BIODIVERSITAS
Volume 26, Number 6, June 2025
Pages: 3071-3079

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260650

Detection of antimicrobial compounds from *Parmotrema tinctorum* lichen from lead contaminated locations

WAHYU AJI MAHARDHIKA[1], LISDAR IDWAN SUDIRMAN[2,♥], SETYANTO TRI WAHYUDI[3]

[1]Program of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Bogor 16680, West Java, Indonesia

[2]Division of Mycology, Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8622642, ♥email: lisdarma@apps.ipb.ac.id

[3]Division of Biophysic, Department of Physics, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Bogor 16680, West Java, Indonesia

Manuscript received: 1 March 2025. Revision accepted: 13 June 2025.

Abstract. *Mahardhika WA, Sudirman LI, Wahyudi ST. 2025. Detection of antimicrobial compounds from* Parmotrema tinctorum *lichen from lead contaminated locations. Biodiversitas 26: 3071-3079. Parmotrema tinctorum* lichen is found in various locations and can be used as a tolerant bioindicator. Based on previous research, it is known that there is a high content of lead (Pb) in the thallus of *P. tinctorum* found in the Mekarsari Fruit Garden. The aim of this study was to determine the relationship between lead concentration and the activity of antimicrobial compounds from thallus *P. tinctorum*. Thallus samples were collected from a Pb-contaminated sites, followed by analysis of Pb content. The thalli were then extracted and tested for antimicrobial activity, followed by partial purification and characterization of the antimicrobial compounds. The highest Pb content was found in zone 3 in Mekarsari (MZ3), amounting to 15.64 ppm, followed by zone 2 (MZ2) with 14.72 ppm, zone 1 (MZ1) with 12.86 ppm, and at Mangrove Batu Bedhil (MBB) as a control with 3.42 ppm. The crude extract was able to inhibit all test microbes, and its activity against *Propionibacterium acnes* showed a significantly different result compared to its activity against the other microbes. In the dilution test of the extract up to 10^4 against *P. acnes*, the MZ3 extract still produced a clear zone, whereas the other extracts showed no activity. The results of TLC-bioautography showed that the MZ3 crude extract had an Rf value of 0.52-0.81, and MBB had an Rf value of 0.68-0.91. The antibacterial test of crude extract and preparative fractions of MZ3 and MBB was able to inhibit *Bacillus subtilis* G and *P. acnes,* however, there was a decrease in antimicrobial activity, presumably due to Pb being adsorbed onto the adsorbent during preparative TLC. Further research is needed to re-evaluate the appropriate adsorbent for purification of active compound containing lead.

Keywords: Antimicrobial activity, lead, *Parmotrema tinctorum*, *Propionibacterium acnes*, TLC-Bioautography

Abbreviations: MZ1: Mekarsari Zone 1, MZ2: Mekarsari Zone 2, MZ3: Mekarsari Zone 3, MBB: Mangrove Batu Bedhil, AA: Antibacterial Activity, AU: Arbitrary Unit, CEMZ3: Crude Extract Mekarsari Zone 3, CEMBB: Crude Extract Belitung Mangrove, PFMZ3: Preparative Fraction Mekarsari Zone 3, FPMBB: Preparative Fraction Mangrove Batu Bedhil

INTRODUCTION

Health problems are one of the important issues to consider in the world. One of these is infections caused by pathogenic bacteria and fungi. Pathogenic bacteria that cause infections, include *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes*, while pathogenic fungi in humans include *Candida albicans, Candida tropicalis, Malassezia furfur, Rhodotorula mucilaginosa*, and *Trichophyton* spp. (Panayidou et al. 2014). These problems can range from mild to severe and can even lead to death. One of the efforts to tackle microbial infection problems is the use of antibiotics. To this day, researchers continue to search for sources of antibiotic-producing compounds from living organisms, one of which is lichen.

Lichens are organisms formed from a symbiosis between algae and/or cyanobacteria with fungi. Lichens can live on tree branches, rocks, and shrubs, and are often used as bioindicators (Gupta et al. 2016; Ellis et al. 2021). Lichens are sensitive to air pollution, which can include heavy metals released by motor vehicles and industries. The presence of heavy metals can affect the growth of especially sensitive lichens. Besides being used as bioindicators, lichens can also be sources of bioactive compounds used for medicine and antibiotics. *Parmotrema tinctorum,* a foliose lichen found in various parts of the world, can act as a bioindicator (Jayalal et al. 2013). This lichen contains bioactive compounds that can be utilized by humans. Besides their role as bioindicators, lichens are a source of bioactive compounds that can be used as medicines and antibiotics. Micheletti et al. (2021) demonstrated that extracts from various lichens, including *Cladonia borealis, Cladonia confusa, Cladonia crispatula, Cladonia furcata, Punctelia canaliculata, Punctelia lichexanthonicum, Pseudoparmelia sphaerospora, Ramalina anceps, Stereocaulon ramulosum, Usnea jamaicensis, Canoparmelia cryptochlorophaea,* and *Concamerella pachyderma*, can inhibit the growth of bacteria, such as*, Enterococcus faecalis, Enterococcus faecium, S. aureus*, and *E. coli.*

P. tinctorum, a foliose lichen that contains bioactive compounds beneficial to humans (Safitri et al. 2020).

Several researchers have proven that *P. tinctorum* has the ability to inhibit plant pathogenic fungi, such as *Colletotrichum* sp., *Fusarium oxysporum* f. sp. sesame, and *Pycularia oryzae* (Tuan et al. 2020). In addition to being antimicrobial, *P. tinctorum* has been shown to have antioxidant activity and the ability to inhibit Aldose Reductase (AR) and carbohydrate-digesting enzymes, it can also be used as functional food for diabetic patients (Salin Raj et al. 2014). Shiromi et al. (2021) indicated that hexane and ethanol extracts of *P. tinctorum* were active against MSSA and MRSA with weak antimicrobial activity. Additionally, *P. tinctorum* extracts show potential as antioxidants. The methanol extract of *P. tinctorum* collected from Bukit Larut, Perak, Malaysia, did not exhibit antibacterial activity against *E. coli* and *B. subtilis* (Rajan et al. 2016).

