ANTIOXIDANT ACTIVITY OF ROASTED KEDAWUNG SEED (Parkia timoriana) USING SCAVENGER FREE RADICAL DPPH METHOD

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ABSTRACT

Kedawung seeds (Parkia timoriana) contain polyphenolics and flavonoids that can provide pharmacological activities, such as antioxidants. In several regions of Indonesia, kedawung seeds are preserved using the roasting method. This study aimed to analyze the effect of roasting on the content of phytochemical compounds and determine their antioxidant activity using the DPPH method. Roasted kedawung seeds were extracted using a maceration method in ethanol. Phytochemical screening was performed to qualitatively analyze the contents of several phytochemical compounds in the extract. The results showed that roasted kedawung seed extract contained alkaloids, flavonoids, saponins, and terpenoids. Antioxidant testing was performed using the DPPH method with ascorbic acid as a positive control. The IC_{50} value of ascorbic acid, P. timoriana seed and roasted seed of P. timoriana measured each 12.3 ppm; 20.6 ppm and 9.8 ppm; which categorized as strong antioxidant compounds. These results indicated an increase in the antioxidant effect after roasted seed processing. It concluded that the roasting process could enhance antioxidant effect of P. timoriana seed

Keywords: *Kedawung seeds, roasted method, antioxidant, DPPH*

INTRODUCTION

Kedawung (*Parkia timoriana*), is one of the tropical plants on Java Island that is commonly used as a traditional medicine. In several regions of Java, kedawung seeds are used to treat nausea, diarrhea, and common fever. The seed is known to contain a variety of phytochemical compounds, including alkaloids, polyphenols, flavonoids, tannins, and several lipid compounds (Suryanti *et al.*, 2022). Previous studies have shown the effectiveness of seed extract as an antidiabetic agent through inhibition of α-glucoside and amylase enzyme (ethyl and methanol extract), an analgetic, anticancer, broad-spectrum antibacterial (ethanolic extract), and antioxidant (methanol and aquos extract) (Sheikh *et al.*, 2016; Angami *et al.*, 2018; Ralte *et al.*, 2022; Suryanti *et al.*, 2022).

This preservation method is commonly used to slow the degradation of compounds and prolong their storage. Plant-based medicine without prior preservation can rot easily. Traditional preservation processes commonly use water-reducing methods such as salting, roasting, and smoking. The most popular method for seed preservation is roasting (Wijaya, 2019). Some studies have confirmed that roasting several seeds can reduce water content and significantly increase polyphenols and flavonoid compounds. This change impacts pharmacological activities, such as antioxidants, which are usually affected by poliphenols groups (Lee *et al.*, 2015; Ajatta *et al.*, 2019; Akomolafe, 2021).

Previous literature shown limited information on influence of roasting application on P. Timoriana seed. This study aimed to investigate the antioxidant activity of roasted P. timoriana seeds. Antioxidant activity was evaluated using the free scavenger DPPH method, while antioxidant activity was evaluated based on the IC_{50} value of both roasted and unroasted P. timorina seeds.

RESEARCH METHODS

This study focused on the antioxidant evaluation of raosted seeds compared to that of unroasted seeds. Qualitative tests of the phytochemical groups were conducted on both extracts. Antioxidant activity was measured using the DPPH method with ascorbic acid as the positive control.

Equipment and Materials

The equipment used in this research were glass laboratorium set (Beaker glass, Erlenmenyer glass, tube reaction etc), spectrophotometry UV-Vis (Shimadzu-1900), analytical digital scales (Ohaus), micro pippet (Ohaus), blue tip, yellow tip, Buchner funnel, Rotary Evaporator (Buchi), waterbath, vacuum Pump dan Oven (Memmert).

The material used were P. Timoriana seed, ethanol, chloroform, lead acetat, HCL, H_2SO_4, NaOH, $AlCl_3$, HNO_3, Wagner reagent, Mayer reagent, gallic acid (Sigma), Dragendroff reagen, $FeCl_3$, aquadest, ascorbic acid (sigma), and DPPH (Sigma)

Research Procedure

1. The P. Timoriana seed roast and extraction of seed

Seeds were collected from West Java (Cirebon Region). The roast of seed proceed using the oven at 105-110ºC for 15 minutes without mixing process. Powders of both unroasted and roasted seeds were extracted using ethanol for 3 × 24 hours. The macerate was filtered using a Buchner funnel and evaporated using a rotary evaporator to condense the extract further.

2. Phytochemical screening

- Terpenoid : 3 mL of extract was dissolved in 2 mL chloroform, and then add 3 drops of concentrated H_2SO_4. Positif will be indicated by a reddish-brown ring in the chloroform phase (Singh and Mathur, 2016)
- Flavonoid: the amount of 3 drops of HCL (2N) were added to 10 mL of the extract. The mixture was then heated to 80 °C for 15 minutes. The reddish-brown color indicates flavonoids (Febriani, Fidrianny and Elfahmi, 2017).
- Alkaloids: 10 drops of Wagner and Mayer's reagents were added to 3 mL of the extract were added to every. Positive results were indicated by brown precipitates (Wagner) and white precipitates (Mayer) (Singh and Mathur, 2016).
- Saponin: 3 mL extract diluted in 2 mL aqueous solution, and then 1 drop of HCL (2N) was added. The mixture was shaken for 20 seconds. Foam that persisted for more than 1 minute (> 1 cm high) indicated saponin within the sample (Auwal *et al.*, 2014).
- Tanin: The amount of 3 drops of lead acetate 1% were added to 5 mL of extract. The white precipitate indicates tannins in the extract (Mitra, Naskar and Chaudhuri, 2021).

