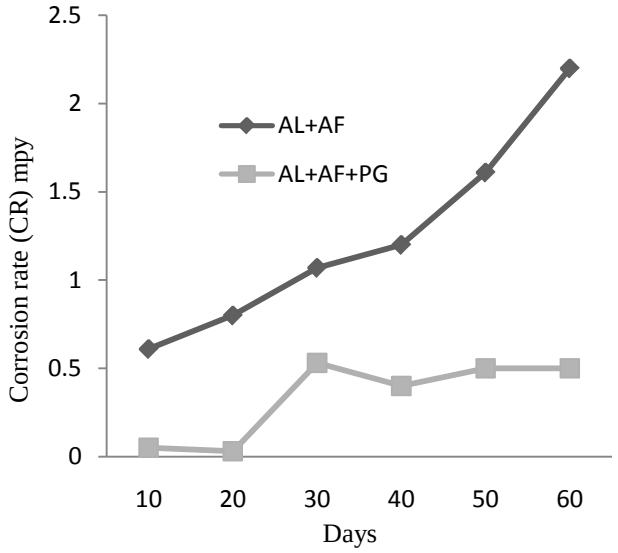
3.3.1. Gravimetric Data

The inhibitive effects of cold water CW extract of *P.guineense* on the corrosion behavior of aluminum in the presence of *A. fumigatus* was studied using gravimetric technique. Figure 1 and Figure 2 shows the weight loss and corrosion rates of aluminum in the presence and absence of the inhibitor respectively. The plots show that *P.guineense* extract effectively retarded aluminum corrosion at the concentration studied. Furthermore, the corrosion rate was found to increase with exposure time.



AL=Aluminum, AF= *A.fumigatus*, and AL+AF+PG= Aluminum+ *A. fumigatus* + *P.guineense*

Figure 1. Weight loss of *A. fumigatus* influenced corrosion of aluminium in the presence and absence of inhibitor *Piper guineense*



AL=Aluminum, AF= *A.fumigatus*, and AL+AF+PG= Aluminum+ *A. fumigatus* + *P.guineense*

Figure 2. Corrosion rate of *A. fumigatus* influenced corrosion of aluminium in the presence and absence of inhibitor *Piper guineense*

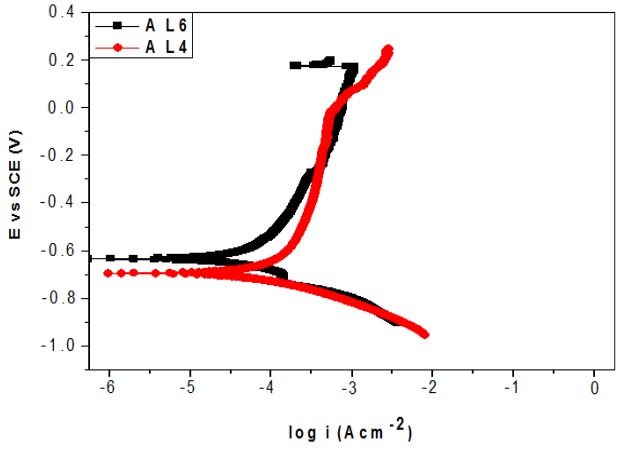
3.3.2. Electrochemical Data

Potentiodynamic polarization studies were carried to ascertain the effects of the fungi on the corrosion behavior of the metals, the influence of the fungi on the anodic and cathodic reactions processes and the influence of the extract on the kinetics of anodic and cathodic partial reactions of the corrosion processes. The electrochemical parameters including the corrosion current density (I_{corr}) and corrosion potential (E_{corr}) in the presence of the inhibitor can be observed from Tafel plot (Figure 3).

Table 4. Polarization data for aluminium in the presence and absence of fungi and *Piper guineense*

Fungi	I_{corr} ($\mu A/cm^2$)	E_{corr} mV Vs SEC	b_a	b_c
Control AL4	153.5	−701.6	89.5	100.3
Piper guineense AL6	78.6	−614.5	80.7	96.6

AL4 = Control
AL6 = Aluminum + *A. fumigatus* with inhibitor *Piper guineense.*



AL4 = Control
AL6 = Aluminum + *A. fumigatus* with inhibitor *Piper guineense*

Figure 3. Potentiodynamic polarization curve for aluminium in the presence of *A. fumigatus* and inhibitor *P.guineense*

