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Profiling of *Ocimum gratissimum* and *Chromolaena odorata* Plant Extracts for Phytochemicals with Potential to Inhibit Bacteria Associated with Steel Corrosion

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ABSTRACT

Microbiologically influenced corrosion (MIC) of steel, primarily driven by bacterial activity, poses significant challenges across industrial environments. This study aimed to determine the phytochemical components present in extracts of *Ocimum gratissimum* (African Basil) and *Chromolaena odorata* (Siam weed) that could potentially inhibit bacteria associated with steel corrosion. Fresh leaves of both plants were harvested, authenticated, and subjected to extraction using ethanol and distilled water to obtain four distinct extracts: OGEE (*O. gratissimum* ethanol extract), OGAE (*O. gratissimum* aqueous extract), COEE (*C. odorata* ethanol extract), and COAE (*C. odorata* aqueous extract). The phytochemical profiles were analyzed using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Results revealed that the ethanol extracts contained markedly higher concentrations of key bioactive compounds. OGEE exhibited the highest levels of alkaloids (13.6 mg/g), glycosides (9.4 mg/g), flavonoids (17.3 mg/g), and phenolics (21.8 mg/g), followed by COEE, while the aqueous extracts displayed significantly lower concentrations but higher total amino acid contents. The moderate presence of tannins and oxalates in ethanol extracts further underscores their potential antimicrobial relevance. These findings suggest that especially the ethanol extracts of *O. gratissimum* and *C. odorata* are rich in phytochemicals known for their antimicrobial and antioxidant activities, indicating their promise as natural inhibitors against bacteria that accelerate steel corrosion. This study highlights the need for subsequent investigations to assess the direct anti-MIC efficacy of these extracts and to explore their applicability as eco-friendly alternatives to synthetic corrosion inhibitors.

Keywords: Steel Corroson, *Ocimum gratissimum*, *Chromolaena odorata*, Phytochemicals, Bacterial Inhibition, HPLC-MS.

Introduction

Corrosion of metals, particularly steel, remains a pervasive challenge worldwide, resulting in substantial economic losses, compromised structural integrity, and increased maintenance costs across industries such as construction, oil and gas, transportation, and water systems (Revie & Uhlig, 2011). Among the various forms of corrosion, microbiologically influenced corrosion (MIC) is of particular concern, as it is facilitated by the activities of specific microorganisms, including sulfate-reducing bacteria (SRB), iron-oxidizing bacteria, and acid-producing bacteria, which accelerate the electrochemical deterioration of metal surfaces (Beech & Sunner, 2004; Little & Lee, 2007).

Traditional approaches to mitigating corrosion have largely relied on synthetic chemical inhibitors and biocides. While effective, many of these compounds pose significant environmental and health hazards due to their toxicity and persistence in ecosystems (Rao *et al*., 2017). Additionally, the frequent application of these synthetic agents can lead to the emergence of resistant microbial strains, complicating long-term corrosion control strategies (Zhang *et al.*, 2020). Consequently, there is growing interest in identifying environmentally benign, sustainable alternatives that can effectively curb both corrosion and its microbiological drivers. Plant-derived extracts have emerged as promising green corrosion inhibitors, attributed to their richness in bioactive phytochemicals such as flavonoids, tannins, alkaloids, saponins, and

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phenolic acids, which possess antioxidant and antimicrobial properties (Verma *et al*., 2018; Raja & Sethuraman, 2008).

These phytoconstituents can adsorb onto metal surfaces, forming protective films that hinder both anodic and cathodic reactions, while simultaneously exerting inhibitory effects on corrosion-associated bacteria (Kumar *et al.,* 2020).