Lichens can serve as environmental bioindicators by absorbing heavy metals. According to Bačkor and Loppi (2009), heavy metals can affect photosynthesis, respiration, and metabolism in lichens. Some algal cells may shrink due to heavy metal exposure, although these metals do not significantly affect the mycobiont. The presence of heavy metals in the thallus can cause changes in color and size in *Usnea diffracta* and *P. tinctorum* (Zulaini et al. 2019). Port et al. (2018) also demonstrated that contamination can alter photobiont content and chlorophyll pigments in the thallus of *P. tinctorum*.

Mekarsari fruit garden is one of the largest fruit gardens located in Bogor, Indonesia. The garden is situated between a highway and industrial factories. Safitri et al. (2020) reported that the level of Pb contamination in the thallus of *P. tinctorum* at Mekarsari Garden ranges from 13.34 to 24.87 ppm at 2019-2020, indicating that the area experienced high levels of Pb pollution and contamination. The study by Noori et al. (2012) proved that heavy metals could enhance the antibacterial activity of *Verbascum speciosum* Schrad. This is suspected to also influence the antimicrobial activity of *P. tinctorum.* The relationship between heavy metal content in *P. tinctorum* thallus and its antimicrobial activity has not been studied or reported to date. The aim of this research was to test the antimicrobial activity of *P. tinctorum* from a Pb-contaminated site against pathogenic bacteria and fungi, followed by characterization of the active compounds.

MATERIALS AND METHODS

Study area

Parmotrema tinctorum samples were collected from Mekarsari Fruit Garden, Cileungsi, Bogor (6.416°S 106.984°E), from three zones: MZ1, MZ2, MZ3, and from the Mangrove Forest at Batu Bedhil Beach, East Belitung Island (MBB), a collection of Prof. Dr. Ir. Lisdar I. Sudirman. MZ1 is the area near the entrance and exit, and is also close to the main road and residential housing. MZ2 is near guava, durian, and palm plantations and is bordered by two industries and densely populated residential areas. MZ3 is an area of durian, palm, starfruit, jackfruit etc. plantations, and bordered by a main road, residential housing, and six industries. Lichen samples were then stored in paper envelopes and transported to the Mycology Laboratory, Institut Pertanian Bogor, Bogor, Indonesia, for drying and extraction.

Procedures

Extraction of bioactive compounds

A total of 9 g of *P. tinctorum* dried thallus was ground using a blender. The resulting powder was transferred to a 250 mL Erlenmeyer flask containing 90 mL of methanol. The mixture was shaken using a rotary shaker at 150 rpm for 24 hours. Next, the supernatant was separated using fritted glass and vacuum pump. The sample filtrate was then evaporated using a rotary evaporator at 40°C. The sample powder was then re-extracted for 24 hours and filtered using a vacuum pump (Kambar et al. 2014; Kekuda et al. 2015).

Lead content analysis

Lead content in the lichen thallus was analyzed using the wet digestion process. The analysis was conducted at the Integrated Laboratory of IPB Baranangsiang. 1 g of thallus was cleaned of impurities and crushed into a fine powder. The sample was dissolved using HNO_3 (nitric acid) and HCl (Hydrochloric acid) heated until the thallus dissolved and the solution became clear. The lead content was then measured using Atomic Absorption Spectrophotometry (AAS) at a wavelength of 283.3 nm (Safitri et al. 2020; Khairunnisa et al. 2021).

Microbial preparation for testing

The bacteria used for antibacterial testing included *B. subtilis*, *P. acnes*, *S. epidermidis*, and *E. coli* (collected from Prof. Lisdar A. Manaf). *E. coli* was cultivated on solid Luria Bertani (LB) media, *B. subtilis* on solid TGY (Tryptone Glucose Yeast) media, *P. acnes* and *S. epidermidis* on NA (Nutrient Agar) media. All cultures were incubated for 24 hours at 37°C. A loopful of bacteria was inoculated into liquid media (LB Broth, TGY Broth, and Nutrient Broth) with a volume of 5 mL and incubated using a shaker for 24 hours. Antifungal test included *C. albicans*, *C. tropicalis*, and *R. mucilaginosa*. The fungi were inoculated on PDA (Potato Dextrose Agar) media and incubated at room temperature (27-29°C) for 24 hours. One loopful fungus was then inoculated into 5 mL of SDA (Sabouraud Dextrose Broth) for subsequent antifungal test and incubated using shaker for 24 hours at 27°C.

Antimicrobial activity screening

Antibacterial activity test of the lichen extract was conducted using the Kirby-Bauer method according to Sudirman (2010). The crude extract (60.000 ppm) was applied to 6 mm diameter paper discs until it was saturated. For the antibacterial test, 30 µg of chloramphenicol was used as positive control, for antifungal test, 50 µg of ketoconazole was used, and methanol was used as the negative control. Before use in the test, the paper discs were sterilized using UV light at 254 nm for 1 hour and then placed onto each test medium. A total of 1 mL of the test microbial suspension was inoculated into 100 mL test media, which contained 0.75% agar, each treatment was

repeated three times. Petri dishes containing the treatments were first incubated at 10°C for 30 minutes to allow the extract to absorb. All Petri dishes containing test bacteria (NA, TGYA, and LBA) were incubated at 37°C for 24 hours, while those containing fungi (SDA) were incubated at room temperature (27-29°C). The inhibition zone diameter was observed and measured, subtracting the paper disc diameter, to determine the antimicrobial activity of each crude extract (Sudirman 2005; Nurliana et al. 2008).

Antimicrobial activity based on extract dilution

The antimicrobial activity assay was conducted using a serial dilution method, referring to Liu et al. (2015) and Lei et al. (2020). This assay aimed to validate the antimicrobial activity based on varying Pb content in each extract. The crude extract was dissolved in methanol to obtain a concentration of 10,000 ppm, followed by serial dilutions (10^1, 10^2, 10^3, 10^4). Each dilution was applied onto a 6 mm diameter paper disc. The antimicrobial assay using the dilution method was performed following the Kirby-Bauer technique. Petri dishes containing the treatments were incubated at 37°C for 24 hours, and the inhibition zones formed at each dilution level were observed.

