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Antibacterial Activity of Endophytic Bacteria Isolated from Purwoceng Roots (*Pimpinella pruatjan* Molk.)

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Abstract

Most antimicrobial agents, antifungus or antibacterial have been isolated from plants. Purwoceng (*Pimpinella pruatjan* Molk.) is one of the potential plants that produced an antibacterial agent. But, the status of purwoceng as an endemic plant in Central Java is "endangered". Based on research before, the endophytic bacteria of the roots can produce an antibacterial agent as well as the plant. This research was performed by purified the endophytic bacteria isolate, the antagonism test as an antibacterial against *Staphylococcus aureus* also performed with agar-well diffusion. There are 4 isolates that have a good antibacterial activity (DG1, GP11, GP12, RP6), 5 isolates have a weak antibacterial activity (DG14, GP9, RP1, RP4, RP5) and 16 isolates did not show antibacterial activity (DG9, DG13, DG15, DG18, DG20, GP1, GP2, GP7, GP8, GP14, RP8, RP9, RP10, RP12, RP13). The data were analyzed descriptively.

Keywords: *Staphylococcus aureus*, antibacterial, Purwoceng

A. Introduction

Purwoceng (*Pimpinella pruatjan* Molk.) is a commercial herbal plant with Coumarin, Sterols, Alkaloids, Saponins, and several types of oligosaccharides in the roots (Darwati et al., 2006). Secondary metabolite compounds produced by purwoceng have abilities such as antimicrobial, anticoagulant, and anti-inflammatory (Darwati et al., 2006, Widayat et al., 2012).

The ability of purwoceng to produce antimicrobials can be used to reduce the growth of pathogenic bacteria. *Staphylococcus aureus* is a type of pathogenic bacteria that often cause infectious diseases in the community (Boucher & Corey, 2008, Hauser & Ozer, 2011; Infection Control Hospital and St.James Team, 2006, Klein et al., 2007), *S. aureus* is a bacterial pathogen that causes the main cause of respiratory infections and is the second cause of the appearance of nosocomial pneumonia bacteremia and cardiovascular infections (Klein et al., 2007). Treatment of infection caused by *S. aureus* is usually by administering drugs such as antibiotics/antimicrobials.

The active compounds of the antimicrobial drugs available on the market are the resulted from plant extraction Elita et al., 2013). The use of secondary metabolite compounds, such as in the purwoceng, as raw material for taking medicine, has considerable obstacles, namely a fairly long plant life cycle and the condition of the purwoceng entering the category of plants in danger or endangered (Rifai et al., 1992, Darwati & Roostika, 2006). Thus, to overcome this obstacle, endophytic bacteria are found in plant tissue (Elita, et al., 2013).

Endophytic bacteria in plants were able to produce the same secondary metabolites as host plants (Elita, et al., 2013, Darwati & Roostika, 2006). Secondary metabolite compounds produced by endophytic bacteria also have antimicrobial activity in pathogenic bacteria (Malfanova et al., 2013). The capacity of endophytic bacteria in the production of secondary metabolites which are the same as their host plants has the potential to facilitate the production of secondary metabolites which can act as antimicrobials (Radji, 2005).

In recent years there have been many studies looking at the potential of endophytic microbes in the production of bioactive compounds from several medicinal plants. However, not many have examined the endophytic microbes of the purwoceng. Therefore, this research is very interesting to do.

B. Literature Review

Based on the research of Hernani & Rostiana (2004), the most active ingredients of Purwoceng are located at the roots. The research of Caropeboka & Lubis (1975) shows that purwoceng root contains coumarin, saponin, and alkaloid derivatives. Research conducted by (Sidik et al., 1975) found the presence of compounds of bergapten, isobergapten, and sphondin, all of which belong to the furanocoumarin group in purwoceng root extract. Hernani & Rostiana (2004) suggest the presence of 4-hydroxy coumarin, umbelliferone, marmesin, and psoralen in purwoceng roots. On the other hand, based on the results of phytochemical tests by the Research Institute for Medicinal and Aromatic Plants (2011) it was reported that in addition to alkaloids, purwoceng root also contains tannins, flavonoids, triterpenoids, steroids, and glycosides.

