Asian J Biol Life Sci., 2025; 14(1):192-197.
https://ajbls.com

Research Article

Amylase-Arbitrated Antibiofilm and Anti-Biocorrosion Efficacy of *Curcuma zanthorrhiza* against *Pseudomonas aeruginosa*

Delna Joseph[1], Kayeen Vadakkan[2], Bince Mani[1,*], Meena Kochappan Cheruvathur[3]

[1]Department of Botany, St. Thomas College Palai (Autonomous), Kottayam, Kerala, INDIA.
[2]Department of Amala Integrated Medical Research, Amala Institute of Medical Sciences, Thrissur, Kerala, INDIA.
[3]Department of Botany, St. Mary's College (Autonomous), Thrissur, Kerala, INDIA.

ABSTRACT

Background: The current research explores the antibiofilm and anti-corrosive effects of the hydromethanolic extract of the *Curcuma zanthorrhiza* rhizome. Biofilm-mediated infections are prevalent and can result in periodontal infection, adenotonsillar hypertrophy, chronic rhinosinusitis, prosthetic joint infection, medical device-related infections, etc. The emergence of biofilms on a metal substrate triggers biocorrosion, and it results in the collapse of buildings and bridges, chemical and oil pipeline breakage, etc. As various bacteria acquired antibiotic resistance to synthetic drugs, it is high time to introduce the antibiofilm and anti-biocorrosive potential of *Curcuma zanthorrhiza* rhizome extract. **Materials and Methods:** The potential of the hydro-methanolic crude extract of *Curcuma zanthorrhiza* against *Pseudomonas aeruginosa* biofilm was evaluated by a microtiter plate assay. Scanning electron microscopic evaluation and EtBr/AO fluorescence assay were also performed. **Results:** Treatment with 40 mg/mL of *Curcuma zanthorrhiza* extract resulted in biofilm inhibition of about 56%. Fluorescence microscopy was used to assess the biofilm inhibition by EtBr/AO staining in *Pseudomonas aeruginosa*. Extract-treated cells appeared in a yellow to orange color, and their cytoplasmic membrane integrity was seen to be lost. Scanning electron microscopy images of the surface morphology evaluation revealed the remarkable biocorrosion inhibition of *Curcuma zanthorrhiza*. Invaginations and grooves in the surface of plant extract-treated steel coupons were minor in comparison with untreated samples. Enzyme kinetics assessment validated the amylase-mediated action of plant extracts in inhibiting biofilm and biocorrosion. **Conclusion:** Analysis of spectrophotometric data revealed the amylase-mediated anti-biofilm and anti-biocorrosion activity of *Curcuma zanthorrhiza*.

Keywords: Biocorrosion, Biofilm, *Curcuma zanthorrhiza*, *Pseudomonas aeruginosa*, Zingiberaceae.

Correspondence:
Dr. Bince Mani
Department of Botany, St. Thomas College Palai (Autonomous), Kottayam-686574, Kerala, INDIA.
Email: binsnm@gmail.com

Received: 04-11-2024;
Revised: 14-01-2025;
Accepted: 21-03-2025.

INTRODUCTION

Curcuma Linn. is one of the largest genera in the family Zingiberaceae. Among the *Curcuma* species, *Curcuma zanthorrhiza* Roxb. is native to southwest India, and it possesses majestic therapeutic efficacy.[1] *Curcuma zanthorrhiza* is popular under the name 'Javanese turmeric,' and it is well-known in Malaysia and Indonesia under the names 'koneng gede' and 'temulawak.' The large, branched rhizome appears pale yellowish brown or pale orange yellow on its outer surface and yellow to deep orange yellow on its inner surface, and it possesses a mild camphoraceous smell and a warm, bitter taste.[2] The therapeutic efficacy of *C. zanthorrhiza* is mainly due to the presence of phytoconstituents such as Xanthorrhizol (XNT), kurlon, artumeron, isofuranogermacene, p-tolyl methyl carbinol, zingiberene, camphene, linalool, curcumin, α-pinene, β-pinene α-thujene and myrcene in its rhizome.[3] Among the therapeutic potentials of *C. zanthorrhiza*, antibiofilm and antibiocorrosive activities are highly significant.