Detection of antimicrobial compounds by TLC-Bioautography

TLC-Bioautography method is used to identify compounds that exhibit antimicrobial activity. Crude extract was applied at 3 cm from the base of analytical TLC plate with silica gel absorbent (Kieselgel G, No 6 F254). The TLC plate was then placed in a chamber with a solvent system of n-butanol, acetic acid, and distilled water in a 3:1:1 (v/v/v) ratio. The plate was observed under UV light at wavelengths of 254 and 366 nm. Bioautography was performed by placing the TLC plate in a sterile incubation box containing a moistened tissue, then coating it with 15 mL of culture media containing *B. subtilis.* Only *B. subtilis* was used in TLC-Bioautography, since colonies of this bacterium were more clearly visible on the TLC plate than those of the other test microorganisms. The plate was incubated at 10°C for 2 hours, followed by 24 hours of incubation at 35°C. Inhibition zones were observed, and the Rf value was calculated by dividing the distance of the clear zone by the distance of the solvent front from the starting point (Sudirman 2005).

Partial purification of active antimicrobial fractions

Partial purification was carried out using preparative TLC with silica gel absorbent (Kieselgel G, MERCK No. 7730.1) at a 50% concentration (Sudirman 2010). A total of 10 µL of crude extract was applied along the silica gel plate and migrated using the same solvent system as in analytical TLC. Active fractions, based on the Rf value from bioautography results, were harvested and extracted using methanol.

Antibacterial activity test of preparative fractions

Antibacterial activity test was conducted in the same way as the crude extract test. This step was only performed on *B. subtilis* and *P. acnes*. Preparative fractions and crude extract, which were used as a comparison, were applied to 6 mm diameter paper discs in increments until saturated while drying with a hairdryer. 30 µg of chloramphenicol as the positive control, and methanol as the negative control.

Absorbance pattern detection of compounds

The absorbance patterns of the crude extract and preparative fraction were detected using a UV-Vis spectrophotometer. The wavelength range used was 200-500 nm (Krisnawati 2018).

Data analysis

The parameters observed were the antimicrobial inhibition zones of the crude extract. Data for each parameter were analyzed using ANOVA, followed by Duncan's multiple range test at a significance level of 5%, with a Completely Randomized Design (CRD) model.

RESULTS AND DISCUSSION

Analysis of Pb contents in thalli

Based on the analysis of *Parmotrema tinctorum* thallus collected from Mekarsari Fruit Garden and the Belitung mangrove forest, all samples were found to contain lead (Pb) (Table 1). The lead content of MZ3 was higher than in the other two zones. This may be because Zone 3 was closer to six industries, highways, and residential areas. MZ2 was near two industries, residential areas, and a highway. MZ1 was located near the highway and the entrance to the Mekarsari fruit garden. The Pb content at the Batu Bedhil Belitung mangrove site (MBB) was lower than that in Mekarsari Fruit Garden, because Belitung Beach was far from residential areas, industries, and highways.

Table 1. Lead (Pb) content in *Parmotrema tinctorum* thalli

Zones	Detail of locations	Pb concentrations (ppm)
MZ1	Gate of Mekarsari, animal and plant garden, highway, residential areas	12.86
MZ2	Lake, garden (durian, palm, guava, coconut, rambutan), highway, residential area, two industries	14.72
MZ3	Garden (durian, palm, starfruit, jackfruit), highway, residential area, six industries	15.64
MBB	Mangrove, beach	3.42

Antimicrobial activity test

Based on the antibacterial test *P. tinctorum* lichen extract was able to inhibit the growth of pathogenic microbes (*E. coli*, *B. subtilis*, *P. acnes*, and *S. epidermidis* and *C. albicans*, *C. tropicalis*, and *R. mucilaginosa*), evidenced by clear zones around the discs (Figure 1 and Table 2). Lichen samples taken from the Belitung mangrove forest (MBB) exhibited lower activity with lower Pb contents compared to those from Mekarsari Fruit Garden. It may be related to lower lead contents in MBB, then higher Pb contents in Mekarsari Fruit Garden, which also had higher Pb content. Based on statistical analysis using ANOVA, the antimicrobial test results showed a significant difference between Mekarsari Fruit Garden, which has a higher Pb content, and MBB, which has a lower Pb content.

Antimicrobial activity based on extract dilution

The antimicrobial activity tests of diluted extracts against *P. acnes* (Table 3). *P. acnes* was selected based on the results of the antimicrobial activity screening, in which this bacteria exhibited a significantly larger and distinct inhibition zone compared to others. Crude extract of MZ3, with a Pb concentration of 15.64 ppm, a clear zone was observed up to a dilution of 10^{-4}. However, MZ2's crude extract, with a Pb concentration of 14.72 ppm, the clear zone was only observed up to 10^{-3}. Similarly, in MZ1's crude extract, with a Pb concentration of 12.86 ppm, the clear zone formed up to 10^{-2}. Meanwhile, in MBB's crude extract, which had the lowest Pb concentration of 3.42 ppm, the clear zone was only observed up to a dilution of 10^{-1}. Based on antimicrobial activity units (AU/mL), the MZ3 extract had the highest value. This test supports the hypothesis that heavy metal concentration influences the antibacterial activity of compounds in *P. tinctorum* thallus.

Detection of antimicrobial compounds using TLC-Bioautography

The detection of antimicrobial compounds in crude *P. tinctorum* extracts from all samples showed inhibition zones marked by clear zone (Figure 3) activity against *B. subtilis*. Only *B. subtilis* was used in TLC-Bioautography, since colonies of this bacterium were more clearly visible on the TLC plate than those of the other test microorganisms. All extract had same Rf value. MZ1 crude extract ranged from 0.47 to 0.81, for MZ2 crude extract from 0.51 to 0.86, for MZ3 crude from 0.52 to 0.81, and for MBB from 0.68 to 0.91. Based on the lead content and antimicrobial activity, crude extracts from MZ3 and the MBB were selected for partial purification.