Plants are closely related to microbes. Microbes are associated with plants in four ways, namely pathogens, symbionts, epiphytes, and endophytes (Iniguez, 2004). Endophytic microbes that are commonly found in plants are fungi and bacteria (Mayerhofer, 2011). Endophytic bacteria can be found in plant tissue, namely roots, stems, flowers, and cotyledons (Carrol, 1988; Zinniel et al., 2002). Initially, endophytic bacteria reside in the rhizosphere, then colonize the plant through cracks in the roots and release protein compounds to facilitate colonization after the competition for nutrients and space is won by these bacteria (Rosenblueth & Martinez, 2006).

Endophytic bacteria provide many benefits for their host, including increased nutrient acquisition, growth, and development through mechanisms such as nitrogen fixation, phytohormone production (Zinniel et al., 2002), stress tolerance, pathogens, and disease resistance (Strobel & Daisy, 2003). Endophytes can protect their hosts from pathogens and insects either directly or indirectly. For example, some endophytic bacteria may produce toxins and secondary metabolites that inhibit other pathogens or insects. These direct and indirect effects can be inferred from an approach based on quantitative genetics (Rasmann & Agrawal, 2009).

The origin of endophytic bacterial isolates, the root conditions of the host plant, and the type of bacteria will cause different abilities to produce a secondary metabolite compound. According to Tan & Zou (2001), endophytic microbes can indeed produce bioactive compounds that are similar in character to or the same as their host. This is due to the evolutionary genetic exchange that occurs between the host and endophytic microbes.

Simamarta (2007) succeeded in isolating 38 isolates of bacteria and endophytic fungi from grafting plants. The results of isolation of endophytic bacteria obtained were able to inhibit the growth of *Escherichia coli*, *Pseudomonas* sp, *Bacillus subtilis,* and inhibit *Candida albicans*, while the endophytic fungi obtained were able to inhibit the growth of *C. albicans* and *B. subtilis* test microbes.

Infection caused by *S. aureus* bacteria is one of the infections that has a huge impact on the world of health. An increasing number of cases of *S. aureus* infection have been reported worldwide. In the United States, 400,000 cases of infection were reported per year in 2003 (Boucher & Corey, 2008). In 1999-2005 there was an increase of 62% of cases of pathogenic infections due to Meticillin-Resistant *S. aureus* (Klein et al., 2007). *S. aureus* is a bacteria found on the skin and nose. These bacteria colonize the skin of 30% of healthy people without causing infection. The infection will occur if there is a wound on the skin so that these bacteria can enter the body. The spread of this disease can occur due to direct contact with people with infection. The use of antibiotics produced by microbes is one of the treatment efforts carried out if the infection is already in a severe condition (Infection Control and Team St.James's Hospital, 2006).

Staphylococcus aureus is a major pathogen that is an important cause of increased resistance to antibiotics. (Lowy, 1998). Some *S. aureus* strains have capsules that inhibit phagocytosis by

polymorphonucleic leukocytes unless specific antibodies are present. Most of the *S. aureus* strains have a coagulase or clotting factor on the surface of the cell wall. Coagulase binding nonenzymatically to fibrinogen causes aggregation in bacteria (Brooks et al., 2004).

C. Methodology

1. Research Design

This research is explorative in nature, which explores the potential of endophytic bacteria from purwoceng root (*Pimpinella pruatjan* Molk.) to produce secondary metabolites as antibacterial against *Staphylococcus aureus*

2. Instruments

Endophytic Bacteria Isolate Subcultures

All endophytic bacteria isolates that have been obtained from previous studies, subculture to TSB (Trypticasein Soy Broth) medium and incubated at room temperature (27^0C) for 24-48 hours. The grown bacterial culture 100 µl was spread on a petri dish containing TSA (Trypticasein Soy Agar) medium using a sterile spreader and then incubated at room temperature (27^0C) for 24 hours. The growing colonies were observed, if the colony was pure (no contamination) then the isolates were stained with Gram and observed using a light microscope then the colony observed and Gram stain results were confirmed by previous research data. After making sure that the isolates obtained were following those obtained, then streaked on the TSA slant medium as pure culture.