The attachment of microbial communities to biotic and abiotic substrates by self-synthesized Extracellular Polymeric Substances (EPS) is known as biofilm. The formation of biofilm aids in enhancing the virulence of bacteria, and bacteria gain much more resistance against adverse environmental conditions.[4] The complete appearance of biofilm results from four stages, such as adhesion to the surface, formation of microcolonies, maturation, and dispersal of biofilm.[5] Inhibition of biofilm is made possible to a certain extent by alleviating quorum sensing, treatment with plant extracts having antibacterial properties, and the application of certain synthetic drugs. Extracellular Polymeric Substances (EPS) have been destabilized and degraded by treatment with

DOI: 10.5530/ajbls.20251428

amylase and protease, as demonstrated in the studies against *Pseudomonas fluorescens*.[6] Hence, the development of a proper biofilm-inhibiting strategy is needed and is also becoming a challenge in the research area.

Beyond the hazardous health problems and infections, the interaction of complex biofilm with metallic surfaces leads to the emergence of corrosion. Biocorrosion is the degradation of metal or nonmetal materials caused by microorganisms that inhabit water, wastewater, or inner piping walls.[7] Biocorrosion is catalyzed by the oxidation of metal ions, which is generated by bacterial catalase enzymes. The role of microbial colonies in enhancing the corrosion of bronze and mild steel in the cooling water system is remarkably high.[8] Electrochemical corrosion occurs when the surface of metal comes into contact with an aqueous environment, and dissolution of iron occurs as a result of the absorption of oxygen onto the surface of the metal. Finally, various corrosion products such as Fe_2O_4, $HFeO_2$, and Fe_2O_3 formed as a result of the oxidation of metals.[9] Microbial metabolites favor the corrosion of metals by altering the acidity, oxygen concentration, pH, and salinity around the surface of metals.[10] Though there is dissolution and oxidation of a zero-valent metal, the formation of biocorrosion is becoming an electrochemical reaction. A complex interaction of microbial consortiums produces biofilm on metal surfaces, which starts to corrode and is called Microbially Influenced Corrosion. Hence, the study of biocorrosion encompasses the collaboration of microbiologists, electrochemists, and material scientists.[11] The ecological and economic impacts of biocorrosion have now become a salient topic under consideration.[10]

Though previous studies have been conducted on sustainable safeguarding of chemical agent-arbitrated corrosion in metal surfaces, the potential of plant extracts in reducing biocorrosion gains much more significance.[12] Research on natural antibiotics against biofilm-mediated biocorrosion has now become indispensable. Despite the complexities and adverse impacts of synthetic antimicrobials on both the environment and humans, phytozymes can regulate the prevention of biocorrosion and biofilm, thereby validating the broad scope of antibacterial research. As a result, studies on the amylolytic effect of a crude hydro-methanol extract of *C. zanthorrhiza* on *Pseudomonas aeruginosa* biofilm have become mandatory. The future intention of this research is to characterize and introduce a biomolecule that has powerful anticorrosive and biofilm potential that has never been explored yet.

MATERIALS AND METHODS

Organism, chemicals and other materials

The anti-biofilm and anti-biocorrosion activity of crude hydromethanolic extract of *C. zanthorrhia* rhizome was evaluated using clinical isolate *Pseudomonas aeruginosa* strain jb9. Sterile 96-well plates and 1X PBS was all bought from Tarsan, India. The clinical isolate of the *P. aeruginosa* strain jb9 was isolated from a sputum sample, and the molecular identification was performed using 16S rRNA sequencing.[13] Conical flasks are purchased from Borosil in India. Spirit lamps and double-distilled water and test samples are also used. Nutrient broth provided from HiMedia in India. Acridine Orange (AO) and Ethylene Bromide (EtBr) were purchased from Sigma Aldrich (USA).

Collection and identification of plant material

Rhizome of *C. zanthorrhiza* obtained from Pampavally, Kottayam district, Kerala, India (9.54421 latitude and 76.78091 longitude). Dr. Bince Mani of St. Thomas College Palai (Autonomous), Kerala, identified and authenticated the specimen. The voucher specimen (RHT 68964) is preserved in the Rapinat Herbarium in Trichy. Plants were carefully taken out without damaging the rhizome, rinsed with running tap water, and stored properly.

