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# Screening of Potential Free Radicals Scavenger and Antibacterial Activities of Purwoceng (*Pimpinella alpina* Molk)

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**Abstract:** Purwoceng (*Pimpinella alpina* Molk) is a traditional medicinal plant used for its aphrodisiac values. This plant was originated Dieng Plateu, Central Java, Indonesia. Purwoceng has been reported to contain steroid, flavonoids, glycoside, saponins, tannins, and phenolic. Based on secondary metabolite compounds of Purwoceng herbs, a research need to be done to determine the other potential free radicals scavenger and antibacterial activities of Purwoceng. The objectives of this research are to screen the potential free radicals scavenger activity of in vitro using DPPH (1,1 diphenyl-2-picryl-hydrazil) radicals and NO• (nitric oxide) radicals, and antibacterial activity of Purwoceng. The extraction is done by a maceration method with petroleum ether, ethyl acetate, and ethanol solvent, respectively. Free radicals scavenger test was performed using DPPH radicals and NO• radicals, while antibacterial activity screening was performed using agar diffusion test. The results showed that ethyl acetate extract of Purwoceng has free radical scavenger activity with $IC_{50}$ 53.07 ppm lower than butylated hydroxytoluene. Ethyl acetate extract and ethanol extract of Purwoceng have antibacterial activity against *Staphyloccus aureus*, *Escherichia coli*, and MG42 bacterial isolate.

**Keywords:** Purwoceng, Radical Scavenger, Antibacterial

## INTRODUCTION

Purwoceng (*Pimpinella alpine* Molk) is one of traditional medicinal plant that has an androgenic effect and used as aphrodisiac. Purwoceng is an endemic plant of Indonesia (Usmiati 2010), that grows at Dieng plateu, Central Java. Purwoceng has been reported to contain steroid, flavonoids, glycoside, saponins, tannins, and phenolic (Ma'mun *et al.* 2011). Based on secondary metabolite compounds of Purwoceng herbs, research needs to be done to discover the other potential activities i.e. free radicals scavenger and antibacterial activities of Purwoceng.

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Many studies had reported the androgenic effect of Purwoceng. Treatment of Purwoceng extract could enhance vitality which is indicated with increasing testosterone and luteinizing hormone (LH) (Nasihun 2009). A chloroform extract of this plant showed the highest aphrodisiac activity compare to other extract and the roots of Purwoceng contain stigmasterol (Suzery *et al.* 2005). Unfortunately, this plant does not have estrogenic activity (Susanti & Dhiani 2012).

Flavonoid and phenolic compounds in this plant may result antioxidant and antibacterial activities. In this research, the antioxidant activity of ethyl acetate extract and the fraction were evaluated in DPPH and nitric oxide assay. The antibacterial activity of these extracts and fractions were accessed using the agar diffusion method.

## MATERIAL AND METHODS

### Plant Material

*Pimpinella alpina* Molk (Purwoceng) was collected from Dieng, Wonosobo, Central Java, Indonesia. The herbs (all parts of the plant) were collected manually and then dried using drying cabinet at 60°C.

### Microorganism Strain

*Staphylococcus aureus* and *Escherichia coli* used in this study were obtained from Microbiology Laboratory University of Muhammadiyah Purwokerto. MG42 bacterial isolate was a Gram positive-isolated bacterial from soil that IS resistant to amoxicillin, cotrimoxazole, and oxytetracyclin.

### Extraction of Plant Material

The powder of *P. alpina* herbs (530 g) were macerated in petroleum ether for 3 days. The residue was then macerated in ethyl acetate for 3 days. Lastly, residue was macerated in ethanol 96%. Each filtrate was evaporated using rotary evaporator and yielded 3 extracts namely petroleum extract, ethyl acetate extract, and ethanol extract.

### DPPH Radical Scavenging Assay

The radical scavenging ability of extract was measured using stable free radical DPPH (Gulluce *et al.* 2006). Methanol solution (5 mL) of extract in various concentration was added to 1 mL (0.4 mM) methanol solution of DPPH. The decrease of absorbance at 516 nm was noted after 30 minutes. The percentage of radicals scavenging was determined the following formula:

$$\text{Scavenging of DPPH (\%)} = \frac{\text{(Absorbance of blank – Absorbance of sample)}}{\text{Absorbance of blank}} \times 100\ \%$$

**Nitric Oxide Scavenging Assay**

A total of 2 mL 10 mM sodium nitroprusside solution was added with 500 µL phosphate buffer saline (pH 7.4), followed by extract and fraction solution in different concentrations in methanol and incubated in 25°C for 150 minutes. The samples from the above were reacted with Griss reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dihidrochloride, and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotisation of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihidrochloride was read at 478 nm.

