

In Vitro Antioxidant Activity of *Zingiber officinale*, *Piper retrofractum*, and Their Combinations

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Abstract

Many plants are used empirically as antioxidant. Plants that are frequently used in Indonesian communities are *Zingiber officinale* and *Piper retrofractum*. The aim of this research was to investigate the in vitro antioxidant activity of single ethanolic extract and the combinations of *Z. officinale* and *P. retrofractum* using free radical scavenging DPPH (1,1-diphenyl-2-picrylhydrazyl) method. *Z. officinale* and *P. retrofractum* were extracted by maceration using 95% ethanol for 3 × 24 hours. Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The concentration of the extract ($\mu\text{g/mL}$) that was required to scavenge 50% of free radicals (IC_{50}) was calculated using the percent scavenging activities of six different extract concentrations. The results showed that the single ethanolic extract of *Z. Officinale* produced the highest antioxidant activity with IC_{50} of 56 $\mu\text{g/mL}$, while the antioxidant activity of the single ethanolic extract of *P. retrofractum* produced an IC_{50} of 3.445 $\mu\text{g/mL}$. The IC_{50} of combination of *Z. officinale* and *P. retrofractum* ethanolic extracts at concentration ratios of 1 : 2, 1 : 1, and 2 : 1 were 148 $\mu\text{g/mL}$, 85 $\mu\text{g/mL}$, and 73 $\mu\text{g/mL}$.

Keywords: *Zingiber officinale*, *Piper retrfractum*, Antioxidant, DPPH

INTRODUCTION

Oxidative stress induced by Reactive Oxygen Species (ROS) is implicated in the pathogenesis of a variety of vascular and neurological diseases, including cancer, atherosclerosis, hypertension, coronary artery diseases, and other diseases. Damage due to free radicals caused by ROS leads to several downstream effects as they can attack lipids, protein or enzymes, carbohydrates, and DNA in cells and tissues. They induce undesirable oxidation, causing membrane damage, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation (Singh *et al.*, 2004). Antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors (Senevirathne *et al.*, 2006).

Plants that can be a potential source of natural antioxidants as *Zingiber officinale* and *Piper retrofractum* (Jagdale *et al.*, (2009); Shirin and Parakash, (2010)). *Z. Officinale* contains gingerol compounds and derivatives such as polyphenols that have antioxidant effects (Ghosh, 2011). *P. retrofractum* contains essential oils such as terpenoids that have been reported as

antioxidants (Grassman, 2005). According to Nakatani, *et al.*, (1986) and Kametani, *et al.*, (2005) compounds that act as antioxidants in *P. Retrofractum* is feruperine. According Dyatmiko (2000) piperine and amide phenol compounds in the *P. retrofractum* has antioxidant activity.

The development of traditional or natural medicine at this time leads to the use of a combination of crops (Beinfeld and Korenglod, 2005). The combination of several types of antioxidants provide better protection against oxidation compared with only one type of antioxidant (Simanjuntak *et al.*, 2008). The present study aimed to evaluate the antioxidant activity of *Z. officinale*, *P. retrofractum* and their combinations use of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) for estimating antioxidant activity.

MATERIALS AND METHODS

Material

Z. officinale, *P. retrofractum*, ethanol, aquades, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (ABCR GmbH, Germany). All chemicals and reagents used were analytical grade.

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Preparation of extracts

Z. officinale and *P. retrofractum* extracted by maceration using 96% ethanol for 3 x 24 hours. The filtered extract was concentrated under reduced pressure to remove the solvent.

Antioxidant assay (DPPH free radical scavenging activity)

The free radical scavenging activity of different concentration of single ethanolic extract and their combinations (1:2), (1:1), and (2:1) from *Z. officinale* and *P. retrofractum* were measured using DPPH. The method modified by Brand-Williams (1995) and James, et al. (2009). Two milliliters of each dilution of single ethanolic extract and their combinations were added to two ml of 100 µM solution of DPPH in ethanol. After 30 minutes absorbance was measured at λ 517nm using a spectrophotometer. A 100 µM solution of DPPH in ethanol was used as DPPH control. All tests were performed in triplicate. Percent inhibition was calculated using the following equation, where Abs (sample absorbance), and Abk (DPPH control absorbance). The mean of three IC₅₀ (concentration causing 50 % inhibition) value of each sample extract was determined graphically.

$$\% \text{ inhibition} = [(Abk - Abs) / Abk] \times 100$$

RESULTS AND DISCUSSION

The concentration of the extract (µg/mL) that was required to scavenge 50% of radicals

(IC₅₀) was calculated by using the percent scavenging activities of six different extract concentrations. The IC₅₀ was calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs. the corresponding scavenging effect. The smaller the IC₅₀ of a compound, the higher activity compound as antioxidant.