Preparation of bioactive fraction

After cleaning and disinfecting, the rhizomes were chopped into small pieces and dried for four weeks at room temperature (32°C). The material was roughly pulverized with an electric grinder and dehydrated in an oven at 50°C for 30 min. Powdered materials are kept in tight containers for future studies. Cold extract of rhizome powder was prepared by soaking 40 grams of powder in 500 mL of hydro-methanol (4:6). Complete extraction is achieved by shaking in a shaking incubator at 50 rpm and 22°C for 48 hr. The extract was filtered using Whatman No. 1 filter paper, and the resultant concentrated filtrate (hydro-methanolic extract) has been subjected to biofilm and biocorrosion assays.[14]

Microtiter Plate assay (MTP) for Biofilm inhibition

The microtiter plate assay was performed using a 96-well, flat-bottom polystyrene titer plate. About 180 µL of Brain Heart Infusion (BHI) broth and 10 µL of *P. aeruginosa*, cultured overnight, were supplied into each well. After that, 10 µL of plant extract (40 mg/mL) was added and incubated at 37°C for 24 hr. After incubation, the wells were rinsed with 0.2 mL Phosphate Buffer Saline (PBS) of pH 7.2 for removing the floating bacteria. Bacterial adhesion was fixed with sodium acetate (2%) and subsequently stained by crystal violet (0.1% w/v). Excess stain was cleaned with deionized water and allowed to dry. The dried plates were then cleaned with 95% ethanol before being measured for optical density at 600 nm by a microtitre plate reader (Thermo).[15] The percentage of biofilm inhibition was calculated using the formula shown below.

$$\text{Percentage of biofilm inhibition} = [(\text{Control OD} - \text{Test OD})/\text{Control OD})] \times 100$$

EtBr/AO fluorescence assay of Biofilm inhibition

The inhibition of biofilms by EtBr/AO staining was evaluated using fluorescence microscopy. *P. aeruginosa* cells (5×10^6 cells/

mL) were treated with 40 mg/mL hydro-methanolic extract in nutrient broth and placed on a coverslip in a 24-well culture plate for 48 hr in a bacteriological incubator at 37°C. After incubation, add 50 µL of 1 mg/mL acridine orange and ethidium bromide to each well, gently mixing. Then the plate was centrifuged at 800 rpm for 2 min, examined within an hour, and observed under a fluorescence microscope with a fluorescent filter.[16]

Bio-corrosion study and Scanning Electron Microscopic (SEM) evaluation

Pseudomonas aeruginosa culture was grown in 250 mL nutrient broth for 48 hr. Stainless steel coupon was tested to evaluate the rate of corrosion by *Pseudomonas aeruginosa* in three conditions. In the first case stainless steel coupon placed in bacterial broth without plant extract. At the same time, in the second treatment stainless steel coupon placed in bacterial broth along with plant extract (40 mg/mL) and the third condition was the control in which the steel coupon is inserted in distilled water where there is no nutrient broth. The steel coupons were placed in a shaking incubator at 100 rpm at 37°C temperature and allowed to incubate for 28 days. 50 mL of nutrient broth and 1 mL of crude extract were replaced every 3 days for treatment 2 and nutrient broth only for treatment 1. After the incubation, samples were subjected to morphological assessment by Scanning Electron Microscopy (SEM).[17]

Evaluation of Amylolytic activity of hydro-methanolic extract

The amylolytic activity of the hydromethanolic extract of *C. zanthorrhiza* was evaluated spectrophotometrically for the determination of enzyme-substrate reactions.[15] Incubate 1 mL of rhizome extract (500, 250, 125, 62.5, and 31.25 µg/mL) with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) at 37°C for 30 min. After 30 min of incubation, 1 mL of DNS reagent was added to terminate further reactions. The above mixture is placed in a water bath at 100°C for 20 min, and absorbance is taken in a UV-vis spectrophotometer (ELICO-SL218) at 540 nm.[18] Enzyme is replaced by distilled water for the control by the same procedures, and the blank solution contains distilled water and DNS reagent. The effect of varying enzyme concentrations with respect to reaction rate was examined at constant temperature (37°C) and pH (8.0).

Statistical Analysis

The obtained data was statistically analyzed using one-way ANOVA (Analysis of Variance) in GraphPad Prism version 5.01 software, followed by Dunnett's post-test.

RESULTS

Microtiter Plate assay (MTP) for Biofilm inhibition

The efficacy of the crude hydro-methanolic extract of *C. zanthorrhiza* rhizome to mitigate biofilm was evaluated by microtiter plate reader. A notable decrease in biofilm was observed according to the treatment with extract. A direct correlation exists between the concentration of *C. zanthorrhiza* crude HME and the percentage of biofilm inhibition. The tested concentration of hydromethanolic extract at 40 mg/mL exhibited 56% inhibition of *P. aeruginosa* biofilm.

