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The Potential of *Tinospora cordifolia* Extracts as Antibacterial Material against *Pseudomonas aeruginosa*

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Abstract

Mitigation and control of bacterial corrosion must have been prioritized for failure anticipation by corrosion associated with microorganisms. Using synthetic inhibitor for material protection from corrosion have problems because it is not eco-friendly. This work conducted preliminary studies on *T. cordifolia* stem extracts at various methanol ratios against *P. aeruginosa* biofilm as a new eco-friendly inhibitor. The *T. cordifolia* stem was extracted by the maceration method with a different ratio of methanol solvent (100, 75 and 50 %). The bacterial activity was assessed using the dilution method (MTT assay) to determine the minimum inhibitory concentration (MIC). The total phenolic and flavonoid contents were determined using Follin-Ciocalteu and aluminum chloride ($AlCl_3$) colorimetric, respectively. Meanwhile, the structure of the active compounds in the extract was identified by using the liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The yields of *T. cordifolia* extracts are 9.45, 7.56 and 8.40 % at 100, 75 and 50 % of methanol ratios, respectively. The total phenolic content (TPC) in crude methanol extract of *T. cordifolia* is 11.20, 9.46 and 6.56 % for 100, 75 and 50 % of methanol ratios. Meanwhile, the flavonoid content (TFC) was obtained is about 5.25, 0.64 and 0.33 % for 100, 75 and 50 % of methanol concentrations, respectively. The *T. cordifolia* extract has antibacterial activity against *P. aeruginosa* in all ratios of methanol concentrations. The minimum inhibitory concentration (MIC) of *T. cordifolia* methanol extract was found at 4096 µg/mL. Methanol solvent concentration with the most active antibacterial activity of the extract was 50 %, followed by 75 and 100 %. The structures of the active compound in the methanolic extract of *T. cordifolia* are a phenolic group, and it is in the alkaloid derivatives (Calopiptin, d-Lirioferine (Lirioferine), Moupinamide, Piperanine, and Yuanhunine).

Keywords: Antibacterial, Bioactive, Flavonoid, Phenolic, *P. aeruginosa*, Plant extracts, *T. cordifolia*

Introduction

Microbiologically influenced corrosion is a type of corrosion that is accelerated or produced by the action of certain microorganisms or metabolic products [1]. Microorganisms are a major cause of corrosion at a variety of industrial sites. Corrosion caused by microbes frequently occurs in marine [2], soil [3,4], and industrial environments [5]. Bacterial biofilms are populations of microorganisms that form on surfaces caused by certain bacteria. *Pseudomonas aeruginosa* is a gram-negative, facultative aerobic bacteria that produce biofilms on metal surfaces [6]. For instance, *P. aeruginosa* causes corrosion on copper alloy and stainless steel due to biofilm formation in the maritime environment [7, 8]. Biofilms of *P. aeruginosa* have been proven to degrade the performance of the pipeline network system used to distribute water [8].

Priority must have been given to mitigating and controlling bacterial corrosion to avoid failures caused by microorganisms. Utilizing inhibitors is critical for preventing material deterioration due to corrosion [9,10]. Several studies reported the effects of heterocyclic compounds consisting of sulfur, nitrogen, and oxygen on corrosion in various acidic environments [12]. Numerous synthetic compounds exhibit excellent corrosion resistance, but most are highly toxic to the environment [13]. The protection of

synthetic corrosion inhibitors and environmental concerns in industries have long been a source of global concern [14]. Due to the known dangers associated with most synthetic corrosion inhibitors, researchers have turned to natural materials as corrosion inhibitors since they are low-cost, abundant, renewable, ecologically acceptable, and eco-friendly [15]. Numerous corrosion inhibitors with compositions ranging from rare earth minerals to organic materials have been synthesized [16].

Due to the diversity of bioactive compounds produced by plants, they are a rich source of antibacterial compounds. Isolating bioactive compounds may aid in the development of more effective pharmacotherapies for *P. aeruginosa* biofilm bacteria. Numerous plant derivatives have been used as green biocides to inhibit microorganism corrosion recently, including *Allium sativum* for *Bacillus subtilis* and *Streptomyces parvus* [17], *Cistus ladanifer* for *P. aeruginosa* [8], Catechin hydrate for *P. aeruginosa* [18], *Aloe vera* and *Morinda citrifolia* for *P. aeruginnosa* [19], *Azadirachta indica* for *Arthrobacter sulfureus* [20], and *Glycyrrhiza glabra* for bacterial consortiums [21]. *T. crispa* is the only natural product used as an inhibitor with bioactive activity to avoid chemical corrosion of mild steel in a 1 M HCl environment [22]. Based on the author's knowledge, the use of *T. cordifolia* stem extract has not been found to prevent biofilm-forming bacteria from causing corrosion in metals.

On the other hand, *T. cordifolia* plants are renowned worldwide for their excellent biological qualities. The trunk, leaves, and bark have been shown to have a variety of medical applications against a variety of bacterial [23]. *T. cordifolia* is a medicinal herb that contains a variety of different compounds. Various bioactive compounds, including phenolics, steroids, sesquiterpenoids, glycosides, and alkaloids [23]. Thus, *T. cordifolia* has been used successfully in the sufficiency of various bacteria so that it has the potential to inhibit corrosion caused by bacteria. In this regard, more research is needed to explore *T. cordifolia* for its potential to inhibit and control corrosion caused by microbes.

