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RESEARCH ARTICLE

Antibacterial properties of *Pyrrosia longifolia* extracts

Rohimatul Khodijah, Yuli Haryani, Hilwan Yuda Teruna, Rudi Hendra

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau, Pekanbaru, Riau, Indonesia

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Correspondence
Rudi Hendra
Department of Chemistry
Faculty of Mathematic & Natural sciences
Indonesia
rudi.hendra@lecturer.unri.ac.id

Abstract

Background: *Pyrrosia longifolia* is an epiphytic plant found in tropical forests. This plant is known to have antioxidant, anti-diabetic, anti-inflammatory, and antibacterial properties and is widely used in traditional medicine. *Pyrrosia longifolia* is one of the species used in traditional medicine, but no evidence of antibacterial activity has been found. **Objective:** The purpose of this study is to determine the antibacterial activity of various *P. longifolia* extracts. **Methods:** The plant was macerated with methanol and separated based on its polarity. The well diffusion method was used to determine the plant's antibacterial activity through the inhibition zones - minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of bacterial growth against seven bacteria pathogens. **Results:** The antibacterial activity of the extracts showed various inhibitions of the bacteria tested. Dichloromethane and ethyl acetate extracts showed intermediate inhibitory activity on *Bacillus subtilis* ATCC 19659, *B. cereus* ATCC 10876, *Salmonella typhimurium* ATCC 142028, and *Vibrio parahaemolyticus* ATCC 17802. **Conclusion:** The extract of *P. longifolia* is susceptible to the test bacteria, and additional testing is necessary to determine its antibacterial activity.

Introduction

The high air humidity during the dry season in Indonesia's tropical climate is ideal for developing bacteria, viruses, fungi, and parasites. These microorganisms can grow highly fertile and have a longer lifespan; the most common organisms are pathogenic. The tropical climate conditions make people more susceptible to contracting bacterial diseases. Bacteria can infect via numerous routes, including food, unsanitary environments, and surrounding objects. Bacteria such as *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella Typhimurium, Listeria monocytogenes*, and *Vibrio parahaemolyticus* are responsible for a variety of diseases such as eye infections, acute diarrhoea, meningitis, endocarditis, and even death. (Desai *et al*., 2019; Esfahanian *et al.,* 2019).

The sources of natural materials guide the development of antibacterial agents. This ensures that the resulting products have minimal side effects and negative bioaccumulation and prevent increased antibacterial resistance. The main advantages of natural plant products (NP) include rich and unique diversity, worldwide distribution and accessibility, the various modes of its antibacterial action mechanism, the proven clinical effectiveness of plant extracts from the origin of its isolate plants and their complementary effects (Porras *et al.,* 2020). The World Health Organisation (WHO) estimated that by 2022, approximately 80% of the world's population would use traditional medicine (Ahmadi, Ahmadi & Ahmadi, 2022). As a source of natural medicine, the synergy of secondary metabolites derived from plants is widely produced and required by the body (Karimi *et al.,* 2010; Altemimi *et al.*, 2017). Due to the country's rich biodiversity, each of Indonesia's regions is repleted with natural products. The use of traditional medicinal plants is derived from a variety of plant species, including ferns.

Pyrrosia is a fern genus found in the tropics and subtropics of Asia and Africa. Its specie is always used as a traditional medicine and has been studied in correlation with its activities. Some have been used as traditional Chinese medicine to treat acute

pyelonephritis, chronic bronchitis, and bronchial asthma (Committee, 2000).

Pyrrosia is a traditional medicine that treats conditions such as cough, jaundice, gingivitis, constipation, and burns. *P. lingua*, *P. petiolosa* (Christ) Ching, *P. sheareri* (Baker) Ching, *P. heterophylla*, *P. gralla* (Gies) Ching, *P. porosa* (C. Presl) Hovenk, and *P. subfurfuracea* are Pyrrosia species with demonstrated bioactivity.
Antitumor, anti-inflammatory, antibacterial, antiviral, antioxidant, and anticancer properties have been attributed to these species. Several studies reported that *P. dividii, P. tonkinensis , P. porosa,* and *P. subfurfuracea* showed antibacterial activity against various pathogenic bacteria. *P. longifolia* (Burm. f.) C.V. Morton is one of the Pyrrosia species found in the Riau region. In some areas of Indonesia and the Pacific Islands, a decoction made from these species' leaves is used to alleviate the pains associated with labour (Hovenkamp, 2003). According to earlier research findings, an ethyl acetate extract from the aerial part of the species exhibited a high level of free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) at an IC_{50} concentration of 28.22 ppm. The study of *Pyrrosia longifolia* (Burm. f.) C.V. Morton against various pathogenic bacteria has not been reported, despite the fact that different species of Pyrrosia have shown different types of antibacterial activity in different studies. In this study, we describe the extraction of the species, including the production of less polar to more polar sub-extracts and the antibacterial activity of the extracts. Previous molecular chemotypes derived from this species were also discussed in relation to the made planta pharmacological claims.