Evaluation of biofilm inhibition by Fluorescence microscopy

Fluorescence staining assay using EtBr/AO staining was used to examine the surface organization of biofilm inhibition of *C. zanthorrhiza* (Figure 1). Treatment with a hydro-methanolic

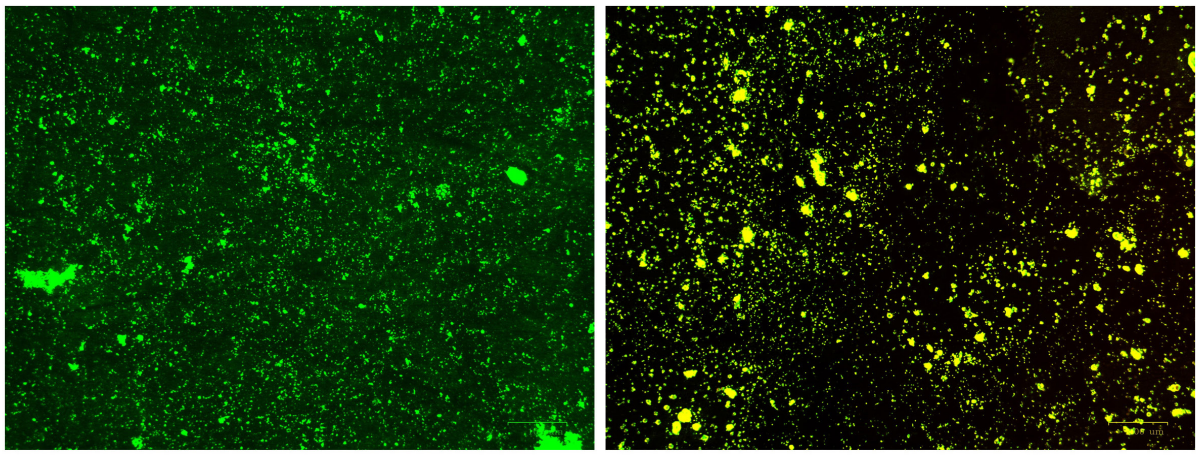

Figure 1: Fluorescence microscopic images of biofilm inhibition. (A) Untreated *P. aeruginosa* culture showing green flurescence. (B) Yellow flurescence from *P. aeruginosa* culture treated with methanolic extract of *C. zanthorrhiza*.

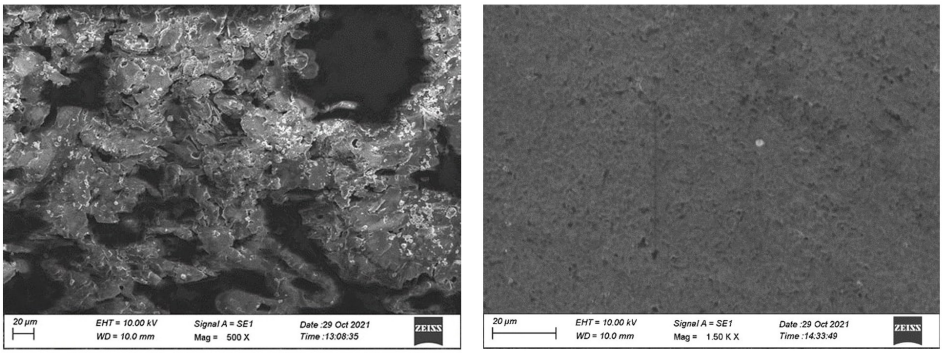

Figure 2: Scanning electron micrographs showing biofilm formation and inhibition. (A) Picture showing the growth of *P. aeruginosa* biofilm and biocorrosion on untreated experimental conditions. (B) Inhibion of *P. aeruginosa* biofilm formation and biocorrosion on treated experimental conditions (hydro-methanolic extract of *C. zanthorrhiza*).

extract of *C. zanthorrhiza* (40 mg/mL) resulted in loosely arranged *P. aeruginosa* cells that were yellow to orange in color. It is due to the uptake of Ethidium Bromide (EtBr) by cells and loss of cytoplasmic membrane integrity (Figure 1B). The untreated sample appeared as densely packed green chromatin because the fluorescent dye is impermeable through viable cell membranes (Figure 1A) and can be used as a fluorescent indicator of dead cells.

Surface morphological evaluation of Bio-corrosion

Scanning Electron Microscopy (SEM) of tested stainless-steel coupons showed the morphological changes of steel coupons of plant extract-treated, untreated, and control, which is shown in Figure 2. The bacterial growth followed by corrosion on the steel coupon was observed in the first treatment. Figure 2A displayed grooves and invaginations that indicate the bacteriological actions. In the second condition, bacteriological broth with 40 mg/mL of hydro-methanolic extract showed minimum bacteriological actions, and steel coupon appeared smooth (Figure 2B). Similarly, the control didn't show either invaginations or grooves on steel coupon and appeared smooth, devoid of bacteriological actions.