**Antibacterial Screening**

The antibacterial screening was conducted with agar diffusion technique. A total of 100 µL of each species ($1 \times 10^8$ CFU/mL) was poured in 20 mL NA. Ten microliter (10 µL) of extract solution in various concentrations was applied to plate. To compare the activity with standard antibiotic, ciprofloxacin (2.5 µg/disc) was used. Disc containing 10 µL dimethylsulfoxide was used as a negative control. The discs were then incubated at 37°C for 24 hours to allow bacterial growth, after which the zones of inhibition of desired growth could be easily measured. The zone of inhibition was considered as an indicator for the antibacterial activity. At the end of the incubation period, the antibacterial activity was evaluated by measuring the inhibition zones in mm.

## RESULTS AND DISCUSSION

**Radical Scavenging Assay**

Two methods have been used to measure the radical scavenging of Purwoceng: DPPH radical scavenging assay and nitric oxid. In these two assays, ethyl acetate extract of Purwoceng had lower activity than the positive controls BHT and quercetin. DPPH radical has been widely used to evaluate the radical scavenging of antioxidant. DPPH is nitrogen centred free radical. The colour is violet and it is converted to yellow colour because of hydrogen or electron donating ability of antioxidants present in tested extract (Sadiq *et al.* 2015). Flavonoid, polyphenol, and tannins were compounds that have antioxidant activity as radical scavenger because these compounds have hydroxyl group in their aromatic structure. Recently, many research reported that compounds in fruits, vegetables, and herbs have antioxidant activity.

Nitric oxide because of its unpaired electron, is classified as a free radical and displays important reactivity with certain types of proteins and other free radicals. In vitro inhibition of nitric oxide radical is also a measure of antioxidant activity. This method is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in buffer saline and measured by Griess reagent (Joseph *et al.* 2010). Colour complex is formed due to the reaction of nitric oxide and Griess reagent and diazotisation of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine. The radical scavenging is measured based on $IC_{50}$ (Tharun & Pindi 2013).

Qualitative identification using thin layer chromatography showed that ethyl acetate extract had flavonoid group compound. The results of radical scavenging activity using two assay were showed in Table 1. The data showed that ethyl acetate extract had antiradical activity but lower than BHT and quercetin.

**Table 1:** The radical scavenging activities of Purwoceng extract.

| Sample | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | DPPH assay | NO |
| Ethyl acetat extract of Purwoceng | 53.07 | 52.60 |
| BHT | 5.97 | 4.31 |
| Quercetin | 0.84 | 1.11 |

*Notes:* DPPH = 1,1 diphenyl-2-picryl-hydrazil*;* NO = nitric oxide.

**Antibacterial Activity Screening**

The antibacterial activities of different extract were tested by agar diffusion method were shown in Table 2. All of the three extracts showed weak antibacterial activity and had lower zone inhibition than positive control ciprofloxacin. Among the three extracts, ethyl acetate extract showed the strongest antibacterial activity against all microorganism tested.

Screening of chemical groups in the extract of Purwoceng revealed presence of alkaloid, tanin, flavonoid, triterpenoid, steroid, dan glycoside (Ma'mun *et al.* 2006). Based on the results of chemical composition, this study showed that antibacterial activity of the three extracts especially the ethyl acetate extract is apparently related to these compounds. Flavonoids is the family compound that is the subject of much antibacterial research. Specific intracellular or surface enzymes are the targets of flavonoid in antibacterial activity (Cushnie & Lamb 2011).

**Table 2:** The antibacterial activities of Purwoceng extracts.

| Microorganism | Zone of inhibition (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Petroleum ether extract | | | Ethyl acetat extract | | | Ethanolic extract | | | DMSO | Ciprofloxacin |
| | 50 mg/mL | 100 mg/mL | 200 mg/mL | 50 mg/mL | 100 mg/mL | 200 mg/mL | 50 mg/mL | 100 mg/mL | 200 mg/mL | | 7.5 µg/disc |
| *Staphylococcus aureus* | 0.00±0.00 | 7.45±0.93 | 10.5±0.95 | 0.00±0.00 | 7.50±1.13 | 9.97±0.25 | 0.00±0.00 | 0.00±0.00 | 9.50±0.60 | 0.00±0.00 | 28.06±0.32 |
| *Escherichia coli* | 0.00±0.00 | 7.67±0.58 | 10.20±0.46 | 7.36±0.15 | 8.10±0.72 | 11.0±0.60 | 0.00±0.00 | 6.60±0.56 | 8.40±0.60 | 0.00±0.00 | 28.56±0.59 |
| MG42 bacterial isolate | 0.00±0.00 | 0.00±0.00 | 9.53±0.96 | 0.00±0.00 | 6.83±0.15 | 9.67±1.76 | 0.00±0.00 | 0.00±0.00 | 7.11±0.39 | 0.00±0.00 | 27.46±0.50 |

## CONCLUSION

It can be concluded from this study that ethyl acetate extract of Purwoceng (*Pimpinella alpina* Molk) had free radical scavenger activity and also showed weak antibacterial activity against *S. aureus*, *E. coli*, and MG42 bacterial isolate.

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