3. Antioxidant test using free scavenger DPPH method

DPPH solution (1 mL, 200 ppm) was added to 2 mL of extract at different concentrations (500, 250, 125, and 62.5 ppm) and stored in the dark for 15 minutes. Ascorbic acid was used as a positive control. After incubation, the absorbance of the mixture was measured using UV-Vis spectrophotometry at 517 nm (Bruck de Souza *et al.*, 2020). The IC_{50} value was calculated by linear regression of the test groups using the Ms Excell software. The percentage inhibition of free radicals was measured using the following equation:

$$\%\ \text{free radical inhibition} = \frac{Blanko\ absorbance - Sample\ Absorbansi}{Absorbansi\ Blanko}\ X\ 100$$

RESULTS AND DISCUSSION

The phytochemical content of plants can be changed according to various external factors, such as human intervention, processing methods, and the environment. Polyphenols and flavonoids have different roles in the prevention of oxidative stress caused by free radical compounds. Over the years, various studies have shown that oxidative stress is among the top factors that cause degenerative disease (Jasmin, Frank and Lisanti, 2012; Schieber and Chandel, 2014). Natural antioxidants have been used as a natural approach to prevent these diseases and could also enhance immunity in humans.

P. timoriana seeds are a plant-based traditional medicine. Some studies have shown that fresh seeds of P. timoriana are rich in essential amino acids, unsaturated lipids, minerals (potassium, magnesium, zinc), polyphenols, tannins, and flavonoids (Angami *et al.*, 2018; Ralte *et al.*, 2022; Suryanti *et al.*, 2022). However, several regions in Java are more used to roasted seeds as ingredients of jamu or spice than the fresh seed of P. timoriana Even then, there is limited information on the phytochemical compound of the roasted seed of P.timoriana. The investigation of phytochemical compounds in the roasted and unroasted seeds of P. timoriana showed positive results in all phytochemical tests. While there was no further information on the quantitative changes in these compounds, this result showed no different compounds in either extract. The results of the phytochemical screening are presented in **Table I**.

Table I. Phytochemical Screening of Roasted Seed Extract

Phytochemical groups	Results	
	Unroasted seed extract	Roasted seed extract
Saponin	+	+
Flavonoid	+	+
Tannin	+	+
Alkaloid	+	+
Terpenoid	+	+

Note :
(+) positive ; (-) negative

The DPPH scavenger test is widely known as a colorimetric test for antioxidants and is reliable, simple, and accurate. This method uses the change in color, which represents the inhibition process of free radicals. The percentage inhibition of free radicals was measured by comparing the absorbance of the positive groups and samples (Ak and Gülçin, 2008; Bruck de Souza *et al.*, 2020). The antioxidant results showed concentration-dependent activity in each concentration group. The IC_{50} values of ascorbic acid, EtOH extract, and roast EtOH extract (roast) each measured at 12.3 ppm; 20.6 ppm and 9.8 ppm; and the results are shown in **Figure 1**.

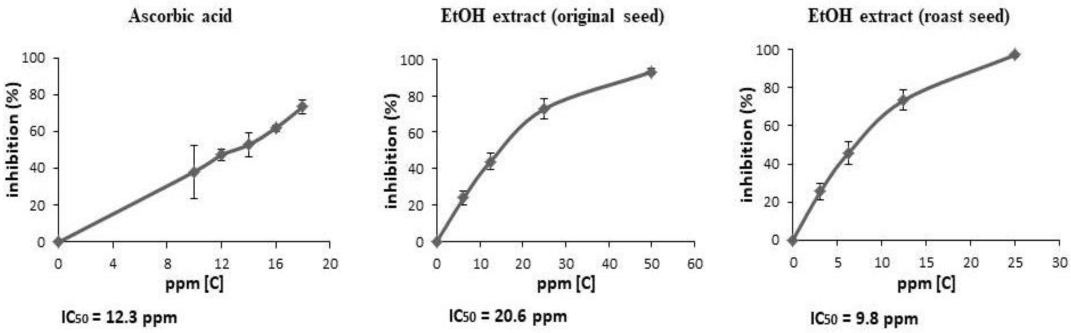

Figure 1. Antioxidant Results of the Extract and Positive Groups Using the Free Radical DPPH Scavenger Method

Several plant preservation methods have been developed. Some tribes use unique methods to preserve the plants that maintain the phytochemical compounds during storage. Roasting is a popular preservation method that uses high temperatures to reduce the water content and avoid the rooting process of plants (Ajatta *et al.*, 2019). This method is usually used in the seed parts of plants, such as pumpkin seeds, coffee, etc. Many studies have shown phytochemical changes after a roasting process that lead to the enhancement of pharmacological activity, such as antioxidants (Lee *et al.*, 2015; Akomolafe, 2021).

Previous studies on P. timoriana showed a strong antioxidant effect in extracts that used a variety of solvents with different polarities (Suryanti *et al.*, 2022). This study also showed strong antioxidant activity in the ethanol extract of seeds, indicating similar results to those of a previous study. The roasted seed also enhanced this effect better than the non-roasted seed and ascorbic acid. During roasting, the hydrating process led to an increase in some polyphenol and flavonoid groups, which further increased the antioxidant effect. This process is assumed to follow a few chemical reactions, such as the Maillard reaction, which initiates a reaction between amino acids and reduced sugars, catalyzed by high temperatures. The products of the Maillard reaction become the dominant antioxidant species, such as polyphenols. (Han *et al.*, 2022; Teng *et al.*, 2023).

This research is limited to the investigation of the antioxidant properties of roasted P. Timiriana seed. Even so, there is a lot of information that can be explored. Hence, for further research, we suggest advanced phytochemical analysis to determine each phytochemical compound.

CONCLUSION

This study concluded that roasting enhances the antioxidant effect of P. timoriana seeds.

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