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In Vitro Antioxidant Activity of Methanolic Extract of *Piper retrofractum* Vahl.

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Abstract. Cabai jamu (Piper retrofractum Vahl.), which is originally from Indonesia and is extensively cultivated and traditionally used in Sumenep, Madura possess high potential medicinal properties. Therefore, providing scientific rationale of their traditional usage would be necessarily required. This research aimed to investigate the antioxidant activity of methanolic extract of P. retrofractum Vahl. using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. This research was carried out using methanol concentrations of 0, 5, 15, 30, 45 and 60 ppm. In addition, ascorbic acid was used as the standard antioxidant. Meanwhile, the parameters measured are the extract yield, percentage of inhibition and IC50. The results showed that the DPPH activity of the extracts was increased in a dose dependent manner, which was found in the range of 0-31.53% as compared to ascorbic acid (0-43.19%). The IC50 values of methanol extract in DPPH radical was obtained to be 101. 74 ppm. Meanwhile, the IC50 value of the ascorbic acid was found to be 66.12 ppm. This result indicates that methanol extract of P. retrofractum possess mild antioxidant activity. Therefore, further investigation using other solvent extracts need to be carried out to evaluate the antioxidant compounds present in the plant extract.

Keywords: Antioxidant, Ascorbic acid, DPPH assay, Piper retrofractum Vahl.

Introduction

Oxygen is extremely important for sustaining life. Most aerobic organisms use oxygen for obtaining their energy *via* cellular respiration [1]. However, this redox-based reaction could generate free radicals, which can be defined as a chemical species possessing an unpaired electron [2]. Therefore, they are highly unstable and could cause, in some extent, oxidative damage to other biomolecules by extracting electrons from them to attain stability [3]. Free radicals, including reactive oxygen species (ROS), possess dual biological role in all living organisms. At low until moderate quantity, they actively mediate cellular responses and immune reactions. Meanwhile, an excess production of ROS generates oxidative stress, which could damage cellular biomolecules (e.g. DNA, lipids, proteins, amines and carbohydrates) and affecting some serious detrimental effects on cell structure and cellular metabolisms [4]. In many reports, oxidative stress might be responsible for some chronic and degenerative ailments such as cancer, severe allergic-based diseases, atherosclerosis, diabetes, arthritis, neurodegenerative diseases and others [5-9]. Therefore, development of antioxidant based drugs offers a good pharmaceutical interest. In addition, natural based antioxidant has also attracted much attention in recent decades.

Some fruits and vegetables have demonstrated their antioxidant activities [10]. These activities are related to their phytochemicals such as phenols, carotenoids, vitamins and flavonoids [11]. Cabai jamu (*Piper retrofractum* Vahl.) is originally from Indonesia and is extensively cultivated in Sumenep, Madura. They possess high potential medicinal properties [12]. However, little information has been reported about their antioxidant properties. Therefore, providing scientific rationale of their traditional usage would be necessarily required. In the present study, the

methanolic extracts of the *P. retrofractum* fruits were used to investigate the antioxidant activity in term of DPPH scavenging capacity.

Material and Methods

Plant material

Plant material was collected from Sumenep Region, Madura Island, Indonesia. The plant was identified following dichotomous key based on "Atlas Tumbuhan Obat" [13] and Flora [14]. The fruits were washed with tap water and dried under shade for a week.

Preparation of methanolic extracts

150 g fruit powder, placed in beaker glass, was soaked in 400 ml methanol and left to macerate in the dark, at ambient temperature for 48 h. The extract was then placed and agitated in a flask for 1 h. Subsequently, the extract was then filtered over Whatman No. 1 filter paper. The extraction process was repeated three times. Finaly, the filtrate was concentrated at 40°C by rotary evaporator. The yield of the extract was measured using:

$$P_r = \frac{B_e}{B_s} \times 100\%$$

where *Pr* is percentage yield of the extract, *Be* represents the weight of the free-dried methanolic extract (gr), while *Bs* represents the original weight of the sample (gr).

Free radical scavenging activity

The free radical scavenging activity of the methanolic extract of *P. retrofractum* was analyzed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) according to [15]. 100 ppm solution of DPPH in methanol was prepared and 1 ml of this solution was added to various concentrations of methanolic extracts (5, 15, 30, 45 and 60 ppm). After 30 minute, absorbance was measured at 517 nm. Ascorbic acid was used as reference material. All the tests were performed in duplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$P_i = \frac{A_b - A_s}{A_b} \times 100\%$$

Where *Pi* is the percentage of inhibition, *Ab* is the absorbance of the control and *As* is the absorbance of the extract/reference. The percentage of inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value. The lower IC_{50} value indicates high antioxidant capacity.