In this work, *T. cordifolia* stems are extracted with various solvent mixtures of methanol. The extract results were then evaluated for their activity properties against *P. aeruginosa*. This study aimed to obtain the active compound from *T. cordifolia* and the minimum inhibitory concentration of these extracts against *P. aeruginosa*. Identification of the flavonoid and phenolic contents and bioactive structure of *T. cordifolia* methanol extracts were also discussed.

Materials and methods

Materials

A stem sample of *T. cordifolia* was obtained from Indonesia Medicinal and Aromatic Crops Research Institute (IMACRI). The plant was identified at the Herbarium Bogoriense Laboratory, Division of Botany - National Research and Innovation Agency (BRIN). The microorganism of *P. aeruginosa* biofilm was obtained from Indonesia Culture Collection (InaCC). Other materials are methanol, Isopropanol (HCl 0.04 M), MTT solution (5 mg/mL), seawater media (Marine Art SF-1), and brain heart infusion (BD Bacto).

Preparation of plant

The plant was sorted from raw material and washed under running tap water. Furthermore, it is chopped into small pieces to make it easier in the powdering process, then dried in an oven at 40 °C for 5 days. It was then crushed with a disc mill (SS 304) and sieved with a size of 60 mesh.

Extractions

The powdered parts (25 g) of *T. cordifolia* were extracted with different ratios of methanol (150 mL) for 3× 24 h at room temperature. In this study, the ratio of methanol to water used is 100, 75 and 50 % (v/v). Each methanolic extract was filtrated with Whatman paper and then evaporated under pressure at 50 °C to yield extract. The extraction yield (%) was calculated as follows:

$$Yield\ (\%) = \frac{weight\ of\ extract\ after\ evaporating\ and\ drying}{dry\ weight\ of\ sample} \times 100 \quad (1)$$

Total phenolic and flavonoid analyzation

The total phenolic content (TPC) was determined using the Follin-Ciocalteu method [24], using gallic acid as a reference. The sample solution (500 µL) and reference solutions of gallic acid (25, 50, 100, 150, and 200 µL) were pipetted into a reaction tube and then added to 4 mL by 250 µL Folin-Ciocalteau, distilled water, and swayed. After 8 min, 750 µL of 20 % Na_2CO_3 was added and homogeneously shaken. The solution result was then allowed to stand for 2 h at room temperature. The absorption was determined at 765 nm. Measurements were repeated 2 times so that the phenol content was obtained as equivalent gallic acid (µg/mL sample). While the total flavonoid content (TFC) was determined by using colorimetric

aluminum chloride ($AlCl_3$) assays [25]. The 4 mg of quercetin and samples were weighed and dissolved in 4 mL of methanol as the main solution (1000 µg/mL). Reference curve measurements: A series of 10, 20, 30, 40 and 50 µg/mL standard solutions were prepared by pipetting standard solutions of quercetin (50, 100, 150, 200 and 250 µL) into in a reaction tube. Meanwhile, sample measurements were generated by pipetting 250 and 500 µL into a reaction tube. Each tube was filled with 150 µL of 5 % $NaNO_2$ and 2 mL of distilled water. The 150 µL of 10 % $AlCl_3$ was added after 5 min. Then after 6 min, 2 mL of 1 M NaOH was added, and the volume of the solution was calibrated to 5 mL using distilled water. After homogenizing the solution, the absorbance was measured by using a UV-Vis spectrophotometer at 510 nm.

Antibacterial assay

T. cordifolia crude extracts were investigated for antibacterial efficacy against *P. aeruginosa* biofilm bacterium. Ciprofloxacin (Sigma Aldrich, Germany) was used as a reference. The antibacterial activity was assessed by determining the minimal inhibitory concentration (MIC) using the microdilution methods with the MTT assay [26]. Each extracted and reference (respectively) were dissolved in dimethylsulfoxide solutions (DMSO) and artificial seawater (Marine Art SF-1). The solution result was then added to artificial seawater and serially diluted twofold in microplates to give a final concentration range of 0, 512 to 6144 µg/mL for extracts and from 0, 8 to 32 µg/mL for references. The 100 µL of inoculums prepared in marine BHI broth media with a series of extract concentrations were pipetted into each well of the 96-well plate. Then 2 µL of the bacterial cell suspension after 24 h cultured was added to the appropriate wells. The plates were sealed with a sterile plate cover and then with a shaker to mix the content of the wells and incubated at room temperature for 24 h. After that, 10 µL of MTT solutions (5 mg/mL) was given to each tube of wells, followed by incubation in dark conditions for 1 h. After incubation, 100 µL of propanol solution (0.04 M HCl) was added to each well. Finally, the absorbance of the cell suspension was measured by using a microplate reader (Bio-Rad xMark) at 595 nm. Viable bacteria changed the clear yellow, transforming it into turbidity and dark brown. The MIC value is the lowest sample concentration that completely inhibited microbial growth and prevented color change. Each experiment was performed independently 3 times.