Screening of Antibacterial Activity of Endophytic Bacteria Isolates Against Staphylococcus aureus

The screening process was carried out by testing bacterial isolates during the stationary phase against pathogenic bacteria *Staphylococcus aureus*. Twenty-five isolates were obtained, tested for antibacterial activity against *Staphylococcus aureus*. This test was carried out using the agar-well diffusion method. The overnight culture of endophytic bacteria from TSA tilt, were taken 1 ose then put into a test tube containing 5 ml of Ringer's solution and homogenized. The turbidity was compared with the standard McFarland 0.5 solution or measured the Optical Density (OD) value using a 0.1 spectrophotometer with a wavelength of 600nm, which means it is equivalent to 10^8 CFU/ml. The bacterial suspension was then taken as much as 100 µl and spread on TSA media and then incubated at room temperature (27^0C) for 24 hours.

On the following day, a pure culture of the target bacteria *Staphylococcus aureus* was taken 1 ose then put into a test tube containing 5 ml of Ringer's solution and homogenized. Turbidity was compared with standard McFarland 0.5 solution. The 200 µl bacterial suspension was put into 20 ml of TSA media then poured into a petri dish and allowed to solidify. The agar slices for the tested bacteria were put into TSA media that had been inoculated with the target bacteria and incubated for 48 hours (the final stationary phase). Observations were made after the incubation period, namely by looking at and measuring the clear zone formed around the well for the test bacteria. Ampicillin disc 30mg/ml was used as a positive control and distilled water as a negative control. Isolates that showed inhibition against the growth of *Staphylococcus aureus* were collected and grown back on TSA slant media for further testing.

3. Technique of Data Analysis

This research was conducted using a completely randomized design (CRD). The data obtained were analyzed descriptively.

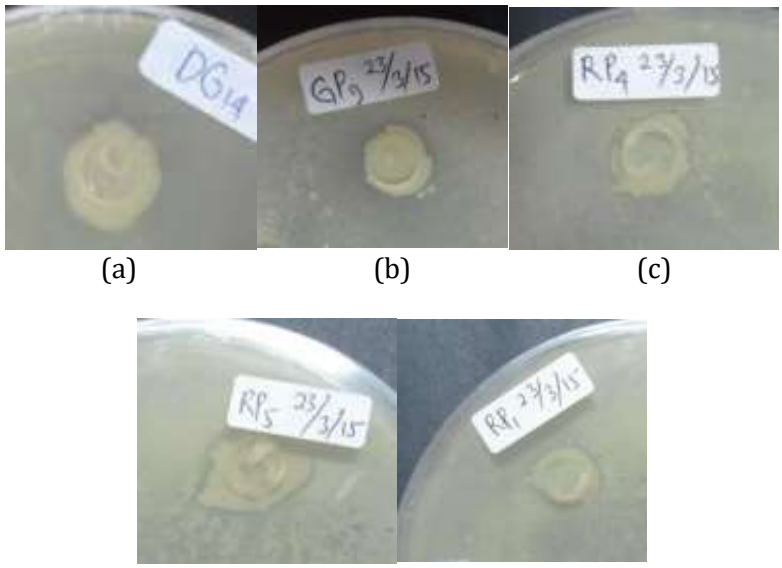
D. Findings and Discussion

1. Findings

(a) (b) (c)

(d) (e) (f)

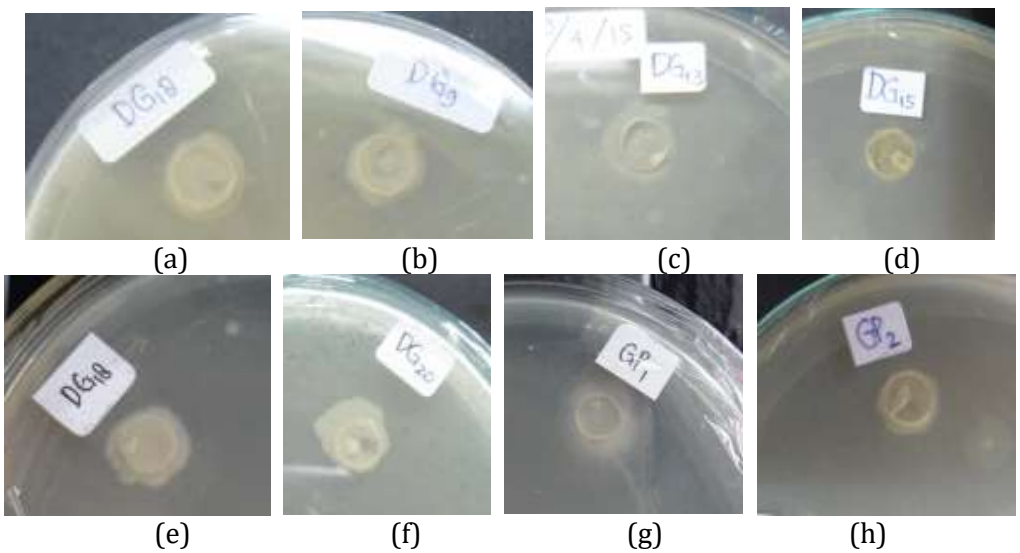
Figure 1. Antibacterial activity test of potential endophytic bacterial isolates from the roots of purwoceng (P. pruatjan Molk.) Against Staphylococcus aureus. (a) isolate DG1, (b) isolate GP11, (c) isolate GP12, (d) isolate RP6, (e) Amphicillin disc 30mg / ml as a positive control, (f) distilled water as a negative control.