Methods

Pyrrosia longifolia (Burm. f.) C.V. Morton was collected in Pekanbaru, Indonesia (0.539166, 101.448843) (Figure 1), and the Head of the Botany laboratory in the Department of Biology at Riau University identified the species. On World Flora online, the accepted name of the plant species was verified. After being air-dried, the aerial portion of the species was ground into a fine powder and then refrigerated until further analysis could be performed.

Figure 1: *Pyrrosia longifolia* (Burm. f.) C.V. Morton in Pekanbaru, Indonesia

Extraction

Maceration in methanol up to 500 g for 24 hours was repeated three times until the maceration no longer produced green results. During the maceration process, the extract was filtered, and the filtrate was kept. The macerate was then concentrated using a rotary evaporator set to 40 °C, resulting in a crude methanol extract. The crude methanol extract was then separated using liquid-liquid extraction to obtain n-hexane, dichloromethane, ethyl acetate, and water extracts. (Afham *et al.*, 2022; Khodijah *et al.*, 2022; Hendra *et al.,* 2020).

Microbial strain

This study utilised seven microorganisms: *Bacillus subtilis* ATCC 1965, *Staphylococcus aureus* ATCC 6538, *B. cereus* ATCC 10876, *Vibrio parahaemolyticus* ATCC 17802, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 8739, and *Salmonella typhimurium* ATCC 142028. The microbial isolates were grown on agar plates at four °C in the laboratory of Biochemistry, Department of Chemistry, Universitas Riau. Before any antimicrobial tests were conducted, the strains were subcultured on fresh agar plates for 24 hours.

Antibacterial activity

The antibacterial activity was determined using the Kirby-Bauer technique and chloramphenicol as a positive control. The final concentration of each extract was 500ppm in 10% DMSO, and the bacterial suspension concentration was standardised to 0.5 McFarland. 100µL of extract solution, positive and negative control were dropped onto the well on the surface of 100µL of bacterial suspension-supplemented Mueller-Hinton agar (MHA). A 24-hour incubation period was carried out at 37° Celsius. The formed clear zone was observed and measured to

make observations. The experiment was carried out three times. (Hendra *et al.*, 2022).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Evaluation

The MIC and MBC of the extracts were done using the method described by Loo and colleagues with few modifications (Loo *et al.,* 2018). The MIC test was conducted in a microtiter plate with 96 wells and broth microdilution, while the MBC test was conducted on MHA plates. Inoculums of bacteria were diluted to 106 CFU/mL. For the MIC test, 100 µL of the extracts stock solution (500 µg/mL) was added to 100µL of MHB (Mueller–Hinton broth). This was accomplished in columns 12 through three. Column 12 of the microtiter plate contained the highest extract concentration, while column three contained the lowest. Column one represented the negative control (only medium), whereas column two represented the positive control (medium and bacterial inoculums). After 24 hours at 37 °C, the density of the plate was measured. The minimal inhibitory concentration (MIC) is the lowest antibacterial concentration known to inhibit bacterial growth. MBC is the lowest antibacterial concentration capable of eliminating bacteria. For the MBC test, the suspension from each microtiter plate was plated onto an MHA plate—24-hour incubation at 37 degrees Celsius. The MBC is the lowest concentration at which no visible growth is observed on an MHA plate.

Statistical analysis

The tests were conducted in triplicate, and the results are expressed as the mean and standard deviation. Graph pad prism version 9 was used to analyse the data, and the Tukey test followed a one-way analysis of variance (ANOVA) to compare the means. The values were regarded as statistically significant.

Results

The antibacterial activity of various extracts from *P. longifolia* (Burm. f.) C.V. Morton was determined against seven pathogenic bacteria: *B. subtilis* ATCC 1965, *S. aureus* ATCC 6538, *B. cereus* ATCC 10876, *V. parahaemolyticus* ATCC 17802, *L. monocytogenes* ATCC 7644, *E. coli* ATCC 8739, and *S. typhimurium* ATCC 142028. Table I provides a summary of the good diffusion assessment of the extracts. The results indicated significant differences between the extracts' effectiveness against pathogenic bacteria.

Table I: Antibacterial Activity of *P. longifolia* (Burm. f.) C.V. Morton Extracts at 500 µg/well

Bacteria	Inhibition zone (cm)				
	***n*-Hexane**	**Dichloromethane**	**Ethyl Acetate**	**Water**	**Chloramphenicol (30 µg/disc)**
Bacillus subtilis	1.38±0.34^{c}	1.176±0.26^{d}	0.84±0.45^{e}	2.24±0.35^{b}	3.31±0.53^{a}
Staphylococcus aureus	0.00±0^{b}	0.00±0^{b}	0.00±0^{b}	0.00±0^{b}	3.44±0.23^{a}
Escherichia coli	0.00±0^{b}	0.00±0^{b}	0.00±0^{b}	0.00±0^{b}	1.92±0.13^{a}
Salmonella typhimurium	0.00±0^{d}	0.89±0.6^{c}	1.02±0.73^{b}	0.73±0.25^{c}	2.27±0.28^{a}
Bacillus cereus	0.00±0^{d}	0.84±0.58^{c}	1.16±0.55^{b}	0.00±0	2.21±0.36^{a}
Listeria monocytogenes	0.00±0^{b}	0.00±0^{b}	0.00±0^{b}	0.00±0^{b}	2.53±0.56^{a}
Vibrio parahaemolyticus	0.00±0^{c}	1.01±0.93^{b}	1.17±0.33^{b}	0.00±0^{c}	2.57±0.55^{a}