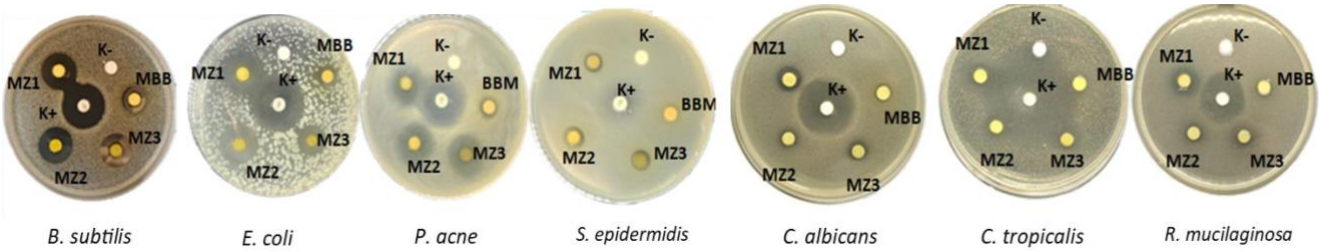

Figure 1. Antimicrobial activity based on inhibition zone of *Parmotrema tinctorum* against pathogenic bacteria and fungi

Table 2. Antimicrobial activity based on inhibition zone of crude extracts of *Parmotrema tinctorum* thallus against pathogenic microbes

Parmotrema tinctorum extracts	Antimicrobial activity (mm)						
	B. subtilis	***E. coli***	***P. acnes***	***S. epidermidis***	***C. albicans***	***C. tropicalis***	***R. mucilaginosa***
MZ1	12.42±0.04^{b}	13.17±0.06^{a}	11.52±0.22^{c}	3.01±0.10^{a}	5.24±0.11^{a}	6.76±0.45^{a}	7.10±0.73^{ac}
MZ2	12.42±0.03^{b}	11.03±1.13^{ab}	12.25±0.08^{b}	6.73±0.31^{b}	3.07±0.62^{c}	7.15±0.44^{b}	5.50±0.40^{b}
MZ3	12.77±0.15^{a}	8.78±1.16^{b}	13.12±0.12^{a}	6.80±0.34^{b}	4.86±0.33^{b}	8.59±1.68^{c}	7.38±0.39^{c}
MBB	7.72±0.06^{c}	5.33±1.01^{c}	3.45±0.06^{d}	1.66±0.43^{c}	2.81±0.59^{d}	5.92±0.15^{d}	3.20±0.53^{d}
Positive controls	23.17±0.01^{d}	27.33±0.02^{d}	25.83±0.02^{d}	13.83±0.01^{d}	28.00±0.00^{e}	45.67±0.03^{e}	21.33±0.01^{e}

Note: Numbers in the same column followed by the same letter are not significantly different according to Duncan's test (α=0.05). Data are the mean values from n = 3. Positive control using chloramphenicol 30 µg for bacteria and ketoconazole 30 µg for fungi

Table 3. Antibacterial activity of *Parmotrema tinctorum* extract against *Propionibacterium acnes*

***Parmotrema tinctorum* extracts**	**Antibacterial activity (inhibition zone, mm and AU/mL)**							
	10-1		**10^{-2}**		**10^{-3}**		**10^{-4}**	
	AA (mm)	**AA (AU/mL)**	**AA (mm)**	**AA (AU/mL)**	**AA (mm)**	**AA (AU/mL)**	**AA (mm)**	**AA (AU/mL)**
MZ1	10.54	8.78	4.30	3.58	0.00	0.00	0.00	0.00
MZ2	11.49	9.57	6.80	5.67	3.15	2.62	0.00	0.00
MZ3	12.23	10.19	8.66	7.21	6.41	5.34	3.42	2.85
MBB	8.51	7.09	0.00	0.00	0.00	0.00	0.00	0.00

Antibacterial activity of crude extracts and preparative fractions

Antibacterial tests revealed both the crude extracts and preparative fractions from MZ3 and MBB had the activity against *P. acnes* and *B. subtilis* (Table 4). However, the preparative fractions formed smaller inhibition zones compared to the crude extracts.

Absorbance pattern detection of extracts

The absorbance patterns of crude extracts and preparative fractions showed varying peak wavelengths (Table 5), ranging from 204-410 nm. The peaks and wavelengths of the crude extracts did not differ significantly, except for the preparative fractions, which had a single peak at 366 nm (MZ3) and 367 nm (MBB). Based on UV-Vis spectrophotometer results, a similar pattern was observed in the crude extracts of *P. tinctorum* from Mekarsari and MBB. However, after preparative fractionation, the peak pattern disappeared. Based on the detection of absorbance patterns using a UV-Vis spectrophotometer, several peaks were observed, showing distinct patterns among the crude extracts of lichen samples. MZ3 had more peaks compared to other zones. Crude extracts of MZ3 and MBB were subjected to preparative TLC. The preparative fractions were then analyzed for their absorbance patterns using a UV-Vis spectrophotometer. The results showed that the peaks and patterns were significantly different from the crude extracts, with the appearance of new peaks. This is presumed to be due to several compounds present in the crude extract that produced peaks being partially removed during the partial purification process.

Table 4. Antibacterial activity from selected crude extract and preparative fractions

***Parmotrema tinctorum* extracts**	**Inhibition zone (mm)**	
	Bacillus subtilis	***Propionibacterium acne***
CEMZ3	9.84	14.44
CEMBB	4.85	3.68
PFMZ3	2.60	2.32
PFMBB	1.40	0.00
Chloramphenicol 30 µg	16.59	10.06