Identification of structure

The structure of active compounds in the extract was identified by liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/ MS) (Water Acquity UPLC I-Class and XEVO G2-XS QTOF). Measurement conditions: column (ACQUITY UPLC® BEH C18 1.7 m × 50 mm) with H_2O + 0.1 % of formic acid (FA) as mobile phase (eluent) for solvent A and ACN + 0.1% FA for solvent B. The sample volume injected is 1 µL with a full scan at 100 - 1200 (m/z).

Results and discussion

Characteristics of *P. aeruginosa*

Generally, *P. aeruginosa* has a moving and rod-shaped characteristic, measuring about 0.6×2 μm^2. Generally, these bacteria have polar flagellums, but sometimes 2 - 3 flagellums. Bacteria are Gram-negative and are seen as single bacteria, paired, and sometimes form short chains. The shape morphology of *P. aeruginosa* in artificial marine media is presented in **Figure 1**.

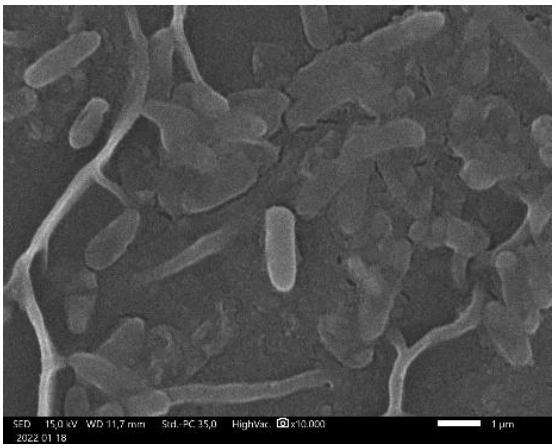


Figure 1 The shape of *P. aeruginosa* (10000×).

P. aeruginosa is a heterotrophic, rod-shaped, motile, gram-negative bacterium between 1 to 5 µm in length and 0.5 to 1.0 µm in width (**Figure 1**). These facultative aerobes reproduce aerobically and anaerobically, using nitrate as the terminal electron acceptor. *P. aeruginosa* can also live anaerobically in the presence of arginine and has a restricted ability for fermentation, which favors very sluggish or no growth. Organisms may utilize over 100 organic compounds as carbon or energy sources. As prototrophs, they can generally develop on a salt-growing media that contains at least 1 energy and carbon source. *P. aeruginosa* thrives optimally at 37 °C but can also live at 4 to 42 °C. PAO1 and PA14 are the 2 most often used laboratory strains that generate genetic resources [27].

Description of *Tinospora cordifolia*

Tinospora is a vine categorized in the Menispermaceae family, which spread in subtropical and tropical regions in Africa and Asia, but there is no information on its spread in Indonesia [28]. This study used *Tinospora cordifolia (T. cordifolia),* as shown in **Figure 2**. The length of the stem can reach 2.5 m, with heart-shaped leaves, the length of the petioles is 7 - 12 cm, has small green flowers. The old stem is browned and has bumps, while the young stem is green and slippery hairless.

Figure 2 The steam of *T. cordifolia*.

More than 65 active chemicals from *Tinospora* plants have been extracted and identified, including furanoditerpene, alkaloids, steroids, lactones, lignans, and flavonoids [28]. According to a review, *Tinospora cordifolia* contained various bioactive chemicals, including steroids, glycosides, alkaloids, and sesquiterpenoids [29]. The plant has antioxidant, antibacterial, and antimicrobial activity.

Extractions

Extraction is the main method for obtaining bioactive chemicals from plant sources. The extraction method is designed to extract the most significant number of target compounds and extracts with the highest bioactive content. The percentage yield of *T. cordifolia* stem extract at various methanol ratios is shown in **Table 1**. The yield of *T. cordifolia* extract was 7.56, 8.40 and 9.45 % at 100, 75 and 50 % methanol ratios, respectively. The results showed that the extraction yield increased with decreasing methanol to water ratios. This decrease could be because the polarity of water higher than methanol, so more polar compounds are soluble in water [30]. The extraction efficiency depends on the extraction time, temperature, extraction method, solvent used, and composition of phytochemicals [29,30]. For example, the extract of *T. crispa* has been claimed that the yield percentage of the sonication method has slightly higher than the maceration method [33]. In their study, the extraction yields were 11 % for water solvent and 3 % for methanol solvent by maceration methods [33]. Meanwhile, the extraction time was chosen 3× 24 h at room temperature in this study, referring to other studies with the same time [32,33]. In addition, the yield extract of *Tinospora* stem obtained in 7 days is not significantly different from 3 days [35].

Table 1 The percentage yield of methanol extract of *T. cordifolia* in various concentrations.

Ratio (methanol: H_2O) (in v/v)	Weight of *T. cordifolia* (g)	Vol. of solutions (mL)	Yield extract of *T. cordifolia* (%)
100:00	25	150	7.56
75:25	25	150	8.40
50:50	25	150	9.45

Total phenolic and flavonoid contents

The influence of methanol ratio on the bioactive compound of *T. cordifolia* extracts was presented in **Table 2**. This investigation revealed a considerable difference in the percentage of bioactive compounds (phenolics and flavonoids) in *T. cordifolia* extracts.

Table 2 The total phenolic and flavonoid contents of *T. cordifolia* stems methanol extract at different concentrations.