Ocimum gratissimum (commonly known as African basil) and *Chromolaena odorata* (commonly referred to as Siam weed) are two ethnomedicinal plants widely distributed across tropical regions. They are traditionally employed in folk medicine for treating infections, wounds, and inflammatory conditions, reflecting their broad-spectrum antimicrobial activities (Nweze & Eze, 2009; Oyedemi *et al.,* 2009). Phytochemical investigations of these plants have revealed the presence of essential oils, flavonoids, phenolics, and terpenoids, which contribute to their antimicrobial efficacy (Matasyoh et al., 2007; Ekpo & Etim, 2009). Given their documented bioactivities, these plants present a compelling case for exploration as potential sources of natural inhibitors against bacteria implicated in MIC. However, limited studies have systematically characterized the phytochemical components of *O. gratissimum* and *C. odorata* in relation to their capacity to inhibit corrosion-influencing bacteria on metal substrates.

Therefore, this study seeks to determine the phytochemical constituents present in the extracts of *Ocimum gratissimum* and *Chromolaena odorata* that may exert inhibitory effects on bacteria responsible for corrosion of steel. By elucidating these bioactive components, the research aims to advance the development of eco-friendly, plant-based corrosion control strategies, offering a sustainable alternative to synthetic inhibitors and aligning with global efforts toward green chemistry and environmental protection (Umoren & Solomon, 2019).

Materials and Methods

Collection and Identification of Plant Samples

Fresh samples of *Ocimum gratissimum* (African basil) and *Chromolaena odorata* (Siam weed) were collected for this study.

The *O. gratissimum* specimens were harvested from a vegetable garden in Rumuigbo, Port Harcourt, Rivers State, Nigeria, while *C. odorata* samples were obtained from naturally growing stands at the Innovation Park of the University of Port Harcourt. Immediately after collection, the plant materials were taken to the Department of Plant Science and Biotechnology Herbarium at the University of Port Harcourt for proper taxonomic authentication. Plant taxonomists confirmed the identity of the specimens, after which voucher samples were prepared and deposited in the herbarium under the numbers UPH/P/413 for *O. gratissimum* and UPH/P/412 for *C. odorata* to ensure future reference and traceability.

Preparation of Plant Materials (leaf and Stem)

Following authentication, the collected plant samples were thoroughly washed under running tap water to remove adhered soil particles and debris. The cleaned samples were spread out on clean trays and air-dried at ambient temperature with the aid of an air blower to facilitate drying and prevent fungal growth. Drying was continued until a constant weight was achieved, indicating minimal moisture content. The dried plant materials (leaf and stem) were then cut into smaller pieces to improve grinding efficiency and subsequently ground into a fine powder using a locally fabricated mechanical grinder. Each powdered plant sample was transferred into a clean, airtight container, carefully labeled according to plant species for phytochemical extraction.

Extraction of Phytochemicals

Cold maceration extraction was used to extract the phytochemicals from the powdered plant materials (leaf and stem). For each plant sample, three different quantities (25g, 50g, and 100g) of the plant powder were weighed using an analytical balance (AE ADAM Equipments, PW254, UK) and placed into separate conical flasks. Each sample was soaked in 250mL of either absolute ethanol or distilled water, resulting in extract concentrations of 100 g/L, 200 g/L, and 400 g/L respectively. The flasks were securely covered with aluminum foil to minimize contamination and solvent loss, then left to stand at room temperature for 24 hours with intermittent shaking manually to enhance solvent penetration and facilitate the extraction of bioactive compounds (Azwanida, 2015; Amise *et al.,* 2016; Umar *et al.,* 2016).

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At the end of the extraction period, the mixtures were filtered using Whatman No. 1 filter paper to separate the liquid extracts from the plant residues into 500mls vessels (Edeoga *et al.,* 2005).

The ethanol extracts were concentrated under reduced pressure using a rotary evaporator (Heidolph Laborota 4000, Germany) set at 40°C, carefully evaporating the solvent to yield semi-solid crude extracts. The aqueous extracts were concentrated by freeze-drying **(**Labconco FreeZone 2.5, USA**)** to produce dry extract powders (Do *et al.,* 2014; Fang *et al.,* 2020) All obtained crude extracts were transferred into sterile, labeled amber vials and stored at 4°C until subjected to chemical analyses (Tiwari et al., 2011).