(a) (b) (c)

(d) (e)

Figure 2. Five endphopytic bacteria isolates that showed weak antibacterial activity against *Staphylococcus aureus.* (a) isolate DG14, (b) isolate GP9, (c) isolate RP4, (d) isolate RP5, (e) isolate RP1



(a) (b) (c) (d)

(e) (f) (g) (h)

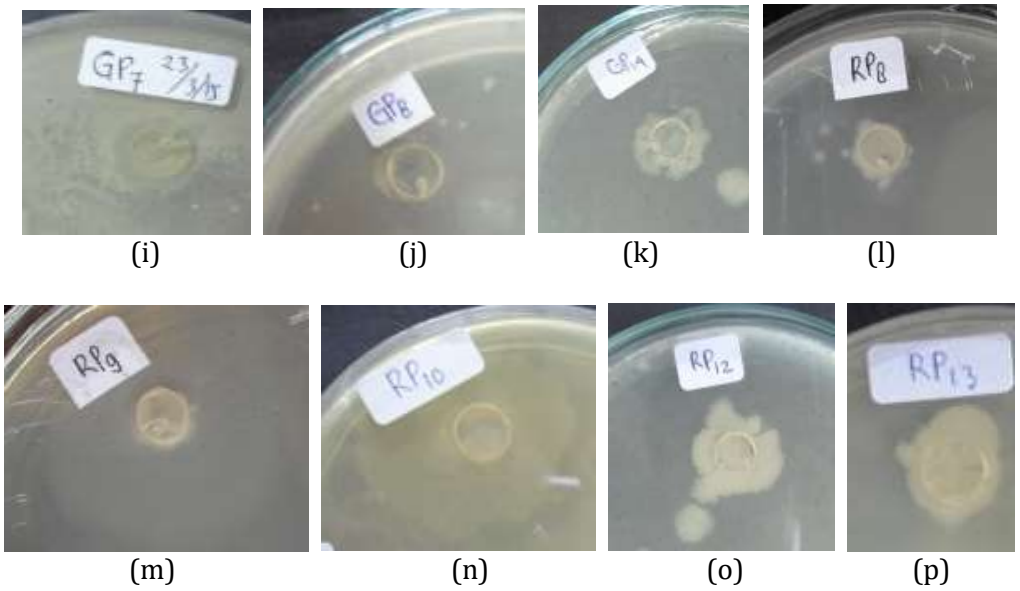


Figure 3. Sixteen endphopytic bacteria isolates that did not show antibacterial activity against *Staphylococcus aureus*. (a) isolate DG18, (b) isolate DG9, (c) isolate DG13, (d) isolate DG15, (e) isolate DG18, (f) isolate DG20, (g) isolate GP1, (h) isolate GP2, (i) isolate GP7, (j) isolate GP8, (k) isolate GP14, (l) isolate RP8, (m) isolate RP9, (n) isolate RP10, (o) isolate RP12, (p) isolate RP13

Table 1. Test for the antibacterial activity of endophytic bacteria isolates from the roots of purwoceng (*P. pruatjan* Molk.) Against *S.aureus*