$^{a\text{-}e}$: Means in the same row with different lowercase letters differed significantly (p<0.05)

At a concentration of 500 µg/well, each extract exhibited various activities against B. subtilis. However, none of the extracts demonstrated antibacterial activity against *S. aureus* ATCC 6538, *E. coli* ATCC 8739, or *L. monocytogenes* ATCC 7638. Additionally, dichloromethane and ethyl acetate exhibited greater antibacterial activity than other extracts but less than chloramphenicol. Moreover, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of dichloromethane and ethyl acetate extracts were determined (MBC). The results indicated that the MIC and MBC of both extracts were greater than 500 µg/mL against all of the bacteria tested (Table II).

Table II: MIC and MBC Values of dichloromethane and ethyl acetate extracts of *P. longifolia* (Burm. f.) C.V. Morton

Bacteria	MIC concentration (µg/mL)		MBC concentration (µg/mL)	
	Dichlormethane	Ethyl acetate	Dichlormethane	Ethyl acetate
B. subtilis ATCC 1965	>500	>500	>500	>500
B. cereus ATCC 10876	>500	>500	>500	>500
V. parahaemolyticus ATCC 17802	>500	>500	>500	>500
S. typhimurium ATCC 142028	>500	>500	>500	>500

Discussion

Treating the species before extracting secondary metabolites is essential because doing so influences the bioactive compounds obtained from the extraction process. The compounds in the sample are susceptible to being quickly broken down by oxidative, enzymatic, or polymerisation processes. (Yenn *et al.,* 2018) The proper selection of an extraction solvent is necessary to isolate the desired bioactive compounds from a plant sample. According to Tong and colleagues (2014), the organic solvents utilised affect the natural products that can be extracted and, as a direct consequence of this, the biological activity of the crude extract produced. In this investigation, the species was removed using organic solvents with varying degrees of polarity (n-hexane, dichloromethane, and ethyl acetate) to distinguish between hydrosoluble molecules and those that were liposoluble (Tong *et al.,* 2014). The antibacterial activity of the extracts revealed that ethyl acetate and dichloromethane extracts possess Gram-positive and Gram-negative bacteria with intermediate susceptibility. This study used the suitable diffusion method to determine an antimicrobial agent's antibacterial activity. To determine the antibacterial activity of the extracts, particularly dichloromethane and ethyl acetate extracts, their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were calculated (Burt, 2004). In terms of extracts' capacity to inhibit the growth of bacteria, dichloromethane, and ethyl acetate extracts are considered to have an intermediate potential. This assessment is based on the inhibition zone category. This showed that the species' dichloromethane and ethyl acetate extracts contained secondary metabolites with antibacterial properties, for instance, flavonoids.

In some cases, flavonoids such as chalcones demonstrated antibacterial activities up to six times more potent than the currently available standard drugs. Against multidrug-resistant gram-negative and gram-positive bacteria, certain synthetic derivatives of flavonoids also exhibited remarkable antibacterial activities, with 20- to 80-fold potency compared to the standard drug (Farhadi *et al.*, 2019). Various publications showed that the species of Pyrrosia contain flavonoids and their glucoside. For instance, kaempferol from *P. petiolosa* (Lang *et al.,* 2021), *P. sheareri* (He *et al.,* 2019); Quercetin from *P. sheareri* (He *et al.,* 2019), *P. calvata* (Chen *et al.,* 2015); rutin from *P. sheareri* (He e*t al.,* 2019). The flavonols, which include Quercetin, myricetrin, morin, galangin, entadanin, rutin, piliostigmol, and their derivatives, are among the most essential classes of flavonoids that demonstrate powerful antibacterial activities. For instance, Quercetin and its derivatives showed significant antibacterial activity against certain bacterial strains (Geoghegan *et al.,* 2010). According to He and colleagues' findings, kampferol inhibited *E. coli* by acting on the cell membranes and the liposomal model. (He *et al.,* 2014) They discovered that the interaction between the model membrane's hydrophobic regions and the model membrane's polar head group could potentially damage the membrane of *E. coli*. To determine which secondary metabolite is accountable for these activities, additional analysis is required to isolate the compounds and determine their mode of action.

Conclusion

According to this study's findings, the species' extracts exhibit diverse responses to pathogenic bacteria. Both dichloromethane and ethyl acetate extracts showed notable antibacterial activity. These results pave the way for future research to isolate and analyse the antibacterial properties of secondary metabolites in active extracts.

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