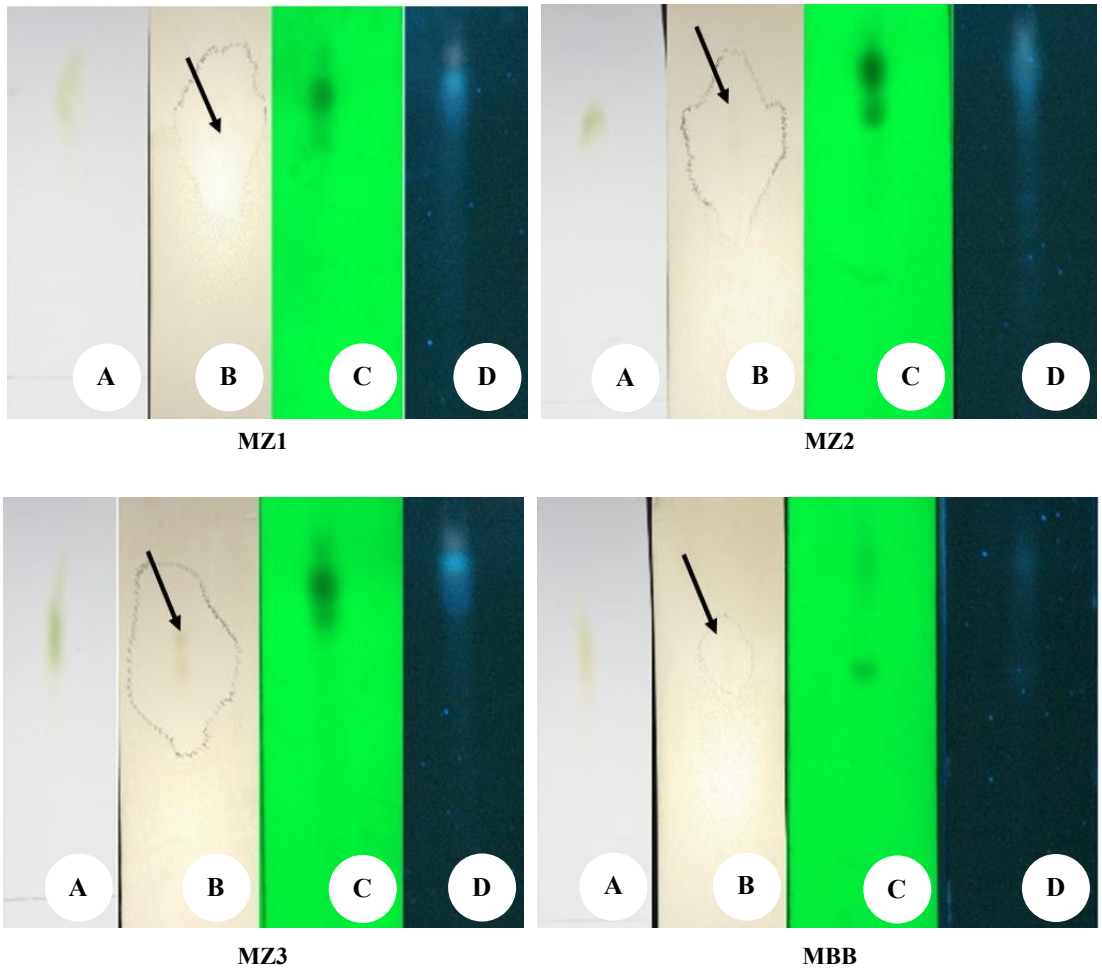

Figure 3. Detection of antimicrobial compounds in *Parmotrema tinctorum* extracts against *Bacillus subtilis*. A. Spot on analytical TLC, B. TLC-bioautography, arrows: inhibition zones, C. Detection fluorescent spots on UV 254 nm, D. Detection fluorescent spots on UV 366 nm

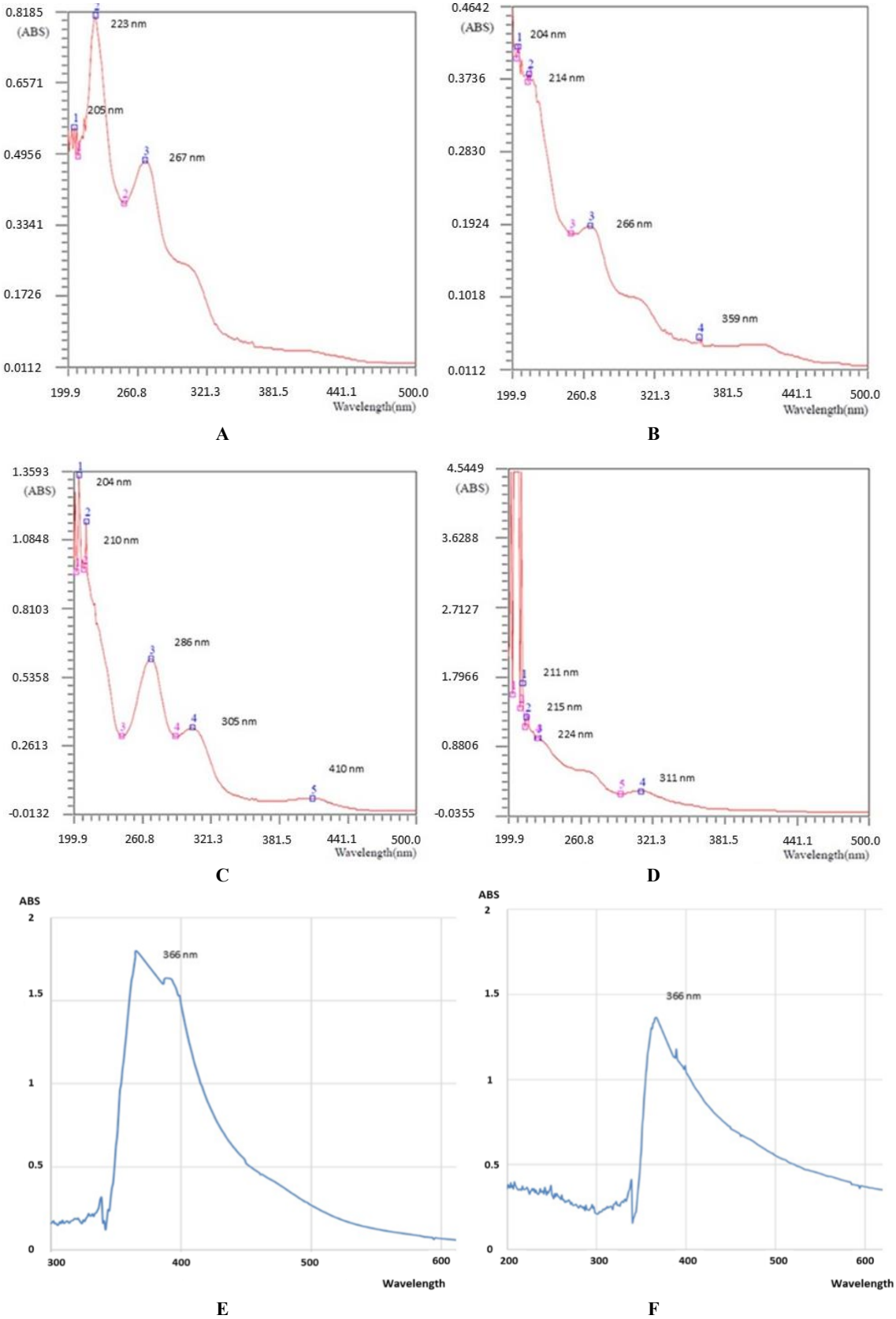

Figure 3. Pattern absorbance of crude extract and preparative fractions detected from 200-500 nm. A. MZ1, B. MZ2, C. MZ3, D. MBB, E. Preparative MZ3, F. Preparative MBB