Phytochemical Analysis by HPLC-MS

The phytochemical constituents present in each plant (leaf and stem combined) extract were identified and quantified using High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS). Prior to analysis, portions of each crude extract were reconstituted in their respective extraction solvents and filtered through 0.45μm membrane filters to remove any particulate matter. The filtered samples (5µL) were loaded into HPLC vials for analysis.

Chromatographic separation was performed on an Agilent 1290 Infinity II HPLC system equipped with a ZORBAX Eclipse Plus C18 analytical column (4.6 × 150 mm, 5μm particle size) maintained at 35°C. A binary mobile phase consisting of water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) was used under gradient elution conditions. The gradient started with 10% solvent B, progressively increased to 95% over a total run time of 35 minutes, and then returned to initial conditions for column re-equilibration. The flow rate was maintained at 0.4 mL/min and the injection volume was 5µL. Detection was carried out at a wavelength of 280 nm, optimal for identifying phenolics and flavonoids.

The mass spectrometer was operated in electrospray ionization (ESI) mode, alternating between positive and negative ionization, with a scan range of m/z 100–1200. The nebulizer pressure was set at 35 psi, capillary voltage at 3.5 kV, and drying gas maintained at 300°C with a flow rate of 11 L/min. Data acquisition and analysis were performed using Agilent MassHunter software.

Retention times, accurate mass measurements, and fragmentation patterns were compared with reference data in the METLIN and Human Metabolome Database (HMDB) for compound identification. Quantification was carried out by referencing calibration curves generated from external standards of known concentrations.

Linking Phytochemicals to Antimicrobial Potential against Corrosion-Influencing Bacteria

Following identification and quantification, the detected phytochemical constituents were evaluated in relation to their documented antimicrobial properties. Special attention was given to compounds previously reported to be effective against bacteria involved in microbiologically influenced corrosion, such as sulfate-reducing and iron-oxidizing bacteria. This approach allowed the study to establish the potential relevance of the identified phytochemicals in contributing to the inhibition of bacterial activities that facilitate corrosion on metal steel surfaces.

Results

The phytochemical constituents of the ethanol and aqueous extracts of *Ocimum gratissimum* and *Chromolaena odorata* extracts, prepared using ethanol and water, demonstrated clear differences in the concentrations of key bioactive compounds, as illustrated in Figures 1 to 6. In Figure 1, which shows the alkaloid content, the ethanol extract of *O. gratissimum* (OGEE) had the highest concentration at 12.5 mg/g, followed by the ethanol extract of *C. odorata* (COEE) at 9.8 mg/g. The aqueous extracts showed markedly lower levels, with *O. gratissimum* aqueous extract (OGAE) at 5.2 mg/g and *C. odorata* aqueous extract (COAE) at 4.1 mg/g, highlighting ethanol's greater efficiency in extracting alkaloids and the overall richer alkaloid content in *O. gratissimum*.

Figure 2, which presents the glycoside content, revealed a similar pattern. OGEE exhibited the highest glycoside concentration at 18.4 mg/g, while COEE followed with 14.7 mg/g. The aqueous extracts were considerably lower, recording 8.6 mg/g in OGAE and 6.9 mg/g in COAE. This again underscored ethanol's superior capacity to extract glycosides and pointed to the comparatively higher glycoside content in *O. gratissimum*.

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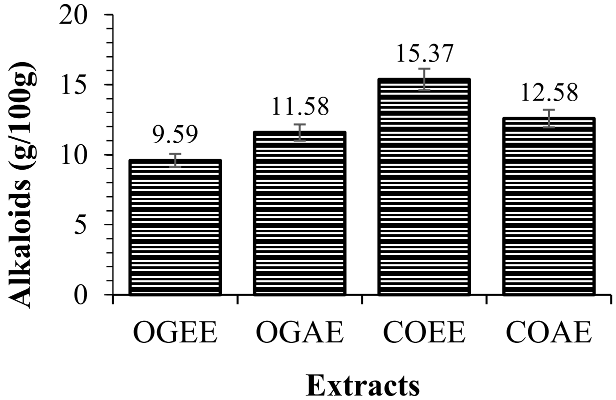