Evaluation of Amylolytic activity of hydro-methanolic extract

Figure 3 shows the correlation of the hydro-methanolic extract of *C. zanthorrhiza* and its total enzyme activity. As the hydro-methanolic extract of *C. zanthorrhiza* contains an amylase enzyme, the total enzyme activity at various concentrations of the extract was determined. Treatment with 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL of plant extract results in total enzyme activity of 62.18 U, 81.676 U, 91.43 U, 101.169 U, and 110.916 U, respectively. The extract at 1.0 mg/mL showed the maximum total enzyme activity (110.916 U) among all tested concentrations. Treatment with 0.4, 0.6, and 0.8 mg/mL of extract resulted in enzyme activity of 81.676, 91.43, and 101.169 U, respectively. At 0.2 mg/mL, the extract had just 62.18 U activity. Hence, it is confirmed that the concentration of hydro-methanolic extract of *C. zanthorrhiza* increases; the total enzyme activity also increases correspondingly.

DISCUSSION

Curcuma zanthorrhiza is a well-known species in the family *Zingiberaceae* that possesses tremendous therapeutic efficacy. The present study emphasized the amylase-mediated reticence of the hydro-methanolic extract of *C. zanthorrhiza* in the emergence of *P. aeruginosa*-mediated biofilm and biocorrosion. Here, the research highlights the potential of the hydromethanolic extract of *C. zanthorrhiza* in inhibiting *P. aeruginosa*-mediated biofilm formation and preventing biocorrosion. As the biofilm is composed of Extra-Polymeric Substances (EPS) that are made of lipids, eDNA, carbohydrates, and proteins, enzymes such as amylase, protease, decarboxy nucleases, and lipase have a leading role in reducing the growth and multiplication of bacterial biofilm.[19] The eco-friendly nature of phytochemical constituents enhances the demand for green inhibition of biocorrosion. Biocorrosion-inhibiting chemical compounds found in plant extracts can act as inhibitor molecules that adhere to the surface and obstruct the active sites.[20]

This study demonstrates the effectiveness of a crude hydromethanolic cold extract of *C. zanthorrhiza* to inhibit *P. aeruginosa* biofilm. A microtitre plate assay showed 56% inhibition of *P. aeruginosa* biofilm by treatment with 40 mg/mL of extract. This finding shall be further supported by an EtBr/AO fluorescence assay and scanning electron microscopic evaluation. Not only *C. zanthorrhiza*, but there are reports regarding the antibiofilm and anti-corrosive potential of phytoconstituents from various plant extracts. Aqueous extract of *Glycyrrhiza glabra* (600 ppm) shows promising anti-biocorrosive activity against *Bacillus subtilis, Streptomyces parvus, Acinetobacter baumannii*, and *Pseudomonas stutzeri* in API 5LX carbon steel by 92%-99%. Phytozymes and bioactive chemicals in the above plant were extracted in 1% HCl, which acts as a protective barrier to the surface of metals and reduces the rate of corrosion.[21]

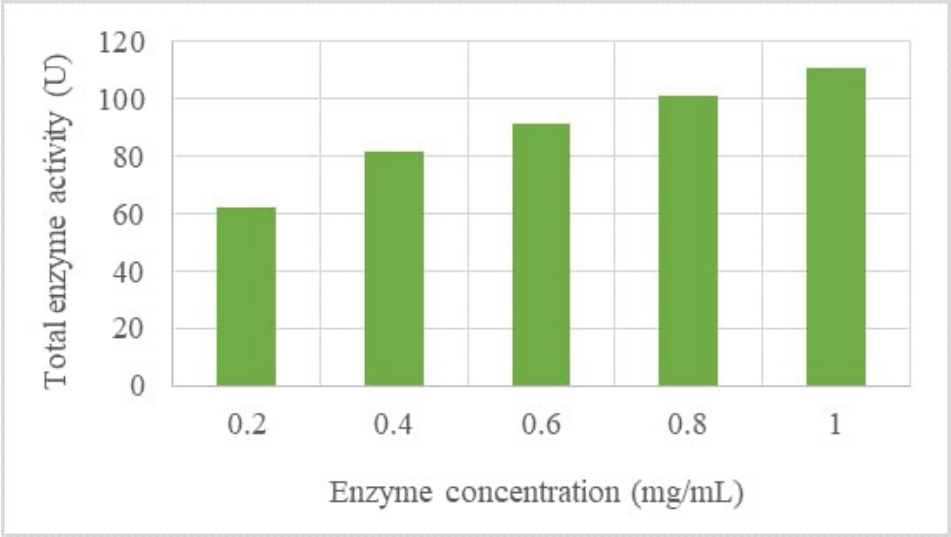

Figure 3: Total enzyme activity of hydro-methanolic extract of *C. zanthorrhiza*.