Discussion

Based on the research conducted, *P. tinctorum* collected from Pb-contaminated environments exhibited greater antimicrobial activity compared to previous studies (Rajan et al. 2016; Swamy et al. 2016; Shiromi et al. 2021). In this study, the methanol extract of *P. tinctorum* showed significant activity against both test bacteria. Based on the results obtained, *P. acnes*, *S. epidermidis*, and *C. tropicalis* showed significantly different results between zones, with *P. acnes* exhibiting the largest clear zone. These three microbes represent the relationship between Pb concentration in each sampling zone and their corresponding antimicrobial activity. Rajan et al. (2016) demonstrated that methanol extract of *P. tinctorum* collected from Bukit Larut, Perak, Malaysia, did not exhibit antibacterial activity against *E. coli* and *B. subtilis*. Previous studies on chemical compounds from *P. tinctorum* have proven that lichen has antibacterial activity against *Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa, Proteus vulgaris, Shigella boydii, S. aureus*, and *Salmonella enterica* using various solvents; however, its activity remains relatively weak (Swamy et al. 2016). Another study reported that *P. tinctorum* extract exhibited low activity against MSSA and MRSA (Shiromi et al. 2021). Based on the literature reviewed, there are no previous reports detailing the lead (Pb) content in *P. tinctorum*. This study is the first to investigate the correlation between Pb content in the thallus and its antimicrobial properties. It is recommended that future researchers analyse the Pb contents in lichen thalli to validate these findings.

Based on the analysis of Pb content in lichen talus at the Mekarsari Fruit Garden, there has been a decrease in Pb content compared to the previous year. The decrease occurred in all three zones: MZ1, MZ2, and MZ3. According to Safitri et al. (2020), lead content in *P. tinctorum* thalli from Mekarsari Fruit Garden during 2018-2019 varied by zone. MZ1, located near the entrance and far from industrial factories, had a Pb concentration of 13.34 ppm, while MZ2 and MZ3 had concentrations of 24.87 ppm and 23.61 ppm, respectively. Lead content analysis in 2022 showed a decrease in Pb levels in *P. tinctorum* thalli, likely due to reduced industrial activities during the COVID-19 pandemic, which affected lead contamination accumulation. *P. tinctorum* from MBB was used as a lower Pb control compared to Mekarsari. However, Pb was present in its thalli. Finding *P. tinctorum* thalli with minimal or no Pb content is challenging due to the widespread contamination of Pb through soil, water, and air. Pb contamination can arise from various sources, including volcanic activity, motor vehicles, factories, mining, irrigation, wastewater treatment, and agro-industrial wastes (Bouida et al. 2022). Pb levels may influence *P. tinctorum* thallus populations. Safitri et al. (2020) demonstrated that thalli in Pb-exposed zones were more abundant than those in zones with lower Pb concentrations. Therefore, future studies are expected to examine *P. tinctorum* with lower Pb levels or thalli free from Pb pollution.

Antimicrobial activity was further evaluated through extract dilution assays to test the hypothesis that the concentration of lead (Pb) in the extract influences its antimicrobial efficacy. Dilution aims to reduce both the concentration of the extract. Dilution also aims to reduce the concentration of lead (Pb), which is one of the heavy metals that is toxic to microorganisms. Based on the results obtained, the crude extract of MZ3 retained antimicrobial activity even at the highest dilution (10^4). This is supported by the high Pb concentration in MZ3, which contrasts sharply with MBB, where the Pb level was the lowest and only a clear zone was observed at a 10^1 dilution. This finding serves as an indicator that heavy metals such as lead (Pb) may have an effect on antimicrobial activity. Heavy metals are known to have oligodynamic effects, meaning they can inhibit microbial growth even at low concentrations. Several heavy metals such as Pb, Fe, Zn, Cu, Bi, Au, and Ag are known to inhibit the growth of pathogenic microbes (Mittapally et al. 2018). Sukul and Sukul (2004) explained that high dilution can affect the physical and biochemical properties of a compound. Roque et al. (2023) demonstrated that the effect of dilution can either increase or decrease the concentration of a substance. These findings strongly support the influence of Pb content in *P. tinctorum* thalli on its antimicrobial activity.

Absorbance pattern analysis using UV-Vis spectrophotometry on crude extracts and preparative TLC fractions revealed several peaks, indicating that the major lichen compounds absorb at specific wavelengths. In the Mekarsari (MZ) and MBB samples, the absorption patterns showed slight differences; however, both exhibited peaks at similar wavelengths. These patterns are consistent with previous studies (Luo et al. 2009; Nguyen et al. 2021), which reported peaks at specific wavelengths. One of the characteristic compounds of *P. tinctorum* is lecanoric acid, which can be detected at wavelengths of 213, 270, and 304 nm (Yoshimura et al. 1994; Luo et al. 2009). According to Chong et al. (2011), the wavelength range of 210-320 nm corresponds to compounds such as amino acids, proteins, peptides, aromatic compounds, phenols, and carboxylic acids. At these three wavelengths, all four samples suggested the presence of lecanoric acid in the thallus of *P. tinctorum*. In the preparative TLC fraction, however, only a single peak was observed, suggesting that compounds absorbing at other wavelengths may have been reduced or partially purified during preparative TLC. Further research, such as analysis using LC-MS or GC-MS, is needed to validate and identify the compounds present in the preparative TLC fraction.

However, the active fraction results did not align with Chan and Chong (2022), where active fractions were expected to enhance antimicrobial activity compared to crude extracts. The result of the preparative fraction was generally lower than that of the crude extract. In addition, the preparative TLC results demonstrated the disappearance of a peak in the crude extract as detected by UV-Vis spectrophotometry, and the appearance of a new peak. These results are likely due to the influence of an unsuitable adsorbent, which may have trapped the lead (Pb) within the adsorbent or Pb cannot migrate, leaving only Pb-free compounds to be harvested (Gupta et al. 2021). Absorbent is one of the important factors in TLC. This factor further supports the hypothesis that Pb plays a role in the antimicrobial activity of the *P. tinctorum* thallus. The

appropriate use adsorbents of TLC and partial purification is crucial for achieving the desired results. In this study, silica gel was used as the adsorbent at stationary phase. Silica gel has the ability to migrate organic compounds based on their polarity. The solvents used as the mobile phase include n-butanol, acetic acid, and distilled water. Stegall et al. (2016) used ethanol: aminosilane: DI H_2O mixture to facilitate Pb+ ion migration on the plate. Some modifications are necessary to allow Pb to migrate along with the organic compounds (Stegall et al. 2016; Gupta et al. 2021). Some hypotheses for the lower activity of preparative fractions are that the concentration of antimicrobial compounds in crude extracts is higher than in fractionation (Eloff 1998; Cowan 1999). Compound stability also plays a role, as antimicrobial compounds are believed to be more stable in the complex composition of crude extracts compared to active fractions or preparative compound (Croteau et al. 2000; Havsteen 2002). Rasoanaivo et al. (2011) also propose the presence of inhibitor compounds that hinder antimicrobial performance in active or preparative fractions, whereas in crude extracts, these inhibitors are potentially masked by other compounds.