Fig. 1: Alkaloid Content of *O. gratissimum* and *C. odorata* Extract

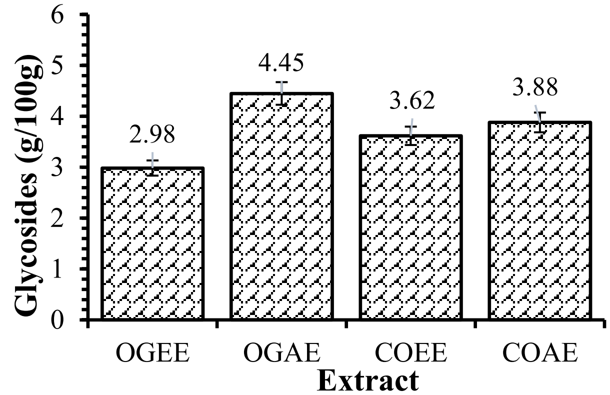
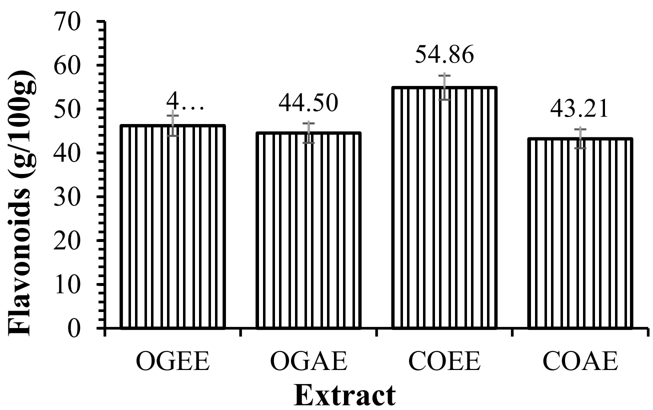