Plant	Ratio (Methanol: H_2O)	Total Phenolic Content (%)	Total Flavonoid Content (%)
T. cordifolia	100:00	11.20 ± 0.34	5.25 ± 0.13
	75:25	9.46 ± 0.24	0.64 ± 0.09
	50:50	6.56 ± 0.17	0.33 ± 0.04

The equation generated from the gallic acid reference curve was $y = 0.1032x + 0.0708$; $r^2 = 0.9996$. The total phenolic content (TPC) value is approximately 11.20, 9.46 and 6.56 % for methanol ratios of 100, 75 and 50 %, respectively, as described in **Table 2**. The total phenolic content value slightly decreased with decreasing ratio of methanol percentage. This reduction may be caused by methanol attracting polar and nonpolar compounds, whereas water only draws polar compounds. Our results align with Anwar and Przybylski [36], who found that the total phenolic content (TPC) decreased when the methanol ratio was lower. The phenolic and flavonoid contents also depend on the plant organs [37]. For example, a study reported that the total phenolic content was greater in flowers than in the leaf [38]. In addition, the phenolic content of *Tinospora cordifolia* in methanolic leaf extract (8.51 mg GAE/g) was lower as compared to the methanolic stem extract (17.48 mg GAE/g) [39]. In our study, the total phenolic content produced from *T. cordifolia* stem extract was 11.20 % for a methanol ratio of 100.

The TFC values of *T. cordifolia* stem in various methanol ratios are shown in **Table 2**. Based on the quercetin standard curve, the linear equation is $y = 0.0073x - 0.0802$ with $r^2 = 0.995$. according to the results, the TFC values of the extracts were 5.25, 0.64 and 0.33 % for 100, 75 and 50 % of methanol ratios, respectively. The TFC value of *T. cordifolia* extract was significantly different with different ratios of methanol. The different flavonoid structures found in plant species can alter the antioxidant properties of flavonoids and their TFC value [37].

Methanol was utilized as the solvent in this investigation to extract *T. cordifolia* stems. Methanol has the potential to inhibit the polyphenol oxidase process, which is responsible for phenolic oxidation. Based on total phenolic and flavonoid content, the 100 % methanol ratio was optimal for extracting bioactive components from *T. cordifolia* stems, as it contained the most phenolics (11.20 %) and flavonoids (5.25 %) were obtained by using this solvent. Meanwhile, 50 % of methanolic solvent showed lower efficiency in extracting phenolic and flavonoid compounds with 6.56 and 0.33 % of these compounds, respectively. However, an antibacterial assay is needed to confirm the activity of these bioactive compounds. Evaluation of the antimicrobial activity of the crude extract of *Tinospora crispa* stem showed different activities. The study showed that the *Tinospora crispa* stem methanol extract had the activity against some bacteria, and no activity was shown in the 3 fungal species tested [33].

Antibacterial activity

The antibacterial activity of *T. cordifolia* crude extract was evaluated against the gram-negative biofilm *P. aeruginosa* using the dilution method. **Figure 3** shows that crude extracts of *T. cordifolia* have antibacterial action in the biofilm *P. aeruginosa* tested. *T. cordifolia* extracts with 100, 75 and 50 %

methanol ratios showed bacterial activity against *P. aeruginosa* with a minimum inhibitory concentration (MIC) of around 4096 µg/mL. The bacterium used in the study showed sensitivity to *T. cordifolia* extracts from different methanol concentration ratios. Of the 3 variations in methanol ratio tested, an excess of 50 % methanol showed optimal values. Antibacterial activity may be caused by various active compounds and molecular characteristics in these concentrations. This study showed that *T. cordifolia* extract has an active compound against the biofilm bacterium *P. aeruginosa*. The antibacterial activity of methanol extract is influenced by the properties and number of active compounds (alkaloids, flavonoids, terpenoids, tannins) and the ability of solvents to produce large amounts of active compounds that affect antibacterial activity. The active compounds against *P. aeruginosa* were indicated from the phenolic group but not from the flavonoid group, as shown in **Table 2**.

The results presented in several bar graphs in **Figure 4** clarify that the MIC was obtained from several extract methanol ratios against *P. aeruginosa* bacteria. All extract ratio methanol showed 4096 µg/mL of MIC against *P. aeruginosa* with a different absorbance. The absorbance values of cell suspension at these concentrations are 1920, 1629, and 0.589 for 100, 75 and 50 % of the methanol ratio, respectively. It is confirmed that the 50 % of methanol extract is more effective than the 100 and 75 % of methanol extracts. Our results show that the MIC obtained from the crude extract of *T. cordifolia* is more effective than other studies. On the other hand, the crude extract of *T. cordifolia* exhibits modest effectiveness against *P. aeruginosa*, with average zones of inhibition of 10.27 ± 2.65 mm at 30 mg/disc [40]. His research also stated that the crude extract of *T. cordifolia* had no inhibitory activity against *P. aeruginosa* in minimum bacteria concentration (MBC). Another study discovered that a crude *T. crispa* methanol extract had a minimum inhibitory concentration (MIC) of 100 mg/mL against *S. aureus* and *B. cereus* [33]. Devprakash *et al.* [41] stated that ethanol extract *T. crispa* has antibacterial activity against *P. aeruginosa* and *E. coli*, but its activity is smaller than that of the reference antibiotic levofloxacin. The differences in the antibacterial activity of extracted compounds might be due to concentration, chemical structure and functional groups of the compounds, and the types of bacteria used [42].