In conclusion, *P. tinctorum* is a cosmopolitan lichen that can be used as a source of secondary metabolites compounds and as a bioindicator of pollutants. The study indicate that *P. tinctorum* from zone 3 had the highest concentration of Pb and showed the strongest antimicrobial activity. This suggests that Pb may play a role in enhancing the antimicrobial properties of the lichen. However, the decreased activity in the preparative fraction compared to the crude extract indicates the potential involvement of other synergistic compounds or Pb-free compound. For future research, further testing is needed to identify a suitable adsorbent to prevent Pb entrapment. Additionally, it is necessary to detect the bioactive compounds present in *P. tinctorum* from MZ3 and MBB using LC-MS/MS to determine their antimicrobial structure, followed by further validation through in silico analysis.

ACKNOWLEDGEMENTS

The author expresses gratitude to Mekarsari Fruit Garden, Bogor, Indonesia, for granting permission for lichen sampling and to UKICIS for granting permission to use analyzed control lichen samples and data from Mangrove Batu Bedhil, Belitung, Indonesia. Additionally, the author would like to thank all the assistants who assisted with this research.

REFERENCES

Bačkor M, Loppi S. 2009. Interactions of lichens with heavy metals. Biol Plant 53: 214-222. DOI: 10.1007/s10535-009-0042-y.

Bouida L, Rafatullah M, Kerrouche A, Qutob M, Alosaimi AM, Alorfi HS, Hussein MA. 2022. A review on cadmium and lead contamination: Sources, fate, mechanism, health effects and remediation methods. Water 14 (21): 3432. DOI: 10.3390/w14213432.

Chan YS, Chong KP. 2022. Bioactive compounds of *Ganoderma boninense* inhibited methicillin-resistant *Staphylococcus aureus* growth by affecting their cell membrane permeability and integrity. Molecules 27 (3): 838. DOI: 10.3390/molecules27030838.

Chong MN, Aryal R, Sidhu J, Tang J, Toze S, Gardner T. 2011. Urban stormwater quality monitoring: From sampling to water quality analysis. Seventh International Conference on Intelligent Sensors, Sensor Networks and Information Processing. Melbourne, Australia, 6-9 December 2011.

Cowan MM. 1999. Plant products as antimicrobial agents. Clin Microbiol Rev 12 (4): 564-582. DOI: 10.1128/CMR.12.4.564.

Croteau R, Kutchan TM, Lewis NG. 2000. Natural Products (Secondary Metabolites). Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD.

Ellis CJ, Asplund J, Benesperi R, Branquinho C, Di Nuzzo L, Hurtado P, Martínez I, Matos P, Nascimbene J, Pinho P, Prieto M, Rocha B, Rodríguez-Arribas C, Thüs H, Giordani P. 2021. Functional traits in lichen ecology: A review of challenge and opportunity. Microorganisms 9 (4): 766. DOI: 10.3390/microorganisms9040766.

Eloff JN. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants?. J Ethnopharmacol 60 (1): 1-8. DOI: 10.1016/S0378-8741(97)00123-2.

Gupta A, Sharma V, Sharma K, Kumar V, Choudhary S, Mankotia P, Kumar B, Mishra H, Moulick A, Ekielski A, Mishra PK. 2021. A review of adsorbents for heavy metal decontamination: Growing approach to wastewater treatment. Materials (Basel) 14 (16): 4702. DOI: 10.3390/ma14164702.

Gupta S, Khare R, Bajpai O, Rai H, Upreti DK, Gupta RK, Sharma PK. 2016. Lichen as bioindicator for monitoring environmental status in western Himalaya, India. Intl J Environ 5: 1. DOI: 10.3126/ije.v5i2.15001.

Havsteen BH. 2002. The biochemistry and medical significance of the flavonoids. Pharmacol Ther 96 (2-3): 67-202. DOI: 10.1016/S0163-7258(02)00298-X.

Jayalal U, Divakar PK, Joshi S, Oh SO, Koh YJ, Hur JS. 2013. The lichen genus *Parmotrema* in South Korea. Mycobiology 41 (1): 25-36. DOI: 10.5941/MYCO.2013.41.1.25.

Kambar Y, Vivek MN, Manasa M, Kekuda TP, Onkarappa R. 2014. Antimicrobial activity of *Ramalina conduplicans* Vain. (Ramalinaceae). Sci Techol Arts Res J 3 (3): 57-62. DOI: 10.4314/star.v3i3.10.

Kekuda TRP, Dhanya R, Dhatri R, Sunita CM, Onkarappa R, Vinayaka KS. 2015. Radical scavenging, antimicrobial and insecticidal efficacy of *Parmotrema cristiferum* and *Dirinaria applanata*. Sci Techol Arts Res J 4 (1): 95-102. DOI: 10.4314/star.v4i1.16.

Khairunnisa K, Alawiyah T, Hidayah N. 2021. Analysis of lead (Pb) heavy metal levels in hair of employees of car showroom'x'with Atomic Absorption Spectrophotometer (AAS) Method. Proc Intl Conf Health Sci 1 (1): 110-118.

Krisnawati M. 2018. Penetapan kadar formalin pada mie basah yang dijual di Pasar Piyungan dengan metode spektrofotometri UV-VIS. Jurnal Kesehatan Madani Medika 9 (2): 62-67. DOI: 10.36569/jmm.v9i2.12. [Indonesian]

Lei S, Zhao R, Sun J, Ran J, Ruan X, Zhu Y. 2020. Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03 isolated from infant's feces. Food Sci Nutr 8 (5): 2214-2222. DOI: 10.1002/fsn3.1428.

Liu G, Ren L, Song Z, Wang C, Sun B. 2015. Purification and characteristics of bifidocin A, a novel bacteriocin produced by *Bifidobacterium animals* BB04 from centenarians' intestine. Food Control 50: 889-895. DOI: 10.1016/j.foodcont.2014.10.049.