No.	Isolates Code	Inhibition Zone (cm)	No.	Isolates Code	Inhibition Zone (cm)
		S. aureus			*S. aureus*
1.	DG1	1,68 cm	15.	GP8	-
2.	DG2	-	16.	GP9	< 0,3 cm
3.	DG3	-	17.	GP11	0,67 cm
4.	DG6	-	18.	GP12	0,62 cm
5.	DG7	-	19.	GP14	-
6.	DG9	-	20.	RP1	< 0,3 cm
7.	DG13	-	21.	RP4	< 0,3 cm
8.	DG14	< 0,3 cm	22.	RP5	< 0,3 cm
9.	DG15	-	23.	RP6	1,21 cm
10.	DG18	-	24.	RP8	-
11.	DG20	-	25.	RP9	-
12.	GP1	-	26.	RP10	-
13.	GP2	-	27.	RP12	-
14.	GP7	-	28.	RP13	-

Note: (-) = did not show the zone of inhibition

2. Discussion

Twenty-five isolates that had an optimal fermentation period in secondary metabolite production were screened for their antibacterial activity against *Staphylococcus aureus*. The results of this test indicated that four endophytic bacteria had strong antibacterial activity, namely DG1, GP11, GP12, and RP6 (Figure 1). Antibacterial activity was characterized by the formation of a clear zone around the agar cut with the diameter of the isolates shown in Table 1. Among the other twenty-five isolates, some isolates showed inhibition but were very weak (DG14, GP9, RP1, RP4, RP5) and the other isolates could not inhibit the growth of *S. aureus* (DG9, DG13, DG15, DG18, DG20, GP1, GP2, GP7, GP8, GP14, RP8, RP9, RP10, RP12, RP13)

Figure 1a shows the inhibition zone produced by the endophytic bacteria DG1 isolate 1,68 cm. When compared with the inhibition zone formed from antibiotic discs, which is Ampicillin has positive control, 1,88 cm, it can be said that DG1 isolate has a strong antibacterial activity. The GP11, GP12 and RP6 isolates respectively inhibited the growth of *S.aureus* and the GP9, RP1, RP4, RP5, RP13 isolates had a small inhibitory zone antibacterial activity against *S.aureus*. Antimicrobial strength can be classified as very strong (clear zone ≥ 2 cm), strong (clear zone ≥ 1 cm), slowing growth (clear zone ≤ 1 cm (Crawford et al. 1993). Thus, it can be said that four

isolates have good antibacterial activity with bacteriostatic ability to inhibit the growth of *S.aureus*.

The capacities of DG1, GP11, GP12, and RP6 6 in their potential as antibacterial agents against *S. aureus* are closely related to the Purwoceng as a bacterial host plant known as a medicinal plant. According to Tan & Zou (2001); Strawel & Daisy (2003), endophytic microbes can produce bioactive compounds that are similar to host plants through a coevolution process.

Endophytic bacteria from the roots of the Purwoceng (*P. pruatjan* Molk.) that have weak potential may need more time incubation to produce the compounds and that do not show antibacterial potential in *S. aureus* may have other potential compounds. Several studies have shown that endophytic microbes can produce antifungal substances (Souza, et al*.,* 2014), antioxidants (Marlinda et al*.,* 2019), anticancer, antivirus, insecticide (Lodewyckx, et al., 2002), and IAA hormone (Yarnaliza et al., 2010)

Based on its effectiveness, the antibacterial activity of endophytic bacteria from the roots of the Purwoceng (*P. pruatjan* Molk.) was categorized as a bacteriostatic group because it can inhibit the growth *S. aureus* that was used. This can be seen from the zone value inhibitors are produced even if the sharpness area is small. Low hurdle this result is described by Jawetz et al., (2007) of the type of bacteria the test bacteria used can replicate and to spread to various extracellular production tissues, in the form of enzymes and toxin. The toxin is partially invited by the plasmid gene and partly by genes on chromosomes.

E. Conclusion

Screening of twenty-five endophytic bacteria isolates from the roots of the Purwoceng (*Pimpinella pruatjan* Molk.) showed that nine isolates has the ability to inhibit bacterial growth of *Staphylococcus aureus* which is 2 isolates has strong ability (DG1 and RP6) and 7 isolates has slowing growth ability (DG14, GP9, GP11, GP12, RP1, RP4, RP5) based on classified antimicrobial strength by Crawford et al. 1993. Antibacterial activity against *S. aureus* based on its effectiveness properties including being bacteriostatic.

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