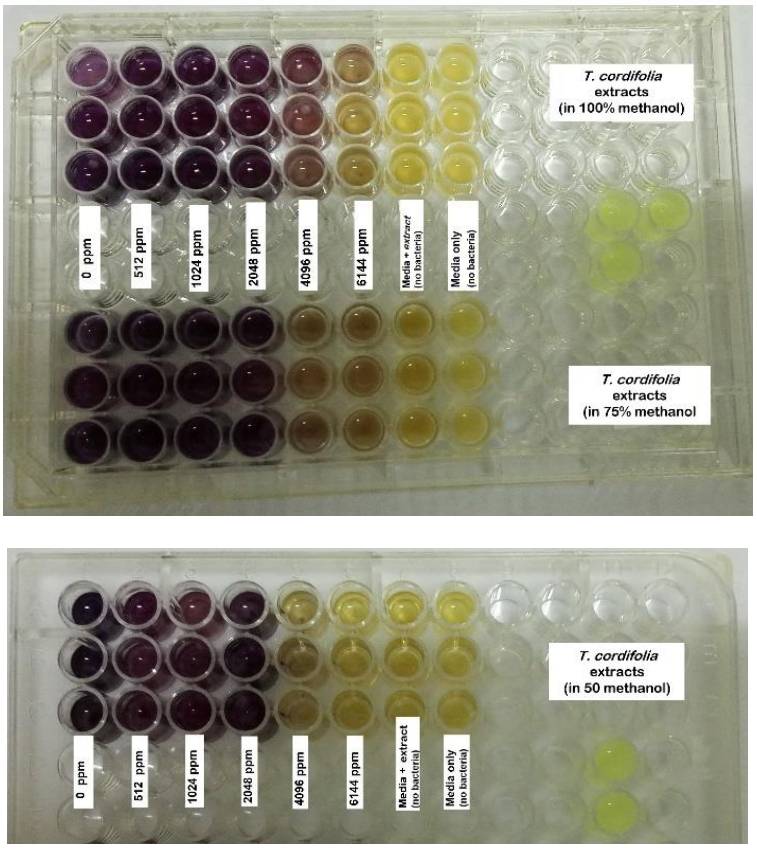
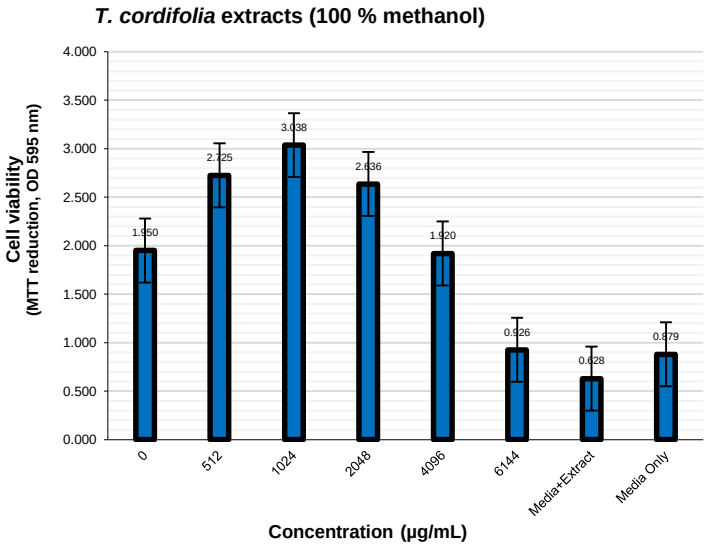
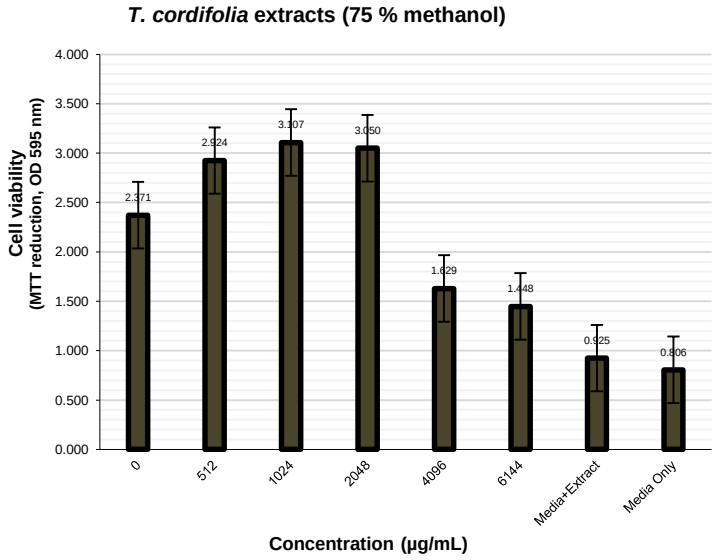