Both the gram-positive and gram-negative bacteria have the potential to form biofilm. The cell walls of gram-negative bacteria possess a high concentration of lipids, which makes them more resistant to chemical compounds and antibiotics. Due to the complex cell wall composition, polar and semi-polar compounds have more difficulty piercing bacterial cells.[22] Hence, the potential of *C. zanthorrhiza* against the gram-negative bacterial strain *P. aeruginosa* gains more significance. Apart from *P. aeruginosa, C. zanthorrhiza* exhibits antibiofilm potential towards gram-negative bacteria and gram-positive bacteria. [23,24] Not only bacterial biofilm, but also fungal biofilm of *Candida* species is susceptible to *C. zanthorrhiza*.[25] Though the hexane fraction of the acetone extract of *C. zanthorrhiza* exhibits significant activity against *P. aeruginosa*, it is necessary to evaluate its antibiofilm potential.[26]

Xanthorrhizol is a bisabolene sesquiterpenoid isolated from the ethyl acetate fraction of the methanol extract of *C. zanthorrhiza*.[27] Coating the wells of a polystyrene microtiter plate with xanthorrhizol can effectively reduce 60% of the adherent cells of *Streptococcus mutans* compared to uncoated wells.[28] Even though the topical application of bactericides such as chlorhexidine, triclosan, cetylpyridinium chloride, etc. can control plaque development and the number of microbiotas in saliva, their frequent use can lead to a change in the oral microbiota and the emergence of resistant strains.[29]

The use of the antibiotics in combination with DNase I dramatically reduced the number of bacteria compared to the use of the antibiotics alone. Yet, research indicates that the dispersion of biofilm by microorganisms may depend on enzymes, notably those that break down polysaccharides. However, biocorrosion can be eventually inhibited by developing a substantial mechanism to prevent the growth of biofilm on metal.

CONCLUSION

Biofilm is a complex matrix consisting of extracellular polysaccharides, proteins, and DNA that shields bacteria from various physical, chemical, and biological challenges and enables them to live in hostile situations. The presence of biofilm on metal surfaces eventually causes deterioration of metal surfaces, which is known as biocorrosion or microbially influenced corrosion. Synthetic drugs that interfere with bacterial metabolism will eventually result in the emergence of drug-resistant strains. Hence, the potential of phytoconstituents to act as an effective antibiofilm as well as an anti-biocorrosion agent is studied. Analysis of the result revealed the highly significant potential of the hydromethanolic extract of *C. zanthorrhiza* (40 mg/mL) against *P. aeruginosa* biofilm with an inhibition of 56%. The result revealed the highly significant potential of the hydromethanolic extract of *C. zanthorrhiza* (40 mg/mL) against *P. aeruginosa* biofilm with an inhibition of 56%. It is then supported by enzyme-like assays and scanning electron microscopy. The total enzymatic activity was evaluated for the determination of the amylolytic nature of hydro-methanolic extract of the *C. zanthorrhiza* against *P. aeruginosa* biofilm, which can ultimately act as an effective anti-biocorrosive agent.

ACKNOWLEDGEMENT

The authors would like to thank Marian Centre for Advanced Research (MCAR), the research division of St. Mary's College in Thrissur, Kerala, India, for providing research infrastructure to enable the completion of this work. I would also like to acknowledge the authorities of St. Thomas College Palai (Autonomous), Kerala, for providing the resources needed for conducting the research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BHI: Brain Heart Infusion; ***C. zanthorrhiza*:** *Curcuma zanthorrhiza*; **DNS:** 3,5-Dinitrosalicylic Acid; **EPS:** Extracellular Polymeric Substances; **EtBr/AO:** Ethidium Bromide/Acridine Orange; **HME:** Hydro-methanolic extract; ***P. aeruginosa:*** *Pseudomonas aeruginosa*; **PBS:** Phosphate Buffered Saline; **RPM:** Revolutions Per Minute; **SEM:** Scanning Electron Microscopy.