Statistical analysis

The experimental data obtained in the present study were expressed as mean ± SD. Linear regression was used to calculate the IC_{50} values. One-way analyses of variance followed by Post-Hoc Tukey test were used. The *P* values of less than 0.05 were adopted as statistically significant.

Result and Discussion

Extraction of *P. retrofractum* fruit

Extraction is a method for separating compounds found in material based on their polarities. The materials could be treated with polar or non-polar solvents. In the present study, we used maceration procedure and methanol as solvent. This technique was performed at room temperature by mixing the grounded *P. retrofractum* fruit with methanol. The mixture was left for several hours with occasional shaking. The overall process was repeated in three times with fresh solvent [16]. Many reports have showed that methanol have been extensively used to extract antioxidant compounds from various plants and plan-based foods [17-18]. Sarker *et al*. [19] reported that methanol has also been used for extracting polar compounds such as phenol, flavonoids, glycosides and several alkaloids. The result of the extraction showed that from 150 gr *P. retrofractum* fruit we were capable of extracting 6.06 gr methanol extract or about 0.0404 % (Table 1).

Table 1. Extraction of *P. retrofractum* fruit using methanol solvent

Solvent	Starting material (gr)	Extract (gr)	Yield (%)
Metanol	150	6,06	0,0404

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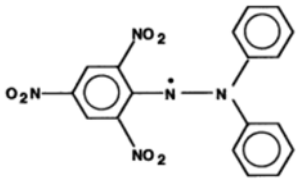
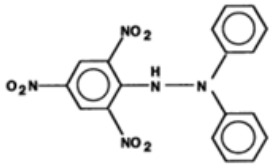
DPPH Radical Scavenging Activity

The *in vitro* antioxidant was performed using DPPH (*diphenylpicrylhydrazyl*) assay. This method was chosen due to their simplicity. DPPH radicals are commonly used for investigating scavenging activity of various phyto-compounds. It is a stable free radical compound and capable of accepting electrons or hydrogen radicals from other compounds to form a stable diamagnetic molecule. DPPH will encounter a proton donating substance such as an antioxidant, where it would be scavenged and thus the absorbance is reduced [20]. The final reaction produced diphenyl pycril hydrazine [21] (Fig. 1).



2,2-diphenyl-1-picryl-hydrazyl



1,1-diphenyl-2-picryl-hidrazin

Figure 1. Chemical structure of DPPH (free radical) and its nonradical form (Molyneux, 2004).

In this present study, the methanolic extract of P. retrofractum fruit were analyzed in comparison with ascorbic acid as antioxidant standard. The results showed that the DPPH activity of the extracts was increased in a dose dependent manner, which was found in the range of 0-31.53% as compared to ascorbic acid (0-43.19%). The IC50 values of methanol extract in DPPH radical was obtained to be 101.74 ppm. Meanwhile, the IC50 value of the ascorbic acid was found to be 66.12 ppm (Table 2). This result indicates that methanol extract of P. retrofractum possess mild antioxidant activity [22]. Therefore, further investigation using other solvent extracts need to be carried out to evaluate the antioxidant compounds present in the plant extract.

Table 2. DPPH radical scavenging activity of methanolic extract of *P. retrofractum* Vahl.

Compounds	Concentration (ppm)	% of Inhibition	IC_{50} (ppm)
Methanol extract of *P. retrofractum*	0	0,000^{d}	101,74
	5	2,298^{d}	
	15	6,239^{cd}	
	30	14,449^{bc}	
	45	18,719^{b}	
	60	31,527^{a}	
Ascorbic Acid	0	0,000^{d}	66,12
	5	3,119^{d}	
	15	3,448^{cd}	
	30	21,346^{bc}	
	45	37,931^{b}	
	60	43,185^{a}	

Note: Values followed by the same letter are not significantly different at α 0,05 (Tukey Test)

Based on the above results, *P. retrofractum* Vahl., which now become one of the important industrial crops in Sumenep, Madura posssess potentially natural antioxidant properties. This finding supports the traditional uses of *P. retrofractum* Vahl. as a medicinal herb mixture by local people [12]. Some previous reports have showed that this plant have been used as an external medicine for several diseases treatment such as rheumatism, influenza, chronic bronchitis, headaches and in some extent, they have a potential activity as an anti-allergy [23-24]. However, the mechanism of their activity as an anti-allergy remains poor.

Conclusion

The DPPH scavenging activity of methanol extract of P. retrofractum fruit in several concentrations (0-60 ppm) is ranging between 0-31.53%. Meanwhile, the IC50 values of methanol extract in DPPH radical was obtained to be 101. 74 ppm. It showed that methanol extract of P. retrofractum fruit possess mild antioxidant activity. Therefore, further investigation using other solvent extracts need to be carried out to evaluate the antioxidant compounds present in the plant extract.

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