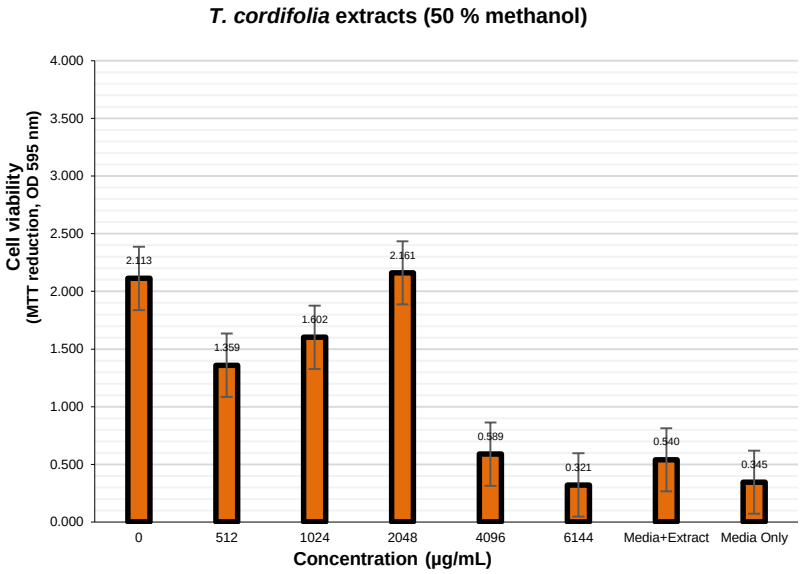
Figure 3 *P. aeruginosa* activity in *T. cordifolia* crude extracts (in 100, 75 and 50 % of methanol).



(a) 100 % methanolic solvent



(b) 75 % methanolic solvent



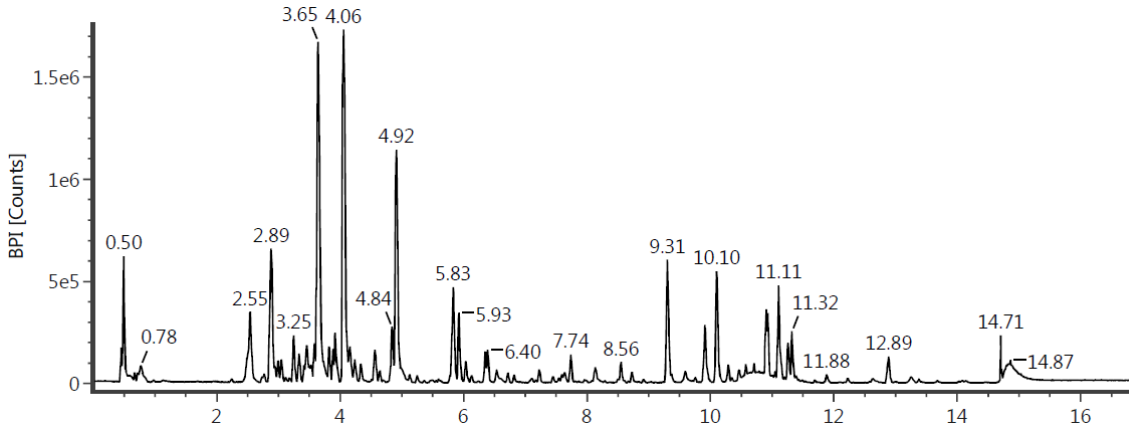
(c) 50 % methanolic solvent

Figure 4 The antibacterial activity of *T. cordifolia* at different concentrations.

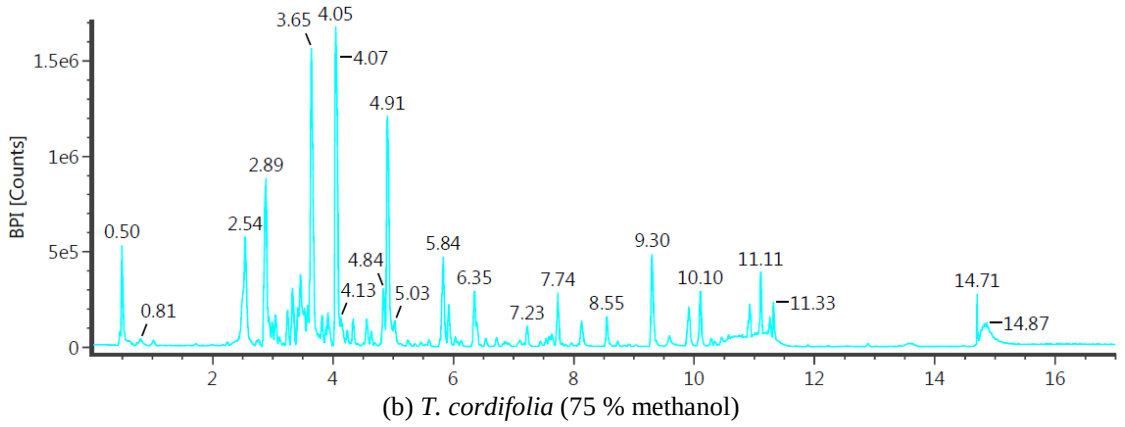
The results (from **Figures 3** and **4**) indicated that 50 % methanol extracts had the highest effectiveness against *P. aeruginosa*, with the lowest MIC value of 4096 µg/mL. This study suggests that the content of *T. cordifolia* stem extract has potent antibacterial properties, so the investigation to prevent corrosion by *P. aeruginosa* must be tried in the subsequent studies. However, the phytochemical and bioactive structure from plant extracts was necessary to investigate the bioactive compounds and study their mechanism of action.