SUMMARY

In this study, the hydro-methanolic extract of *C. zanthorrhiza* was subjected to biofilm and biocorrosion assays. EtBr/AO fluorescence assay and scanning electron microscopic evaluation showed the remarkable potential of *C. zanthorrhiza* to inhibit microbial corrosion and biofilm formation by *P. aeruginosa*. Enzyme kinetics studies indicated the presence of an amylase-like compound in the hydro-methanolic extract of *C. zanthorrhiza*, which can act as a powerful antibiofilm and antibiocorrosive agent. These results suggest that *C. zanthorrhiza* extract could be used as an eco-friendly strategy to prevent biocorrosion and remove biofilm in a number of industrial settings, providing a long-term alternative to chemical treatments.

REFERENCES

1. Skornickova J, Sabu M. The Identity and Distribution of *Curcuma zanthorrhiza* Roxb. (Zingiberaceae). Gard Bull Singapore 2005;57:199-210.
2. Halim MRA, Tan MSMZ, Ismail S, Mahmud R. Standardization and phytochemical studies of *Curcuma xanthorrhiza* Roxb. Int J Pharm Pharm Sci 2012;4:606-10.
3. Kasai H, Yamane Y, Ikegami-Kawai M, Sudo H. Analysis of Compounds of *Curcuma* Rhizome Using Mass Spectrometry and Investigation of the Antioxidant Activity of Rhizome Extracts. Med Aromat Plants 2019;8:1-7. https://doi.org/10.35248/2167-0412.19.8.336.
4. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. Microorganisms 2023:11:1-32. https://doi.org/10.3390/microorganisms11061614.
5. Guzmán-Soto I, McTiernan C, Gonzalez-Gomez M, Ross A, Gupta K, Suuronen EJ, *et al.* Mimicking biofilm formation and development: Recent progress in *in vitro* and *in vivo* biofilm models. iScience 2021;24:1-51. https://doi.org/10.1016/j.isci.2021.102443.
6. Molobela IP, Cloete TE, Beukes M. Protease and amylase enzymes for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by *Pseudomonas fluorescens* bacteria. Afr J Microbiol Res 2010;4:1515-24.
7. Pal MK, Lavanya M. Microbial Influenced Corrosion: Understanding Bioadhesion and Biofilm Formation. J Bio Tribo Corros 2022;8:1-13. https://doi.org/10.1007/s40735-022-00677-x.
8. Narenkumar J, AlSalhi MS, Prakash AA, Abilaji S, Devanesan S, Rajasekar A, *et al.* Impact and Role of Bacterial Communities on Biocorrosion of Metals Used in the Processing Industry. ACS omega 2019;4:21353-60. https://doi.org/10.1021/acsomega.9b02954.
9. Telegdi J, Shaban A, Vastag G. Biocorrosion-Steel. In: K Wandelt,. Encyclopedia of Interfacial Chemistry: Surface Science and Electrochemistry 2018;7:28-42. https://doi.org/10.3389/fenvs.2022.924842.
10. Palaniappan B, Toleti SR. Characterization of microfouling and corrosive bacterial community of a firewater distribution system. J Biosci Bioeng 2015;57:1-7. https://doi.org/10.1016/j.jbiosc.2015.08.009.
11. Lou Y, Chang W, Cui T, Qian H, Huang L, Ma L, *et al.* Microbiologically influenced corrosion inhibition of carbon steel via biomineralization induced by *Shewanella putrefaciens*. npj mater Degrad 2021;5:1-11. https://doi.org/10.1038/s41529-021-00206-0.
12. Manh TD, Huynh TL, Thi BV, Lee S, Yi J, Nguyen Dang N. Corrosion inhibition of mild steel in hydrochloric acid environments containing *Sonneratia caseolaris* leaf extract. ACS omega 2022;7:8874-86. https://doi.org/10.1021/acsomega.1c07237.
13. Vadakkan K, Hemapriya J, Anbarasu A, Ramaiah S, Vijayanand, S. Quorum quenching by 2-Hydroxyanisole extracted from *Solanum torvum* on *Pseudomonas aeruginosa* and its inhibitory action upon LasR protein. Gene Rep 2020;21:1-9. https://doi.org/10.1016/j.genrep.2020.100802.
14. Degu A, Kefale B, Alemayehu D, Tegegne GT. Evaluation of the Antidiarrheal Activity of Hydromethanol Crude Extracts of *Ruta chalepensis* and *Vernonia amygdalina* in Mice. Evid Based Complement Alternat Med 2020; 2020:1-6. https://doi.org/10.1155/2020/8318713.