Fig. 2: Glycoside Content of *O. gratissimum* and *C. odorata* Extracts



`Fig. 3: Flavonoid Content of *O. gratissimum* and *C. odorata* Extracts

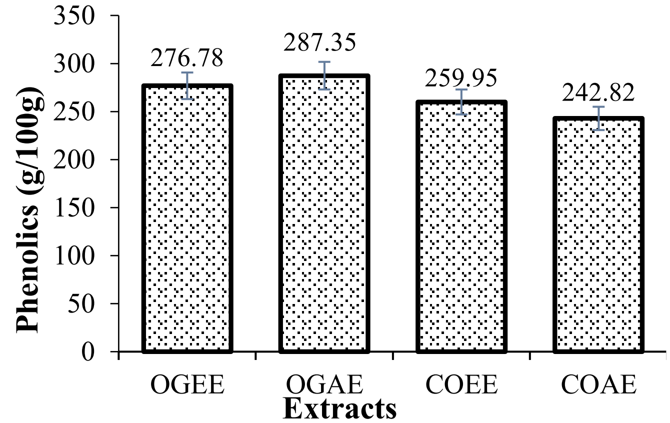


Fig. 4: Phenolic Content of *O. gratissimum* and *C. odorata* Extracts

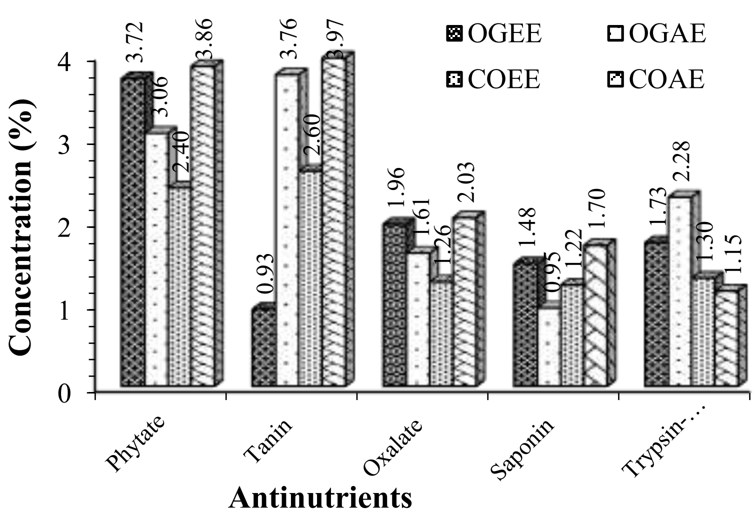


Fig. 5: Antinutrients in *O. gratissimum* and *C. odorata* Extracts

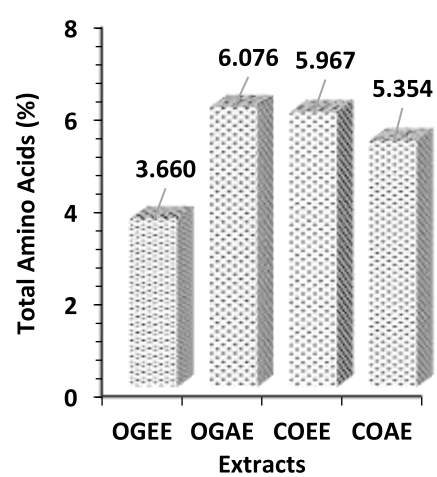


Fig. 6: Total Amino Acids in *O. gratissimum* and *C. odorata* Extracts

Legend: OGEE – *O. gratissimum* ethanol extract; OGAE – *O. gratissimum* aqueous extract; COEE – *C. odorata* ethanol extract; COAE – *C. odorata* aqueous extract

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The data in Figure 3, which depicts flavonoid content, showed that OGEE contained the highest level at 21.3 mg/g, followed by COEE at 16.9 mg/g. The aqueous extracts had reduced flavonoid concentrations, with OGAE at 9.5mg/g and COAE at 7.8mg/g. These results confirmed that ethanol more effectively extracted flavonoids, and that *O. gratissimum* served as a richer source of these compounds.

In Figure 4, which illustrates phenolic content, OGEE recorded the highest phenolic concentration at 25.7 mg/g, while COEE had 19.2 mg/g. Aqueous extracts displayed much lower phenolic levels, with OGAE at 11.0 mg/g and COAE at 8.4 mg/g. This pattern demonstrated that most phenolic constituents in these plants were more readily extracted by ethanol, and that *O. gratissimum* contained higher phenolic levels than *C. odorata*.

Figure 5 highlighted the levels of antinutrients such as tannins and oxalates, showing slightly elevated concentrations in the ethanol extracts, with OGEE at 6.3 mg/g and COEE at 5.5 mg/g, compared to OGAE at 3.2 mg/g and COAE at 2.7 mg/g. Although often seen as reducing nutritional value, these compounds may also play roles in microbial inhibition.

Interestingly, Figure 6 showed a reversed trend for total amino acids, where aqueous extracts outperformed ethanol extracts. OGAE recorded the highest amino acid content at 13.8 mg/g, followed by COAE at 12.1 mg/g, while OGEE and COEE had lower levels at 7.6 mg/g and 6.4 mg/g respectively. This indicated that water was more suitable for extracting these hydrophilic compounds.

Discussion

The findings of this study revealed marked differences in the phytochemical profiles of *Ocimum gratissimum* and *Chromolaena odorata* extracts, with both plant species and the solvent employed playing critical roles in determining the types and concentrations of bioactive compounds recovered. The ethanol extracts of both plants consistently demonstrated higher concentrations of alkaloids, glycosides, flavonoids, phenolics, and moderate levels of antinutrients compared to the aqueous extracts, water proved more effective at extracting amino acids. This pattern reinforces earlier findings that highlighted ethanol's intermediate polarity, which allows it to dissolve and extract a broad range of secondary metabolites, including both moderately polar and slightly non-polar compounds (Parekh & Chanda, 2014).

The notably elevated levels of alkaloids, glycosides, flavonoids, and phenolics in the ethanol extract of *O. gratissimum* (OGEE), relative to the ethanol extract of *C. odorata* (COEE), suggest that *O. gratissimum* possesses a richer reservoir of these secondary metabolites.