Structure of *Tinospora cordifolia*

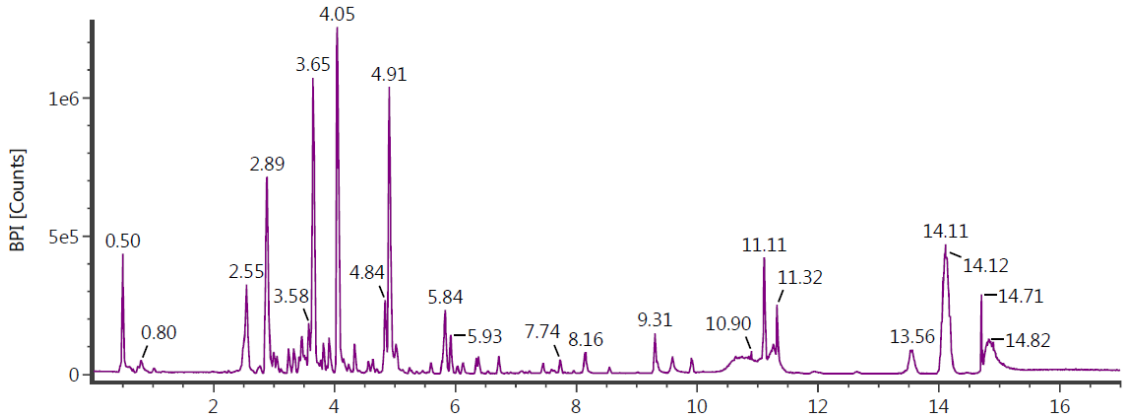
The LC-MS / MS analysis results described the difference in compound content of methanol extract at different ratios. These differences are described by a chromatogram of the peaks of compounds with different molecular weights. The analysis results of molecular weight with LC-MS/MS show that the active compounds are found at 100, 75 and 50 % of methanolic ratios, as shown in **Figure 5**. The bioactive content of all concentration ratios of methanol has the same compounds, namely Calopiptin, d-Lirioferine (Lirioferine), Moupinamide, Piperanine, and Yuanhunine, with a difference in peak intensity (**Figure 5**).



(a) *T. cordifolia* (100 % of methanol)



(b) *T. cordifolia* (75 % methanol)



(c) *T. cordifolia* (50 % methanol)

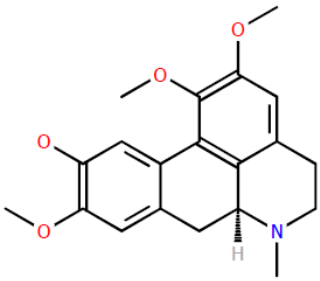
Figure 5 Chromatogram results of *T. cordifolia* at different ratios of methanol to water (in v/v).

From **Figure 5**, there are various compounds with different peak intensity levels. Peak compound was detected at 100 and 75 % methanol ratios, while 50 % was not detected at 8.5 of retention time. Moreover, certain compounds were detected only at 100 % methanol ratio during the retention time of 12.89, but not at 75 and 50 % methanol ratios. In the contras, there are active compounds with high peak intensities only at a methanol ratio of 50 %, specifically at retention times 13.56 and 14.11. The difference in the presence of these compounds may be caused by the different polarities of the extracted active compounds. Compounds at 50 % methanolic extract may be more polar than compounds at 75 and 100 % methanol extracts. Meanwhile, compounds are only found at 100 % methanol; this compound tends to have lower polarity than other compounds. This difference is because the water solvent is more polar than the methanol solvent [42,43].

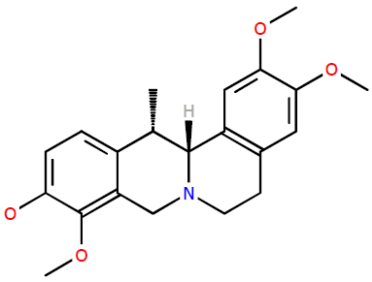
The active structures could be obtained from the chromatogram at a certain retention time (RT) with different amounts (peaks). For example, the Moupinamide compounds ($C_{18}H_{19}NO_4$) were obtained at a retention time of about 4.06 min with varying peak heights for each methanol concentration ratio. Detailed results of the main active compounds of *T. cordifolia* extract at different methanol ratios are presented in **Table 3**, and the structure of *T. cordifolia* compounds is in **Figure 6**.

Table 3 The characteristics and formula of *T. cordifolia* at different ratios of methanol to water.