Luo H, Yamamoto Y, A Kim J, Jung JS, Koh YJ, Hur JS. 2009. Lecanoric acid, a secondary lichen substance with antioxidant properties from *Umbilicaria antarctica* in maritime Antarctica (King George Island). Polar Biol 32: 1033-1040. DOI: 10.1007/s00300-009-0602-9.

Micheletti AC, Honda NK, Ravaglia LM, Matayoshi T, Spielmann AA. 2021. Antibacterial potencial of 12 lichen species. An Acad Bras Cienc 93 (4): S0001-37652021000700904. DOI: 10.1590/0001-3765202120191194.

Mittapally S, Taranum R, Parveen S. 2018. Metal ions as antibacterial agents. J Drug Delivery Therapeutics 8 (6): 411-419. DOI: 10.22270/jddt.v8i6-s.2063.

Nguyen TTT, Vo TTD, Tran YH, Truong DT, Phan DC, Le PH. 2021. Photoprotective activity of lichen extracts and isolated compounds in *Parmotrema tinctorum*. Biointerface Res Appl Chem 11 (5): 12653-12661. DOI: 10.33263/BRIAC115.1265312661.

Noori M, Malayeri B, Moosaei M, Pakzad R, Piriye MH. 2012. Effects of heavy metals on the antibacterial properties of *Verbascum speciosum* Schard. Rev Cient UDO Agric 12 (2): 463-471.

Nurliana SM, Sudirman LI, Sanjaya AW. 2008. Pengujian awal aktivitas antibakteri dari minyak pliek u dan pliek u: Makanan tradisional Aceh. Jurnal Kedokteran Hewan 2 (2): 150-156. [Indonesian]

Panayidou S, Ioannidou E, Apidianakis Y. 2014. Human pathogenic bacteria, fungi, and viruses in *Drosophila*: Disease modeling, lessons, and shortcomings. Virulence 5 (2): 253-269. DOI: 10.4161/viru.27524.

Port RK, Käffer MI, Schmitt JL. 2018. Morphophysiological variation and metal concentration in the thallus of *Parmotrema tinctorum* (Despr. ex Nyl.) Hale between urban and forest areas in the subtropical region of Brazil. Environ Sci and Pollution Res 25: 33667-33677.

Raj PS, Prathapan A, Sebastian J, Antony AK, Riya MP, Rani MR, Biju H, Priya S, Raghu KG. 2014. *Parmotrema tinctorum* exhibits antioxidant, antiglycation and inhibitory activities against aldose reductase and carbohydrate digestive enzymes: An in vitro study. Nat Prod Res 28 (18): 1480-1484. DOI: 10.1080/14786419.2014.909420.

Rajan VP, Gunasekaran S, Ramanathan S, Murugaiyah V, Samsudin MW, Din LB. 2016. Biological activities of four *Parmotrema* species of Malaysian origin and their chemical constituents. J Appl Pharm Sci 6 (8): 036-043. DOI: 10.7324/JAPS.2016.60806.

Rasoanaivo P, Wright CW, Willcox ML, Gilbert B. 2011. Whole plant extracts versus single compounds for the treatment of malaria: Synergy and positive interactions. Malar J 10 (Suppl 1): S4. DOI: 10.1186/1475-2875-10-S1-S4.

Roque C, Andrade É, Duarte L, Costa M, Tavares APM, Madeira PP. 2023. How does dilution affect the conductivity, the propensity to aggregate and the biological activity of enzymes?. J Mol Liq 382: 121926. DOI: 10.1016/j.molliq.2023.121926.

Safitri RR, Sudirman LI, Koesmaryono Y. 2020. Exploration of *Parmotrema tinctorum* and *Leptogium* sp. lichens as bioindikator of air quality in Mekarsari Fruit Park. IOP Conf Ser: Earth Environ Sci 457: 012006. DOI: 10.1088/1755-1315/457/1/012006.

Shiromi PSAI, Hewawasam RP, Jayalal RGU, Rathnayake H, Wijayaratne WMDGB, Wanniarachchi D. 2021. Chemical composition and antimicrobial activity of two Sri Lankan lichens, *Parmotrema rampoddense*, and *Parmotrema tinctorum* against Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus.* Evid Based Complement Alternat Med 2021: 9985325. DOI: 10.1155/2021/9985325.

Stegall SL, Ashraf KM, Moye JR, Higgins DA, Collinson MM. 2016. Separation of transition and heavy metals using stationary phase gradients and thin layer chromatography. J Chromatogr A 1446: 141-148. DOI: 10.1016/j.chroma.2016.04.005.

Sudirman LI. 2005. Detection of antimicrobial compounds isolated from several tropical *Lentinus* by bioautographic method. Hayati J Biosci 12 (2): 67-67. DOI: 10.4308/hjb.12.2.67.

Sudirman LI. 2010. Partial purification of antimicrobial compounds isolated from mycelia of tropical *Lentinus cladopus* LC4. HAYATI J Biosci 17 (2): 63-67. DOI: 10.4308/hjb.17.2.63.

Sukul NC, Sukul A. 2004. High Dilution Effects: Physical and Biochemical Basis. Kluwer Academic Publishers, Norwell, USA.

Swamy CT, Gayathri D, Devaraja TN. 2016. Antibacterial activity of lichens *Parmotrema tinctorum* and *Pyxine sorediata* and their secondary metabolites. Intl J Adv Life Sci 9 (3): 373-380.

Tuan NT, Dam NP, Van Hieu MV, Trang DTX, Danh LT, Men TT, De TQ, Bach LT, Kanaori K. 2020. Chemical constituents of the lichen *Parmotrema tinctorum* and their antifungal activity. Chem Nat Compd 56: 315-317. DOI: 10.1007/s10600-020-03017-y.

Yoshimura I, Kinoshita Y, Yamamoto Y, Huneck S, Yamada Y. 1994. Analysis of secondary metabolites from lichen by high performance liquid chromatography with a photodiode array detector. Phytochem Anal 5 (4): 197-205. DOI: 10.1002/pca.2800050405.

Zulaini AAM, Muhammad N, Asman S, Hashim NH, Jusoh S, Abas A, Yusof H, Din L. 2019. Evaluation of transplanted lichens, *Parmotrema tinctorum* and *Usnea diffracta* as bioindicator on heavy metals accumulation in southern peninsular Malaysia. J Sustain Sci Manag 14 (4): 1-13.