Table I showed the free radical scavenging activity (IC₅₀) of different concentration of single ethanolic extract and their combinations (1:2), (1:1), and (2:1) from *Z. officinale* and *P. Retrofractum*. The results showed that the single ethanolic extract of *Z. Officinalle* showed the biggest antioxidant activity (IC₅₀ 56 µg/mL), while the antioxidant activity (IC₅₀) of the single ethanolic extract of *P. retrofractum* was 3.445 µg/mL. Antioxidant activity (IC₅₀) combination of *Z. officinale* and *P. retrofractum* ethanolic extract (1:2), (1:1), and (2:1) were 148 µg/mL, 85 µg/mL, and 73 µg/mL. The combination of higher types of antioxidants (*Z. officinale* IC₅₀ 56 µg/mL) with lower types (*P. Retrofractum* IC₅₀ 3445 µg/mL) provide better protection as antioxidant compared with only one type of lower antioxidant. The observed differential scavenging activities of the extracts against the DPPH system could be due to the presence of different compounds in the extract. According to Djeridane, et al., (2006), the antioxidants in the combination extract makes the antioxidant activity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants.

Table I. In vitro antioxidant activity of various extract sample

Extract Sample	IC ₅₀ (µg/mL)	Mean IC ₅₀ (µg/mL)
<i>Z. officinale</i> (single extract)	58	56
	55	
	56	
<i>P. retrofractum</i> (single extract)	3517	3445
	3570	
	3246	
<i>Z. officinale</i> and <i>P. Retrofractum</i> (Combination 1:2)	144	147
	156	
	144	
<i>Z. officinale</i> and <i>P. Retrofractum</i> (Combination 1:1)	79	85
	81	
	96	
<i>Z. officinale</i> and <i>P. Retrofractum</i> (Combination 2:1)	73	73
	74	
	70	

The free radical scavenging activity was investigated in DPPH assay. Scavenging of DPPH free radical is the basis of a common antioxidant assay (Sharma and Bhat, 2009). The molecule of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical because of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour in ethanol solution (Molyneux, 2004).

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour, although there would be expected to be a residual pale yellow colour from the picryl group still present (Molyneux, 2004), thus we have estimated the antioxidant activity through free radical scavenging of single ethanolic extract and their combinations (1:2), (1:1), and (2:1) from *Z. officinale* and *P. Retrofratum*. Antioxidant activities of plant extracts were usually linked to their phenolic

content. *Z. officinale* contains gingerol compounds and derivatives such as polyphenols that have antioxidant effects (Ghosh, 2011) and according to Dyatmiko (2000) piperine and amidephenol compounds in the *P. Retrofratum* has antioxidant activity. DPPH scavenging activity of phenolics is positively correlated with the number of hydroxyl groups. The phenolic compounds contribute significantly to the antioxidant capacity of the plants, especially phenolic acids and flavonoids (Djeridane *et al.*, (2006); Ignat *et al.*, 2011)). Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones (Djeridane *et al.*, 2006). The antioxidant activity of phenolic compounds depends on the structure, the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings (Balasundram *et al.*, 2006). Antioxidants combat oxidative stress by working to neutralise excess free radicals and stopping them from starting the chain reactions that contribute to various diseases and premature aging (Singh *et al.*, 2004).

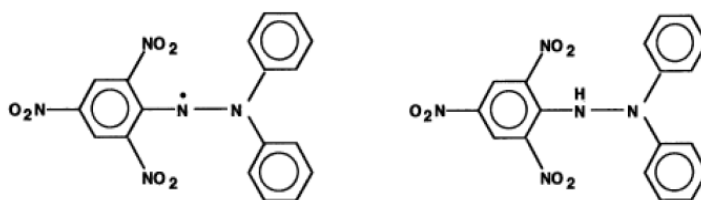


Figure 1. Structure free radical and nonradical DPPH

CONCLUSION

The results showed that the single ethanolic extract of *Z. Officinale* showed the biggest antioxidant activity (IC_{50} 56 $\mu\text{g/mL}$), while the antioxidant activity (IC_{50}) of the single ethanolic extract of *P. retrofratum* was 3.445 $\mu\text{g/mL}$. Antioxidant activity (IC_{50}) combination of *Z. officinale* and *P. retrofratum* ethanolic extract (1:2), (1 : 1), and (2 : 1) were 148 $\mu\text{g/mL}$, 85 $\mu\text{g/mL}$, and 73 $\mu\text{g/mL}$.

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