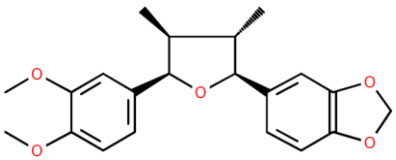
***T. cordifolia* crude Extracts**	**Component name**	**Formula**	**Identification status**	**Observed (m/z)**	**Neutral mass (Da)**	**Observed RT (min)**	**Detector counts**
100 % of methanol (v/v)	d-Lirioferine (Lirioferine)	$C_{20}H_{23}NO_4$	Identified	342.1694	341.16271	2.89	889508
	Yuanhunine	$C_{21}H_{25}NO_4$	Identified	356.1851	355.17836	3.65	1824893
	Moupinamide	$C_{18}H_{19}NO_4$	Identified	314.1390	313.13141	4.06	1787796
	Calopiptin	$C_{21}H_{24}O_5$	Identified	357.1695	356.16237	4.92	1204290
	Piperanine	$C_{17}H_{21}NO_3$	Identified	310.1438	287.15214	5.84	517534
75 % of methanol (v/v)	d-Lirioferine (Lirioferine)	$C_{20}H_{23}NO_4$	Identified	342.1695	341.16271	2.88	1111327
	Yuanhunine	$C_{21}H_{25}NO_4$	Identified	356.1853	355.17836	3.65	1735570
	Moupinamide	$C_{18}H_{19}NO_4$	Identified	314.1386	313.13141	4.06	1659976
	Calopiptin	$C_{21}H_{24}O_5$	Identified	357.1700	356.16237	4.92	1247302
	Piperanine	$C_{17}H_{21}NO_3$	Identified	310.1442	287.15214	5.84	522899
50 % of methanol (v/v)	d-Lirioferine (Lirioferine)	$C_{20}H_{23}NO_4$	Identified	342.1699	341.16271	2.89	965569
	Yuanhunine	$C_{21}H_{25}NO_4$	Identified	356.1856	355.17836	3.66	1092196
	Moupinamide	$C_{18}H_{19}NO_4$	Identified	314.1389	313.13141	4.06	1209341
	Calopiptin	$C_{21}H_{24}O_5$	Identified	357.1694	356.16237	4.92	1080507



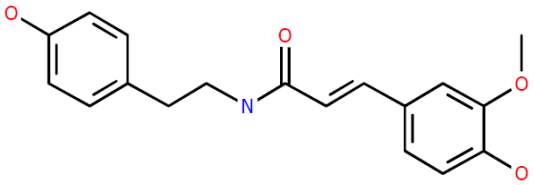
(a). d-Lirioferine (Lirioferine)



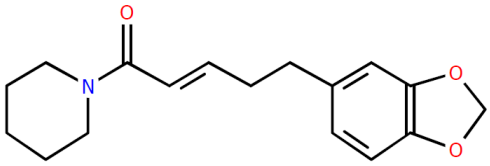
(b). Yuanhunine



(c). Calopiptin



(d). Moupinamide



(e). Piperanine

Figure 6 Structures of *T. cordifolia* compounds.

The development of several bioactive compounds from some plants could be used for various purposes both in the medical field and other fields. Due to the bioactive content in the *T. cordifolia* plant, this plant has the potential as an antioxidant and antimicrobial. *T. cordifolia* is made up of a large number of secondary metabolites. Numerous research on the contents of *T. crispa* has resulted in the isolation and identification of over 65 compounds, including terpenoids, phenolics, furanoditerpen, steroids, lactones, flavonoids, alkaloids, and lignans [44,45]. In our study, the results of structure compounds from *T. cordifolia* extract are a phenolic aromatic group in derivative alkaloid compounds with N bonds, as shown in **Figure 6**.

Although several studies have been performed to reveal the antibacterial activity of *T. cordifolia* in the medical field, it is still very rarely used to prevent corrosion problems caused by microorganisms. So for the next study, further scientific-based research is needed to explore the chemical characterization and pharmacological evaluation of *T. cordifolia* to prevent bacteria from causing biofilms and corrosion of metals.

Conclusions

Preliminary studies have been conducted on *T. cordifolia* stem extracts at various methanol ratios against *P. aeruginosa* biofilm. The extract yields are 9.45, 7.56 and 8.40 % at 100, 75 and 50 % methanol ratios. The total phenolic content (TPC) in the crude methanol extract of *T. cordifolia* is 11.20, 9.46 and 6.56 % for 100, 75 and 50 % of methanol concentrations, respectively. Meanwhile, the flavonoid content (TFC) was obtained at 100, 75 and 50 % of methanol concentrations is about 5.25, 0.64 and 0.33 %, respectively. The *T. cordifolia* extract has antibacterial activity against *P. aeruginosa* in all ratios of methanol concentrations. The minimum inhibitory concentration (MIC) of *T. cordifolia* methanol extract was found at 4096 µg/mL. The effectiveness of the antibacterial activity against *P. aeruginosa* was as follows: 50 % > 75 % > 100 %. The results of the structure compounds from *T. cordifolia* extract are a phenolic aromatic group in the form of derivative alkaloid compounds. The structures of active compounds in *T. cordifolia* methanol extract are Calopiptin, d-Lirioferine (Lirioferine), Moupinamide, Piperanine, and Yuanhunine with formulas $C_{21}H_{24}O_5$, $C_{20}H_{23}NO_4$, $C_{18}H_{19}NO_4$, $C_{17}H_{21}NO_3$, and $C_{21}H_{25}NO_4$, respectively. The next process, such as fractionation and purification, must be done to obtain active materials that play a role against *P. aeruginosa* bacteria biofilms before application to control microbiologically influenced corrosion.

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