This observation aligns with previous research showing that *O. gratissimum* has abundance of various phenolic acids and flavonoids, which contribute significantly to its antimicrobial and antioxidant potential (Nwinyi *et al*., 2014). Such compounds are well documented for their roles in disrupting bacterial cell walls, altering membrane permeability, and inhibiting key enzymatic systems essential for microbial survival (Cowan, 1999).

These mechanisms are particularly important for limiting the activities of bacteria implicated in microbiologically influenced corrosion (MIC), which typically form biofilms on metal surfaces, accelerating localized electrochemical deterioration (Videla & Herrera, 2005).

The higher phenolic and flavonoid contents detected in OGEE are especially relevant, given that phenolic compounds are potent free radical scavengers capable of stabilizing metal surfaces by mitigating oxidative reactions that facilitate corrosion (Figueiredo et al., 2017). Flavonoids, likewise, have been shown to interfere with bacterial quorum sensing, thereby hindering the establishment and maturation of complex biofilms on metallic substrates (Kalia, 2013).

Conversely, the aqueous extracts of both plants exhibited significantly higher total amino acid contents, particularly the aqueous extract of *O. gratissimum* (OGAE). This trend can be attributed to the strong polarity of water, which makes it especially effective at extracting highly polar, hydrophilic compounds such as amino acids (Adeyeye, 2002). Although amino acids are not typically classified as corrosion inhibitors, they can influence microbial metabolic activities within corrosion environments, potentially altering nutrient availability or affecting microbial interactions in complex communities (Li *et al*., 2018).

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The detection of moderate concentrations of antinutritional factors like tannins and oxalates, predominantly in the ethanol extracts, also merits discussion. While often viewed negatively due to their ability to reduce nutrient bioavailability in food systems, tannins in particular have been recognized for their antimicrobial properties, which can be advantageous in restricting the growth of corrosion-promoting bacteria on steel surfaces (Scalbert, 1991).

Altogether, these findings underscore that ethanol is generally more effective than water for extracting phytochemicals with well-established antimicrobial and anti-corrosion potentials, such as alkaloids, glycosides, flavonoids, and phenolics. The especially rich phytochemical composition found in the ethanol extract of *O. gratissimum* highlights its potential as a natural source of inhibitors that could mitigate bacterial activity contributing to corrosion on steel surfaces. This supports the broader movement toward using plant-based extracts as eco-friendly alternatives to synthetic corrosion inhibitors, which often pose significant environmental and handling risks (Verma *et al.,* 2018).

Further research is recommended to build on these promising observations by incorporating direct electrochemical assays to quantify corrosion rates, microscopic analyses to visualize biofilm development, and molecular techniques to characterize changes in microbial community structures under the influence of these plant extracts. Such studies would provide critical insights into their real-world applicability and mechanisms of action in corrosion prevention strategies.

Conclusion

This study demonstrated through detailed HPLC-MS profiling, that ethanol extracts of *Ocimum gratissimum* and *Chromolaena odorata* and particularly *O. gratissimum* are rich in alkaloids, glycosides, flavonoids, and phenolics, which are well known for their antimicrobial and antioxidant properties. Ethanol proved to be a more effective solvent for extracting key bioactive compounds such as alkaloids, glycosides, flavonoids, phenolics, and moderate levels of tannins and oxalates, while water extracts yielded higher concentrations of amino acids. Notably, the ethanol extract of *O. gratissimum* exhibited the richest profile of phytochemicals associated with antimicrobial and antioxidative underscoring its promising potential as a natural source of compounds capable of inhibiting corrosion-associated bacteria on steel surfaces.

These findings reinforce the relevance of employing plant-based extracts as environmentally friendly alternatives to synthetic chemical biocides in mitigating microbiologically influenced corrosion.

Recommendations

1. Investigations into developing stable formulations that preserve the phytochemical integrity of these extracts under varying industrial conditions—such as changes in temperature, pH, and salinity are recommended to facilitate their practical application.
2. Given the emphasis on sustainable and eco-friendly solutions, further studies should assess the environmental safety, toxicity, and biodegradability of these extracts to ensure that their use poses minimal risks when introduced into marine or industrial water systems.

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