15. Karayamparambil B, Vadakkan K, Thomas S. Amylolytic-dependent biofilm inhibition of *H. forrestti* var. forrestii rhizome extract against *S. aureus* pathogens. Journal of Natural Pesticide Research 2024;8:2-6. https://doi.org/10.1016/j.napere.2024.100072.
16. Bogachev MI, Volkov VY, Markelov OA, Trizna EY, Baydamshina DR, Melnikov V, *et al.* Fast and simple tool for the quantification of biofilm-embedded cells sub-populations from fluorescent microscopic images. PLoS One 2018;13:1-24. https://doi.org/10.1371/journal.pone.0193267.
17. Krishnan M, Subramanian H, Dahms H-U, Sivanandham V, Seeni P, Gopalan S, *et al.*, Biogenic corrosion inhibitor on mild steel protection in concentrated HCl medium. Scientific Reports, 2018;8(1):1-16. https://doi.org/10.1038/s41598-018-20718-1.
18. Jain A, Jain R, Jain S. Determination of Salivary Amylase Activity. In: Basic Techniques in Biochemistry, Microbiology and Molecular Biology. Springer Protocols Handbooks. Humana, New York, NY. 2020. https://doi.org/10.1007/978-1-4939-9861-6_52.
19. Di Martinom P. Extracellular polymeric substances, a key element in understanding biofilm phenotype. AIMS Microbiol 2018;4:274-88. https://doi.org/10.3934/microbiol.2018.2.274.
20. Pradipta I, Kong D, Joash T. Natural organic antioxidants from green tea inhibit corrosion of steel reinforcing bars embedded in mortar. Constr Build Mater 2019;227:351-62. https://doi.org/10.1016/j.conbuildmat.2019.117058.
21. Parthipan P, Cheng L, Rajasekar A. *Glycyrrhiza glabra* extract as an eco-friendly inhibitor for microbiologically influenced corrosion of API 5LX carbon steel in oil well produced water environments. J Mol Liq 2021;333:1-10. https://doi.org/10.1016/j.molliq.2021.115952.
22. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules 2020;25:1-23. https://doi.org/10.3390/molecules25061340.
23. Fitri M, Nazar K, Meidyawati R, Azmi R. Antibacterial effect of xanthorrhizol (*Curcuma zanthorrhiza* Roxb.) against the biofilm of *Fusobacterium nucleatum*. Int J Appl Pharm 2020;12:57-61. https://doi/10.22159/ijap.2020.v12s2.PP-06.
24. Warmasari NWM, Ernawati DK, Indrayani AW, Dewi NWS, Jawi IM. Antibacterial activity from Temulawak extract (*Curcuma xanthorrhiza* Roxb) on growth inhibition of *Staphylococcus epidermidis in vitro*. Journal Epidemiologi Kesehatan Komunitas 2020;5:1-7. https://doi.org/10.14710/jekk.v5i1.6909.
25. Rukayadi Y, Han S, Yong D, Hwang, JK. *In vitro* activity of xanthorrhizol against *Candida glabrata, Candida guilliermondii*, and *Candida parapsilosis* biofilms. Med Mycol J 2011;49:1-9. https://doi.org/10.3109/13693786.2010.492482.
26. Diastuti H, Syah YM, Juliawaty LD, Singgih M. Antibacterial *Curcuma xanthorrhiza* extract and fractions. J Math Sci 2014;46:224-34. https://doi.org/10.5614/j.math.fund.sci.2014.46.3.2.
27. Hwang JK, Shim JS, Baek N, Pyun YR. Xanthorrhizol: a potential antibacterial agent from *Curcuma zanthorrhiza* against *Streptococcus mutans*. Planta Med 2000;66:196-7. https://doi.org/10.1002/jobm.200510088.
28. Rukayadi Y, Hwang JK. Effect of coating the wells of a polystyrene microtiter plate with xanthorrhizol on the biofilm formation of *Streptococcus mutans*. J Basic Microbiol 2006;46:410-5. https://doi.org/10.1002/jobm.200510088.
29. Tetz GV, Artemenko NK, Tetz VV. Effect of DNase and antibiotics on biofilm characteristics. Antimicrob Agents Chemother 2018;53:1204-9. https://doi.org/10.1128/aac.00471-08.

Cite this article: Joseph D, Vadakkan K, Mani B, Cheruvathur MK. Amylase-Arbitrated Antibiofilm and Anti-Biocorrosion Efficacy of *Curcuma zanthorrhiza* against *Pseudomonas aeruginosa*. Asian J Biol Life Sci. 2025;14(1):192-7.