4. Discussion

The results of the phytochemical screening and percentage amount of key phytochemical constituents of *P. guineense* seed extract is in line with the findings of [23] and [12]. They isolated alkaloids, flavonoids, saponins and tannin from the seeds extracts of *P. guineense*. Also the phytochemical screening of *P.guineense* and *A. melegueta* conducted by [24] showed that the seeds of these plants contained alkaloids, flavonoids, tannin, saponin, steroids, terpenes, phenols and cardiac glycosides. Oguzie *et al.*, [5] reported that the alkaloidal constituents responsible for the pungency and peppery flavour are collectively called capsaicinoids. For instance, components with phenolic structures such as phenylpropenes are known to be very active against microorganisms. The high concentration of saponin present in the extracts act on the microbial cells as detergents, just as reported for quaternary ammonium salts [5], dissolving lipids and thus causing the loss of cellular contents. The tannin moieties, with their protein-binding abilities, could interact with bacic constituents of proteins present in cell walls, cell membranes and cytoplasm with resultant inhibition of key metabolic functions of the cells. The above effects could have contributed to the observed fungal growth inhibition. Echo *et al.*, [24] observed that these phytochemicals exhibit a wide range of biological effects as a consequence of their antioxidant properties. Okoye and Ebeledike [23] also observed that flavoids possess antioxidant activity and equally anti-inflammatory. Kubmarawa *et al.*, [25] reported the importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains. Nwaiwu and Imo [29] also reported the antifungal properties of the essential oil of *P.guineense* on food-borne fungi. It is therefore possible that the antimicrobial properties of these extracts might have played some roles in inhibiting the growth of the fungi and subsequent inhibition of the corrosion processes.

Figure 1 and Figure 2 shows gravimetric results of the effects of *P.guineense* on the influence of fungi on Aluminium. Oguzie *et al.*, [5] studied the anticorrosion effects of the ethanol extract of *Capsicum frutescens* on the low carbon in acidic media using gravimetric, impedence and polarization techniques. They observed that *C.frutescens* effectively inhibited both corrosion and growth of SRB due to the action of the phytochemical constituents present therein which included alkaloids, tannins and saponins (which are also present in *P.guineense*). It is therefore possible that the inhibition of the corrosion and fungal growth observed might have been as a result of the presence of these phytochemicals in the seed extracts. The authors also stated that saponin possesses fungicidal activities against *A. fumigatus* species. Although the detailed mechanism of the fungicidal actions of *P. guineense* were not extensively investigated, actions of the observed growth inhibition abilities could be attributed to lipids dissolving ability of saponin moieties [5] which results in loss of cellular content as well as the protein binding abilities of tannins which facilitates interference with key metabolic functions of the cells. Oguzie *et al.*, [12] also studied the anticorrosion effect of *P.guineense* leave extract on corrosion associated sulfate – reducing bacteria (SRB), *Desulfotomaculum* species.

P.guineense was found to be excellent inhibitor for both corrosion and SRB growth. They also attributed both effects to phytochemical constituents present in the extract.

From the polarization curve it can be observed that the cathodic reaction was inhibited by *P.guineese*. This was observed in the shift of E_{corr} towards the more negative potential. Similar observations have been reported by [30] in their study of corrosion and microbial (SRB) growth inhibiting effects of *P. guineense* extract. Ahamed *et al*., [19] stated that if displacement in E_{corr} is > 85mV, the inhibitor can be seen as a cathodic or anodic type inhibitor and if the displacement is < 85mV the inhibitor can be seen as mixed type. In this study, the E_{corr} value is less than 85mV, which indicates that the inhibitor is a mixed type inhibitor showing more inhibition at the cathodic than at the anodic sites.

5. Conclusions

This study has established that the seed extract of *P.guineense* can inhibit the fungal corrosion of aluminum as well as the growth of *A.fumigatus*. The antifungal effect of the cold water extract is attributed to the absorption of the phytochemical constituents of the extract which disrupt the growth and essential metabolic functions of the fungus. For example the high concentration of saponin in the extract acts on the microbial cells as detergents. Secondly components with phenolic structures such as phenylpropene are known to be very active against microorganism. The polarization measurements show that the corrosion inhibition followed a mixed-type mechanism. The results obtained in this study are consistent with our hypothesis that the phytochemical constituents of *P. guineense* seed extract can be exploited for fungal –influenced corrosion of metals and inhibition of fungal growth.

Conflict of Interest

The authors declare that there is no conflict of interest.

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